

GenomeLab GeXP Genetic Analysis System

User Guide

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Notes and Warnings

This section provides safety information and instructions for the hardware and accessories of the system. It includes the following topics:

- Safety Symbols
- Chemical Precautions
- Electrical Safety
- Moving Parts
- Laser Safety

The following information describes the notes and warnings used in this document.

Note: Contains supplemental or explanatory information concerning the current topic or procedural step.

IMPORTANT Used whenever an action or circumstance may potentially cause personal injury. Mechanical damage may also result. Also can contain information about a possible software program failure, draw attention to a specific software setting or point out that a loss of data may occur if information stated within the paragraph is not adhered to or if procedures are executed incorrectly.

CAUTION: Used to prevent equipment damage. To disregard that caution may cause mechanical damage, however, personal injury is not likely.

WARNING! If the equipment is used in a manner not specified by SCIEX, the protection provided by the equipment may be impaired.

Safety Symbols

The symbols below and on the GenomeLab GeXP system should remind you to read and understand all safety instructions before attempting installation, operation, maintenance, or repair to the GenomeLab GeXP system.

-	
	HIGH VOLTAGE: Paragraphs marked by this symbol indicate that a potential hazard to your personal safety exists from a high voltage source.
	ATTENTION SAFETY SYMBOL: This symbol calls attention to important information to read, or is accompanied by another symbol indicating a particular safety hazard. The information is located either on the label with the symbol or in the GenomeLab GeXP system documentation.
456162-B	BIOHAZARD: Paragraphs marked by this symbol indicate that a potential hazard to your personal safety exists from a biological source.
	LASER LIGHT: Paragraphs marked by this symbol indicate that a potential hazard to your personal safety exists from a laser source.
CAUTION SHARP OBJECTS - REFER SERVICING AND MAINTENANCE TO QUALIFIED SERVICE PERSONNEL. 456853-AB	SHARP OBJECTS: Paragraphs marked by this symbol indicate that a potential hazard to your personal safety exists from unblunted corners or other appendages on the outside or inside of the equipment.
	HOT SURFACE: Paragraphs marked by this symbol indicate that a potential hazard to your personal safety exists from heated surfaces or other appendages on the outside or inside of the equipment.
	PROTECTIVE EARTH OR GROUND TERMINAL: This symbol identifies the location of the protective earth or ground terminal plug on the equipment.
	OFF POSITION OF PRINCIPAL POWER SWITCH: This symbol graphically represents the equipment main power push-button switch when it is in the off position.

Д	ON POSITION OF PRINCIPAL POWER SWITCH: This symbol graphically represents the equipment main power push-button switch when it is in the on position.
CAUTION: TO PREVENT POSSIBLE SYSTEM DAMAGE, ALWAYS EXECUTE "RELEASE CAPILLARY ARRAY" COMMAND PRIOR TO REMOVING ARRAY FITTING. INSTALL ARRAY FITTING WITH FINGER GRIP POINTING DOWNWARD. PRINTED IN U.S.A. 607783-AA	RELEASE CAPILLARY ARRAY COMMAND: Carefully follow the instructions for releasing the capillary array before removing the array fitting.

Chemical Precautions



WARNING! Ionizing Radiation Hazard, Biohazard, or Toxic Chemical Hazard. Determine whether decontamination is required before cleaning or maintenance. If radioactive materials, biological agents, or toxic chemicals have been used with the system, then the customer must decontaminate the system before cleaning or maintenance.



WARNING! Environmental Hazard. Do not dispose of system components in municipal waste. Follow local regulations when disposing of components.

- Determine which chemicals have been used in the system prior to service and regular maintenance. For the health and safety precautions that must be followed for a chemical, refer to the document: *Safety Data Sheet*. For storage information, refer to the document: *Certificate of Analysis*. To find a SCIEX *Safety Data Sheet* or *Certificate of Analysis*, go to sciex.com/tech-regulatory.
- Always wear assigned personal protective equipment, including powder-free gloves, safety glasses, and a laboratory coat.

Note: Nitrile or neoprene gloves are recommended.

- Work in a well-ventilated area or fume hood.
- Avoid ignition sources when working with flammable materials, such as isopropanol, methanol, and other flammable solvents.
- Take care in the use and disposal of any chemicals. There is a potential risk of personal injury if proper procedures for handling and disposal of chemicals are not followed.
- Avoid skin contact with chemicals during cleaning, and wash hands after use.

- Collect all spent liquids and dispose of them as hazardous waste.
- Comply with all of the local regulations for the storage, handling, and disposal of biohazardous, toxic, and radioactive materials.

Electrical Safety

To reduce risk of electrical shock, all devices employ a three-wire electrical cable and plug to connect the equipment to earth ground.

- Ensure that the wall outlet receptacle is properly wired and earth grounded.
- DO NOT use a three-to-two wire plug adapter.
- DO NOT use a two-wire extension cord or a two-wire multiple-outlet power strip.
- Disconnect power to the system before performing maintenance.
- DO NOT remove any panels; panels should be removed only by qualified service personnel.

Moving Parts

Moving parts are limited to the sample handling system. Plate movement is safety interlocked through the sample access cover. To avoid injury due to moving parts, observe the following:

- · Keep loose clothing and hair away from the plate area.
- Never attempt to physically restrict movement of the plate assembly.

Laser Safety

The GenomeLab GeXP system uses a Class 3B laser. The "3B" classification means that "direct intrabeam viewing of this type of laser is always hazardous to personnel."
The laser and several other integral components are housed in a sealed container that together comprise the laser assembly. The laser assembly has no user serviceable parts. Service of the laser assembly is restricted to certified SCIEX field engineers.
During normal operation of the system, laser light is not accessible to the user. Therefore, the overall laser classification of the GenomeLab GeXP system is "Class 1," defined as "lasers which are safe under reasonably foreseeable conditions of operation."
To prevent users from potentially harmful laser light, observe all safety warnings (for label locations, refer to the figure: Figure 1-1) and NEVER REMOVE THE OUTER CASING OF THE LASER ASSEMBLY.

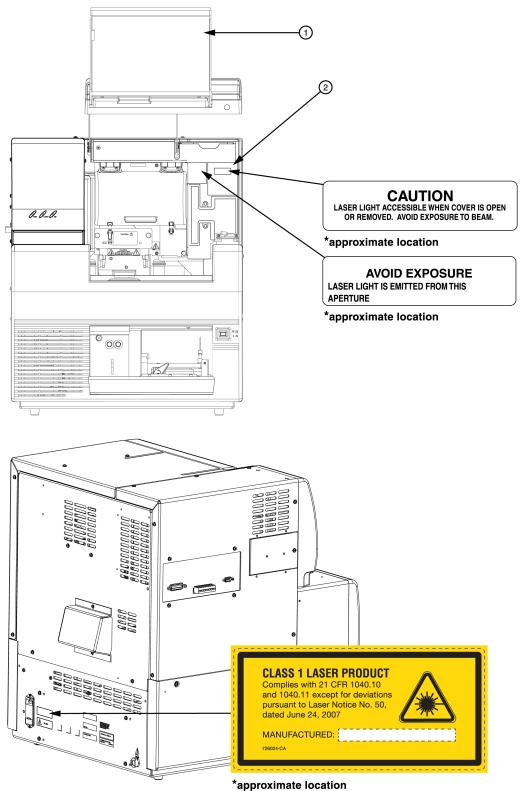


Figure 1-1 Laser Label Locations

ltem	Description
1	Capillary access cover (extended)
2	Laser assembly cover

Electromagnetic Environment

Electromagnetic Compatibility

Basic Electromagnetic Environment: Environment existing at locations characterized by being supplied directly at low voltage from the public mains network.

The equipment is intended for use in a basic electromagnetic environment.

Make sure that a compatible electromagnetic environment for the equipment can be maintained so that the device will operate as intended. If the power supply line is subject to high electrical noise, then install a surge protector.

Electromagnetic Interference

Group 1 Equipment: This equipment is classified as industrial, scientific, and medical (ISM) equipment that might use RF energy for internal operation.

Class A Equipment: Equipment which is suitable for use in all establishments other than domestic and those directly connected to a low voltage power supply network which supplies buildings used for domestic purposes. [Derived from CISPR 11:2009, 5.3] Class A equipment shall meet Class A limits.

CAUTION: Potential Radio Interference. This equipment is not intended for use in residential environments and may not provide adequate protection to radio reception in such environments.

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC (Federal Communications Commission) Compliance Rules.

These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the operator's manual, can cause harmful interference to radio communications.

Operation of this equipment in a residential area is likely to cause harmful interference in which case you will be required to correct the interference, at your own expense. Changes or modifications not expressly approved by the manufacturer could void your authority to operate the equipment.

System Operation and Electromagnetic Interference

Note: The following information addresses the electromagnetic interference (EMI) effect on system performance and provides recommended mitigations.

Under the test conditions specified by the European normative electromagnetic compatibility standard EN 61326-1, the GenomeLab GeXP system may exhibit temporary degradations in performance in accordance with the table below.

Because the environmental circumstances contributing to the problem can vary, several different mitigation techniques have been provided that should help eliminate or reduce the interference.

Test Condition	Effect on Performance	Mitigation
Radio Frequency Field Interference (RFI) system exposed to electromagnetic field strengths of greater than or equal to 3 V/m at multiple frequency bands.	 May cause temporary degradation of data accuracy. In some cases, significant noise may appear in the measured 	 Always run duplicates and controls. Evaluate peaks carefully for anomalous results.
	 results. Separation data will return to normal performance once exposure is removed. 	 Move location of the system by several meters.
	May cause temporary loss of capillary temperature	 Change the orientation of the system by 90 degrees.
	control resulting in lower than expected capillary temperature. The system logs the effect in the run log. It will return to	 Avoid using transmitters or cellular phones within 1 m of the GenomeLab GeXP system. Review the temperatures
	normal performance once exposure is removed.	in the run log for any anomalies and run again as
	May cause temporary loss of capillary temperature control resulting in run not being initiated because temperature cannot reach desired level.	necessary.

Troubleshooting Performance

The GenomeLab GeXP system requires three grounded electrical receptacles. The power line to the laboratory should be connected directly from a main power line transformer of a power source that is known to be clear of erratic power loads, spikes, and electromagnetic interference.

Power lines used with the GenomeLab GeXP system must have an adequate reserve capacity to allow for start-up loads and the additional equipment of this system. SCIEX recommends that the normal loading not exceed 50% of the nominal capacity of the power lines.

Installation of the GenomeLab GeXP system controller on a local network is not required for the system to operate. Installation, support, and administration of local networks or internet connections are the responsibility of the user. Network and internet connections can expose the system to security risks, potential loss of data, and possible system damage caused by hackers, software viruses, or other external factors. It is the user's responsibility to ensure that appropriate security measures, anti-virus software, firewalls, and procedures have been implemented.

Disposal and Recycling

rtant to understand and follow all laws regarding the safe er disposal of electrical instrumentation.
bol of a crossed-out wheeled bin on the product is required lance with the Waste Electrical and Electronic Equipment Directive of the European Union. The presence of this on the product indicates that the device:
out on the European Market after August 13, 2005.
to be disposed using the municipal waste collection system member state of the European Union.
ucts under the requirement of WEEE directive, please rour dealer or local SCIEX office for the proper nination information and take-back program, which will the proper collection, treatment, recovery, recycling, and losal of the device.

RoHS Notice

These labels and materials declaration table (the Table of Hazardous Substance's Name and Concentration) are to meet People's Republic of China Electronic Industry Standard SJ/ T11364-2006 Marking for Control of Pollution Caused by Electronic Information Products" requirements.

使 使 使 の の の の の の の の の の の の の	CHINA RoHS CAUTION LABEL: This logo indicates that this electronic information product contains certain toxic or hazardous substances or elements, and can be used safely during its environmental protection use period. The number in the middle of the logo indicates the environmental protection use period for the product. The outer circle indicates that the product can be recycled. The logo also signifies that the product should be recycled immediately after its environmental protection use period has expired. The date on the label indicates the date of manufacture.
Addagenergy	CHINA RoHS ENVIRONMENTAL LABEL: This logo indicates that the product does not contain any toxic or hazardous substances or elements. The "e" stands for electrical, electronic and environmental electronic information products. This logo indicates that this electronic information product does not contact any toxic or hazardous substances or elements, and is green and environmental. The outer circle indicates that the product can be recycled. The logo also signifies that the product can be recycled after being discarded, and should not be casually discarded.

Other Instrument Labels

456259-BA	CE MARK: A "CE" mark indicates that a product has been assessed before being placed on the market, and has been found to meet European Union safety, health, and /or environmental protection requirements.
\otimes	RCM MARK: The RCM mark is intended for use on products that comply with Australian Communications Media Authority (ACMA) EMC requirements.
c (US US 169502 605175-AB	CSA/INTERNATIONAL CERTIFIED LABEL 169502: This label indicates recognition by a Nationally Recognized Testing Laboratory (NRTL) that the GenomeLab GeXP system has met the relevant product safety standards.

About This Guide

This guide is intended for use with the GenomeLab GeXP Genetic Analysis System. This guide is divided into the following sections:

- 1. Foreword, this section, describes the purpose of this guide, provides a list of its contents, discusses the use of the notes used in the document, tells how to get help, and provides service contact information.
- 2. Getting Started discusses the purpose and functional description of the system, provides an overview of the three main components (chemistry, hardware, and software) compose the system, and details the safety features relevant to the system. It also provides step-by-step procedures for operating the system.
- 3. Sample Setup Module provides an overview of the module, including its menu options, toolbars, and dialogs. It also shows you how to work with sample plates using this module, such as opening, setting up, exporting, and importing sample plates.
- 4. Run Module provides an overview of the Run module, including its menu options, toolbars, and dialogs. It also shows you how to run sample plates and set the display options using this module.
- 5. Sequence Analysis Module provides an overview of the module, including its menu options, toolbars, and dialogs. It shows you how to use this module to run a sequence analysis, and to set up its parameters, result properties, and display options. It also describes how to work with, report, and export sequence analysis results.
- 6. Sequence Investigator Module provides an overview of the module, including its menu options, toolbars, and dialogs. It also shows you how to use this module to investigate and compare sequence analysis results.
- 7. Fragment Analysis Module provides an overview of the module, including its menu options, toolbars, and dialogs. It shows you how to use this module to work with Studies, to run a fragment analysis, and to set up its parameters, result properties, and display options. It also describes how to work with, report, and export fragment analysis results.
- 8. Gene Expression describes how to use the GenomeLab GeXP system and the associated software to perform gene expressing analysis.
- 9. Data Manager Module provides an overview of the module, including its menu options, toolbars, and dialogs. It provides procedures for database activities, such as setting up and managing databases, exporting/importing data, and managing user accounts.

Foreword

10. Maintenance and Diagnostics provides routine maintenance, replenishment, diagnostic, and biological waste disposal procedures. It also lists the consumable materials used in the system.

This chapter discusses the purpose and functional description of the system, provides an overview of the three main components (chemistry, hardware, and software) composing the system, and gives details about the safety features relevant to the system. It also provides step-by-step procedures for operating the system.

Purpose of the System

The purpose of the GenomeLab GeXP system is two-fold:

- To determine the nucleotide sequence of any given DNA sample
- To estimate sizes of DNA fragments

Functional Description

The GenomeLab GeXP system is fully automated and capable of determining the base sequence and fragment length of DNA samples that have been prepared with SCIEX dyelabeled reagents. Four-color, dye-labeled terminator chemistry kits are used to process samples for base sequence analysis. Generation of samples for fragment length analysis is performed using one of two techniques:

- Dye-labeled primers for micro-satellite, gene expression AFLP, and other similar applications
- Four-color dye-labeled terminator chemistry, for the single nucleotide polymorphism application

The dual-rail GenomeLab GeXP system incorporates two plate holders and accepts two 96-well plates at one time. Each row of eight samples (sample set), containing labeled DNA fragments, is automatically denatured and then separated by capillary electrophoresis. The separation gel is automatically replaced in the eight capillaries after each separation. The separation gel supply is an easily replaced cartridge with a capacity sufficient for two full microplates.

The GenomeLab GeXP system performs detection using laser-induced fluorescence in four spectral channels. It automatically processes the four-channel raw data sets generated by each of the eight capillaries to produce high-quality base sequences or fragment lists after separation.

Raw and analyzed data are stored in a database and can also be exported in file formats compatible with common analysis applications.

Hardware

The hardware performs sample handling as well as tasks associated with the separation and detection phases of electrophoresis.

The following figure shows the GenomeLab GeXP system. The following sections describe the user-accessible hardware components.

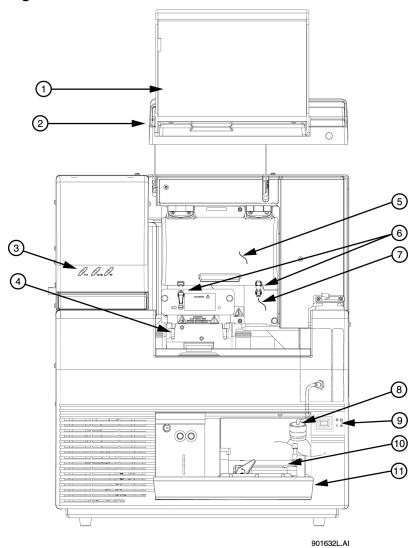


Figure 3-1 Hardware Overview

ltem	Description
1	Sample access cover (extended)

Item	Description
2	Capillary access cover (extended)
3	Status indicators
4	Plate holders/sample transport
5	Capillary temperature control cover
6	Rubber latches
7	Manifold access cover
8	Gel waste bottle
9	Power switch
10	Gel pump
11	Gel pump/gel cartridge access cover

Sample Access Cover

Provides access to the buffer plate, wetting tray, and sample plate.

Capillary Access Cover

Provides access to the capillary array through the capillary temperature control cover.

Capillary Temperature Control Cover

Provides an enclosed environment for the capillary temperature control and allows access to the capillary array.

Capillary Array

Used to produce raw data for sequencing and fragment analysis, is 33 cm long and has an i.d. of 75 μ m. The capillary array has three components: electrode block (inlet), eight capillaries, and array fitting (outlet).

The electrode block is the DNA sample inlet side of the array. It holds eight hollow, stainlesssteel electrodes. Each stainless-steel electrode holds a capillary in the center. These electrodes are designed for immersion into an entire row (8 wells) of a 96-well microplate (8 rows × 12 columns).

The capillaries pass through and exit the array fitting. When the fitting is installed, the ends of the capillaries are inserted into the gel/buffer manifold reservoir.

Getting Started

The array fitting contains the detection window and is the outlet side of the array. The detection window of the fitting exposes the eight capillaries to laser excitation. The external polyimide coating of the capillaries has been removed for this purpose.

Note: When the capillary array is not installed (for example, during shipping and storage), the manifold plug is inserted into the manifold (array fitting outlet) to prevent any gel inside of the GenomeLab GeXP system from drying.

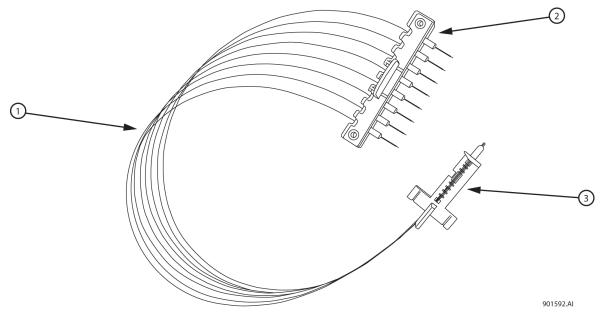


Figure 3-2 Capillary Array

ltem	Description
1	Capillaries
2	Electrode block (inlet)
3	Array fitting (outlet)

Plenum

The GenomeLab GeXP system is supplied with a capillary heater plenum suitable for use with a 33 cm capillary array. This short plenum assembly has a capillary array routing notch and a conical depression on the back.

Note: Refer to the caution label on the front of the plenum assembly for positive identification.

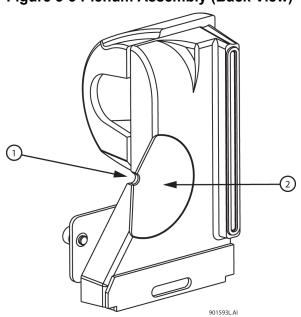


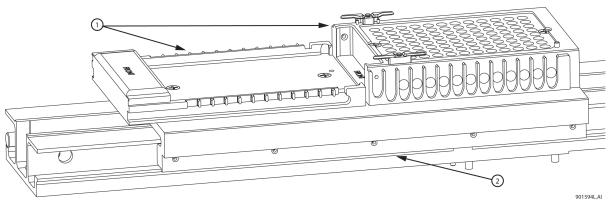
Figure 3-3 Plenum Assembly (Back View)

ltem	Description
1	Routing notch
2	Conical depression

Sample Transport and Plate Holders

The sample transport contains the plate holders used to hold the sample plate, buffer plate with buffer evaporation cover, and wetting tray.

Figure 3-4 Sample Transport and Plate Holders

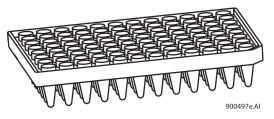


ltem	Description
1	Plate holders
2	Part of sample transport

Sample Plate

The sample plate is used to hold the samples for separation. The plate is a V-bottom, thermal cycler-compatible, polypropylene plate containing 96 wells (8 rows × 12 columns). The wells have a 200 μ L capacity.

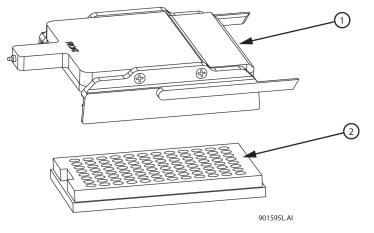
Figure 3-5 Sample Plate



Buffer Plate and Buffer Evaporation Cover

The buffer plate holds the DNA separation buffer used during a sample run. The plate is a flat-bottom, polystyrene, nonsterile plate containing 96 wells. When placed over the buffer plate, an evaporation cover maintains the proper buffer level (250 μ L to 300 μ L) in the buffer plate by preventing the buffer from evaporating. The cover slips over the buffer plate position of the sample transport. As the GenomeLab GeXP system advances through the sample plate during a run, the evaporation cover is pushed back far enough to expose the next row of buffer wells to use in the next sample set run.





Item	Description
1	Buffer evaporation cover
2	Buffer plate

Wetting Tray

The wetting tray is used to immerse the ends of the capillaries in deionized water. When properly filled, the capillaries can be maintained for approximately seven days in the wetting tray without attention. During continuous use of the GenomeLab GeXP system, the wetting tray should be replenished with deionized water after a 96-well microplate has been run or prior to a sample plate run.

The wetting tray is also used to rinse capillary tips and provide a receptacle for separation medium that was purged from the capillaries.

CAUTION: Potential System Damage. Do not process more than one 96-well plate for each wetting tray without replenishing the wetting tray.

For details, refer to the section: Replace the Wetting Tray.

Figure 3-7 Wetting Tray



Gel Waste Bottle

The gel waste bottle is used to capture and store the waste gel that is pushed out of the manifold during the purge function. The bottle can hold the equivalent of 25 gel cartridges. To prevent overflow, observe the gel level in the waste bottle.

Figure 3-8 Gel Waste Bottle



Gel Pump/Gel Cartridge Access Cover

Provides access to the gel pump and gel cartridge.

Component	Description
Gel pump	Used to replenish the capillaries with fresh gel (from the cartridge) after each sample set and two 96-well plate runs.
Gel cartridge	Contains the fresh DNA separation gel. The separation gel is used for both sequence and fragment analysis. For an example of the 20 ml gel cartridge on the dual-rail system, refer to the figure: Figure 3-9.
Gel pump plug	When the gel cartridge is not installed (for example, during shipping and storage) the gel pump plug is inserted into the pump barrel to prevent gel from drying in the system.

Table 3-1 Gel Pump, Gel Cartridge, and Gel Pump Plug

Figure 3-9 Gel Pump 2 Щ \mathbb{D} 0. . 0. . O. ④ 5 **D**:: 000 0 C ୯ D \bigcirc 901635L.AI

Item	Description
1	Gel pump/gel cartridge access cover
2	Cartridge locking lever
3	Cartridge barrel
4	Cartridge locking lever
5	Cartridge barrel

Status Indicator Lights

The GenomeLab GeXP system has the following three status indicators: **PWR** (power), **LASER**, and **HV** (high voltage).

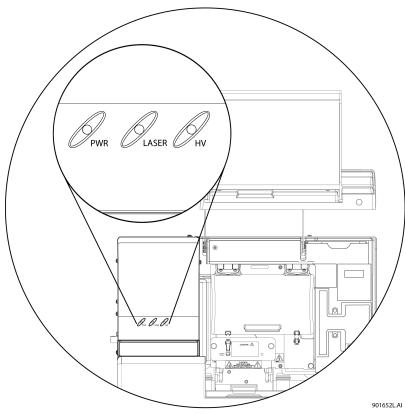


Figure 3-10 Location of Status Indicator Lights

Indicator	Description
PWR	The green light is illuminated when the GenomeLab GeXP system is powered on. The green light is not illuminated when the GenomeLab GeXP system is powered off.
LASER	The green light is illuminated when the lasers are turned on during a run. The light is not illuminated when the lasers are turned off.
HV	The green light is illuminated when high voltage is applied during injection or separation. The light is not illuminated when high voltage is turned off.

Software

The software provides the interface for manual or automatic (pre-programmed) control of the system, data capture, and basic data analysis.

The GenomeLab GeXP system provides an interface that enables the user to open programs and initiate commands by clicking buttons or selecting menu options.

When the software is opened for the first time, the user must select whether to activate the 21 CFR Part 11 tools. These tools enable features in the software that support compliance with 21 CFR Part 11.

IMPORTANT This selection is made only once, when the software is started for the first time, and cannot be changed.

Refer to the section: Initial Startup - Select a 21 CFR Part 11 Option.

Initial Startup - Select a 21 CFR Part 11 Option

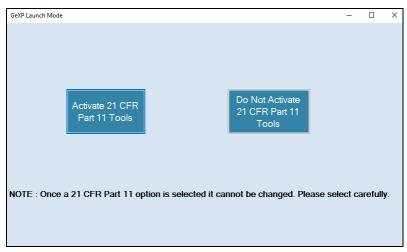
If the 21 CFR Part 11 tools are not activated, all users can create and edit methods and data in the GenomeLab System software.

If the 21 CFR Part 11 tools are activated, access to functions in the GenomeLab System software is controlled by roles that are set by the software Administrator, an audit trail supplies a log of the main operations that occur in the software, and users can sign reports with a digital signature.

IMPORTANT This selection is made only once when the software is started for the first time and cannot be changed.

 On the computer desktop, double-click the GenomeLab System icon The GeXP Launch Mode window opens.

Figure 3-11 GeXP Launch Mode Window



- 2. To activate the 21 CFR Part 11 tools, do this:
 - a. Click Activate 21 CFR Part 11 Tools.

The Login window opens.

- b. In the User Name field, type Admin.
- c. In the **Password** field, type **GeXP@Win10**, and then click **Login**.

Note: These are the default login values. Changing the password for the Administrator role is recommended. Refer to the section: Edit a User.

The GeXP Home Page opens.

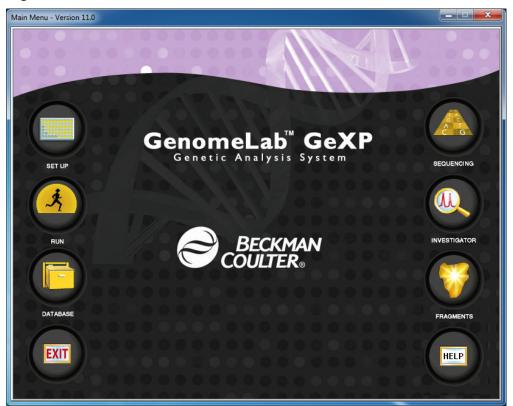
- d. Refer to the section: 21 CFR Part 11 Tools.
- 3. To use the software without the 21 CFR Part 11 tools, do this:
 - a. Click Do Not Activate 21 CFR Part 11 Tools.
 - b. Click **Exit**. The GenomeLab GeXP Genetic Analysis System Main Menu opens.
 - c. Refer to the section: GeXP Main Menu.

GeXP Main Menu

The Main Menu provides access to the software modules. To open a module, click its icon. For a description of the software modules, refer to the section: Software Module Descriptions.

After opening a software module, the Main Menu collapses to the Windows taskbar or as a shortcut toolbar. Refer to the section: Main Menu Collapse Options.

Figure 3-12 Main Menu



Main Menu Collapse Options

After opening the Main Menu, you can set its collapse features using the menu. To do this, right-click the Main Menu title bar and select your option from the menu.



	Collapse to Toolbar			
8	Restore			
	Move			
	Size			
-	Minimize			
	Maximize			
×	Close Alt+F4			
	Always Collapse To Toolbar			
•	Always Collapse To Taskbar			

Option	Description
Collapse to Toolbar	Select this option to immediately collapse the Main Menu to show the Main Menu toolbar.
Always Collapse to Toolbar	Select this option to set the Main Menu to collapse to the Main Menu toolbar every time you open a module from the Main Menu. Selecting Always Collapse to Toolbar makes it the default setting, instead of Always Collapse to Taskbar .
Always Collapse to Taskbar	Select this option to set the Main Menu to collapse to the Windows taskbar every time you open a module from the Main Menu. Selecting Always Collapse to Taskbar makes it the default setting, instead of Always Collapse to Toolbar . From any application, you can access the Main Menu by left-clicking the Main Menu button, located on the Windows taskbar.

Main Menu Toolbar

Provides access to all GenomeLab System software modules from any active application window. By default, the Main Menu toolbar provides a miniature version of software module icons as a horizontal toolbar.

Figure 3-14 Main Menu Horizontal Toolbar



To move the toolbar, click the title bar and hold the left mouse button while dragging the toolbar to another location. When given enough space, the toolbar becomes a two-column vertical toolbar.

Figure 3-15 Main Menu Vertical Toolbar



The toolbar resizes to fit in any location in the active application window. To move the toolbar to a more convenient location, click the title bar and hold the left mouse button while dragging the toolbar to one of the four edges of the application window.

The Main Menu toolbar icons are miniature versions of the ones provided in the expanded Main Menu. Clicking one of these icons opens the corresponding software module. Refer to the section: Software Module Descriptions.

Software Module Descriptions

The following table shows the shortcut icons available on the Main Menu and describes the module that opens when you click the corresponding shortcut icon.

lcon	Module Description
	Sample Setup Module
	Create, save, and modify sample plates. Use sample plates to assign methods to control sample sets and determine the sequence of methods used to produce data.
, ž	Run module
	Run sample plates and control individual functions of the GenomeLab GeXP system.
	Sequence Analysis module
	View, analyze, compare, manipulate, and print base sequence data produced by sample runs.
<u>M</u>	Sequence Investigator module
	Compare sequences and create a consensus to compare against a reference.
\$	Fragment Analysis module
	View, analyze, compare, manipulate, and print fragment data produced by sample runs.
	Data Manager module
	Modify and print database files
EXIT	Exit
	Close the GenomeLab System software.

Table 3-2 Software Module Descriptions

Operating the System

Perform the tasks involved in analyzing or managing samples and sample data.

Although the following procedures are independent from setting up and starting the system, perform the procedures below in the sequence provided.

Before you begin, confirm that the capillary is ready for use. Refer to the section: Remove and Replace the Capillary Array. Install a new gel waste bottle. Refer to the section: Replace the Gel Waste Bottle for Dual-Rail System.

Reagents and Materials

Table 3-3 Materials from SCIEX Required for Set	equence Analysis and Fragment Analysis
---	--

Material	Part Number	Sequence Analysis	Fragment Analysis
Separation gel, 10 mL, for single-rail systems	608010	\checkmark	~
Separation gel, 20 mL, for dual-rail systems	391438	\checkmark	~
Buffer microplates, 96-well nonsterile	609844	\checkmark	~
DNA separation capillary array	608087	\checkmark	~
GenomeLab Separation Buffer	608012	\checkmark	~
Sample microtiter plate (96-well)	609801	\checkmark	~
DTCS Quick Start Kit	608120	\checkmark	
DNA Size Standard 400 Kit	608098	_	~
DNA Size Standard 600 Kit	608095		~
DNA Size Standard 80 Kit	608395		~
GenomeLab GeXP Start Kit	A85017	_	~
GenomeLab SNPstart Primer Extension Kit	A23201	_	~
Human STR Primer Set Kit	A20100	_	~
Sample Loading Solution	608082	_	~

Table 3-3 Materials from SCIEX Required for Sequence Analysis and Fragment Analysis (continued)

Material	Part Number	Sequence Analysis	Fragment Analysis
Thermo-Start <i>Taq</i> DNA Polymerase	A85022	—	~

Table 3-4 Additional Materials Required for Sequence Analysis and Fragment Analysis

Material	Sequence Analysis	Fragment Analysis
100 mM Na ₂ -EDTA pH 8.0 (500 mM) (Sigma E7889)	~	—
3 M Sodium Acetate pH 5.2 (Sigma S7899)	~	—
(Molecular biology grade) Sterile dH ₂ O, 95% (v/v) ethanol/dH ₂ O, 70% (v/v) ethanol/ dH ₂ O	~	—
Interrogation primers, purified PCR products, shrimp alkaline phosphatase, and exonuclease I	_	~
Labeled primers (Sigma or Integrated DNA Technologies)	—	~
PCR enzyme and buffer	—	~
1 M Tris-HCl, pH 8.0 (Thermo Fisher 15568025)	—	~
AmpliTaq Gold DNA polymerase with gold buffer and MgCl ₂ (Thermo Fisher 4311806)	—	~
DNase/RNase-free distilled water (Thermo Fisher 10977015)	_	~
THE RNA Storage Solution (Thermo Fisher AM7000)	—	\checkmark

Additional required materials:

• Sterile tubes - 0.5 mL microfuge tubes, 0.2 mL thin wall thermal cycling tubes or plates

Getting Started

- Aerosol Duster (CRC 05185)
- Alpha Mini Swab from Texwipe (VWR TWTX754B)

Safety

Refer to the Safety Data Sheets (SDS), available at sciex.com/tech-regulatory, for information about the proper handling of materials and reagents. Always follow standard laboratory safety guidelines. For hazardous substance information, refer to the section: Hazardous Substance Information.

Customer-Supplied Equipment and Supplies

- Aerosol-resistant tips for P10, P20, P100, P200, and P1000 pipettors
- Aluminum foil seal
- Microplate centrifuge
- Microtube centrifuge
- Microtubes: 2.0 mL, 1.5 mL, and 0.65 mL
- Non-frost-free freezers (–80 °C and –20 °C)
- Optically clear flat cap strips for RT-PCR tubes
 - Optical Flat 8-Cap Strips for PCR Tubes (BioRad TCS0803)
 - Thermowell GOLD PCR 1 x 8 Optically Clear Flat Cap Strips, for RT-PCR (Corning 3742)
- Pipettors: P10, P20, P100, P200, and P1000
- Regular tips for P10, P20, P100, P200, and P1000 pipettors
- · Thermal cycler with heated lid for 96-well plates
- Vortex mixer

Prepare a Sample

Prepare a sample for sequence or fragment analysis using the instructions in the Dye Terminator Cycle Sequencing (DTCS) Kit, GenomeLab GeXP Start Kit, or PCR reagent manufacturer's kit for fragment analysis.

Start the System

- 1. Turn on the computer and wait for Windows to start.
- 2. Turn on the GenomeLab GeXP system.
- 3. From the Windows desktop, double-click the GenomeLab System icon

As an alternative, select **Start > GenomeLab System > Control Center**.

The system initializes and after several seconds shows the Main Menu.

Create a Database and Project Folder Open the Data Manager Module

On the Main Menu, click the **DATABASE** icon and verify that the Data Manager main window opens.

Create a Database

To create a database:

- In the Data Manager window, select File > New Database. The New Database dialog opens.
- 2. Enter the name for this new database.
- 3. Select Set As Working Database.
- 4. Click OK.

Create and Name a Project Folder

To create and name a project folder:

- In the Data Manager window, highlight the database where the project will reside and select File > New.
- 2. Highlight the new project and select **File > Rename**.
- 3. Enter a descriptive name for this project and press **Enter**.
- 4. Select **File > Exit** to close the Data Manager module.

Set Up a Sample

- 1. To set up a sample, on the Main Menu, click the **SET UP** icon and verify that the Sample Plate Selection dialog opens.
- 2. Select Create a new sample plate, and click OK.
- 3. Set up the sample plates by naming the required cells.
- 4. Add any associated notes or property information.
- 5. Select a method from the list at the bottom of the sample set.
- 6. Select the appropriate analysis parameters (as well as report and export parameters, if needed).

Getting Started

- 7. Enter the barcode in the **Barcode** field.
- 8. Select **File > Save As**. Enter a name for the sample plate.
- 9. Select the project you just created from the **Project Name** menu, and click **OK**.

Run a Sample Start the Run Module

On the Main Menu, click the **RUN** icon *A* and verify that the Run main window opens.

Monitor the Capillary Array

Monitor the capillary life and usage in the Status Monitor window on the Life tab. If necessary, perform the procedure in the section: Remove and Replace the Capillary Array, and then return here.

Install a Gel Cartridge

Monitor the gel life and usage in the Status Monitor window on the Life tab. If necessary, perform the procedure in the section: Remove and Replace a Gel Cartridge/Gel Pump Plug for Dual-Rail System, and then return here.

Install the Gel Waste Bottle

If necessary, perform the procedure in the section: Replace the Gel Waste Bottle for Dual-Rail System, and then return here.

Prepare Plates for a Run

- 1. Load samples into the sample plate and cover with mineral oil.
- 2. Load buffer (250 μ L to 300 μ L) into the buffer plate.
- 3. Fill the wetting tray with deionized water to the level indicator marker.

CAUTION: Potential System Damage. NEVER allow the liquid level to rise into the eight cannula recesses of the wetting tray lid, nor drop below the fill level indicator line. The top surface of the wetting tray lid must remain clean and dry under any and all circumstances.

Note: Periodically check the liquid level in the wetting tray.

CAUTION: Potential System Damage. Do not process more than one 96-well plate for each wetting tray without replenishing the wetting tray.

Start the Sample Plate Run for Dual-Rail System

1. Select **Run > Start Sample Plate**.

The Sample Plate Run Confirmation dialog opens.

Figure 3-16 Sample Plate Run Confirmation Dialog

Sample Plate Run Confirmation	×
Left Plate Operator Name: Save to Project: Default	Right Plate Operator Name: Save to Project: Default
Plate Loaded O Run this plate first	Plate Loaded C Run this plate first
Sample Plate Barcode:	Sample Plate Barcode:
Sample Plate Name:	Sample Plate Name:
View/Edit this sample plate configuration	View/Edit this sample plate configuration
Start Load Plates Replace Gel Cartric	lge Details Cancel Help

- 2. Select the required project on the appropriate side (left or right) for each prepared sample plate, and then click **OK**.
- 3. Click Load Plates.

Figure 3-17 Access Plates Dialog

Access Plates	X
If you are going to load new plates, please prepare your plates before clicking on	Start
Start. You have 15 minutes to change plates once you click on Start.	Cancel
This time limit is critical so that the capillaries are not adversely affected by prolonged exposure to air.	Help

- 4. Click **Start** to continue.
- 5. Install the plate, select **Plate Loaded** for the left and/or right plate, as applicable, and then select the side to immerse the capillaries.

Note: Prepare the new plates before proceeding. Once the capillaries have been exposed to air, you will have 15 min to load the plates.

Figure 3-18 Capillaries Exposed Dialog

Capillaries Exposed	×	Capillaries Exposed	×
PLEASE WAIT ! STO	Do not open sample door.	Go You may now open the and load plates.	sample door
Capillaries exposed to air:	Load	Capillaries exposed to air:	Load
	Cancel		Cancel
Time Remaining	Alarm Off	Time Remaining	Alarm Off
min: sec: 14 53	Help	min: sec: 13 53	Help
Left Plate	Right Plate	Left Plate Right Pla	ite
Plate Loaded	Plate Loaded	Plate Loaded 🔲 Plate	e Loaded
Immerse Capillaries (requires wetting tray on the left side)	Immerse Capillaries C (requires wetting tray on the right side)	 (requires wetting tray (requires wetting tray 	erse Capillaries uires wetting tray ne right side)

- 6. Click **Load** to continue.
- 7. Click **Start** to begin the run.

Start the Sample Plate Run for Single-Rail System

 Select Run > Start Sample Plate. The Select Sample Plate To Run dialog opens.

Figure 3-19 Select Sample Plate To Run Dialog

Select Sample Plate To Run	×
Database: MLEE UNIT 178	ОК
Start Date: 08-22-2001 Enable	Cancel
End Date: 08-22-2001 <u>*</u> <u>R</u> efresh	Help
Name Date-Time	00514
DefaultSamplePlate 12/02/98 17:00:	UUPM
•	
Project Name: Default	

2. Select the required project and sample plate, and then click **OK**.

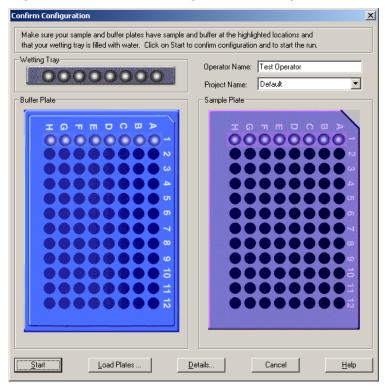


Figure 3-20 Confirm Configuration Dialog

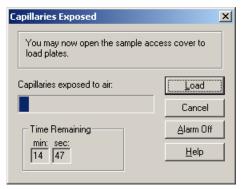
- 3. Verify the sample and buffer set locations.
- 4. Click Load Plates.

Figure 3-21 Unload Plates Dialog

Unload Plates	×
Please prepare your plates before clicking on Unload. You have 15 minutes to change plates once you click on Unload. This time limit is critical so that the capillaries are not adversely affacted by	<u>U</u> nload Cancel
prolonged exposure to air.	<u>H</u> elp
The capillaries will automatically be immersed in the Wetting Tray after the plates are loaded.	

5. Click Unload.

Figure 3-22 Capillaries Exposed Dialog



6. Install the sample plate, buffer plate, and wetting tray into their appropriate positions, and then click **Load**.

Note: Refer to the sections: Load the Sample Plate - Single, Load the Buffer Plate and Evaporation Cover - Single, and Install the Wetting Tray.

7. Click **Start** to begin the run.

Overview

Use the Sample Setup module to create, save, and modify methods and sample plates. Methods consist of sequential events needed to perform a DNA separation. Sample plates provide a way to name and organize samples.

The sample plate definition contains the 96-well plate locations of the samples as well as the method assigned to each sample. The capillary array must run the same separation parameters across eight samples, one column at a time. Each column that contains samples is called a sample set. The separation parameters applied to each sample set are specified in the method. Method parameters can be changed to create new methods.

Main Window

The main window for the Sample Setup module has the following sections.

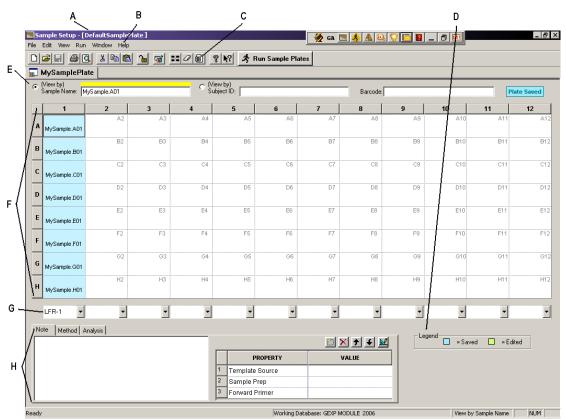


Figure 4-1 Main Window, Sample Setup Module

Item	Description
A	Title Bar: Shows the module name (Sample Setup) and the active sample plate (DefaultSamplePlate).
В	Menu Bar: Refer to the section: Menu Bar.
С	Toolbar: Refer to the section: Toolbar.
D	Legend: Shows the color coding information (Saved or Edited) of the individual cells.
E	Sample Name and Subject ID of the currently active cell. The radio button toggles the sample plate cell labels between Sample Name and Subject ID. Use the Barcode field to enter the barcode for the current plate.
F	Sample Plate: Shows 96 cells that correspond to a 96-well sample plate. (Sample plate columns are numbered 1 through 12. Rows are labeled A through H . This naming convention uniquely identifies each sample location.)

ltem	Description
G	 Currently active (selected) method. Use this list to select a predefined method to the corresponding samples in the column above. Selecting a method assigns it to all samples in a column (sample set). Frag methods are used for all fragment analysis applications other than SNP primer extension
	LFR method is used for long fast-read sequencing on the 33 cm array
	SNP method is used for the SNP primer extension application
Н	Note tab: Shows the Note sub-window, which can be used to describe or apply a property set to single or multiple samples.
	 Method tab: Shows the Method sub-window, which shows the parameters defined for the selected method and allows you to edit or create separation methods.
	Analysis tab: Shows the Analysis sub-window, which can be used to:
	Enable or disable the automatic analysis mode.
	 Select and edit a Sequence Analysis or Fragment Analysis parameter set.
	 Enable or disable automatic report printing and edit print report options.
	 Enable or disable automatic exporting of data and change export options.

Edit Property Values

Attach information and a property set to the highlighted samples. This informational note will accompany the sample throughout its lifetime.



Figure 4-2 Note Sub-Window

Note Method Analysis				
			🛅 🗙 🗲 🖌 🔛	
		PROPERTY	VALUE	-
	1	Template Source		
	2	Sample Prep		
	3	Forward Primer		·

Edit Sample Properties

Sort and remove sample properties.

- 1. To select a field in an existing property set, left-click the cell.
- 2. Next, use the **Tab** key to move your selection from cell to cell. The **Tab** key moves the focus from property to value and then to the next row and the arrow keys to move in the direction selected.
- 3. Press **Enter** to move the focus down the table one cell at a time, within the same column.
 - To select an entire column for sorting, click its header. The selected column is highlighted.
 - To select a row, click its number. As with the column selection, the selected row is highlighted.
 - To select consecutive rows, left-click and drag the cursor through the blocks. Click the toolbar arrow buttons to move the row up or down one cell.
 - To delete the contents of the rows, select the desired rows and click **Delete**. A warning message appears, prompting you to confirm deletion. Click **Yes** to delete the contents or **No** to return to the editor.

View the Method Tab

The Method sub-window shows the parameters of the method for the selected samples. Click **Edit** to modify one or all of the parameters of the existing method or to create a new method.

Note: If the 21 CFR Part 11 tools are in use, then the edit function is only available to users with Write access.

Figure 4-3 Sample Setup Method Sub-Window

Note Method Analysis		
Method Name: LFR-b	– Denature Temperature: 90 °C Duration: 120 sec.	Separate Voltage: 6.0 kV Duration: 60.0 min.
Capillary Temperature: 57 °C Wait for Temp: Yes	– Inject Voltage: 2.0 kV Duration: 15 sec.	Pause: 0 min. Edit

Analysis Tab

Use the Analysis tab to enable or disable **Perform Analysis** mode. When enabled, this mode causes the GenomeLab System software to analyze the sample at the end of the run. Set the **Perform Analysis** option for each sample separately, row/column, or entire plate.

Figure 4-4 Analysis Tab, Sample Setup Module

Note Method Analysis		
Analysis	Reports	Export
Perform Analysis	Print Report	Export Data
Parameter Set:		
equenceAnalysisParameters	Edit Print Format For Plate	Edit Export Options For Plate

- 1. Select the required Analysis Parameter Set for each sample from the list.
- 2. Select each sample separately, row/column, or entire plate.
- 3. Enable or disable automatic printing of reports or edit the print report parameters.
- Click Edit Print Format For Plate. The Report Format dialog shows the current report settings for the sample plate. Print Format applies to all samples. Print Report applies to individual samples.
- 5. Enable or disable automatic exporting of data and/or change export options.
- Click Edit Export Options For Plate. The Export dialog shows the current settings for the sample plate. Export Format applies to all samples. Export Report applies to individual samples.

Note: New sequence or fragment analysis parameters must be created in their respective analysis modules.

Menu Bar

The following example shows the menu bar for the Sample Setup module.

Figure 4-5 Sample Setup Module Menu Bar

<u>File Edit View R</u>un <u>W</u>indow <u>H</u>elp

The following topics describe each of these menus and their options.

File Menu

Click File to show the following menu.

Figure 4-6 File Menu, Sample Setup Module

File	
New	Ctrl+N
<u>O</u> pen	Ctrl+O
⊆lose	
Save	Ctrl+S
Save <u>A</u> s	
Import	
Export	
Propert <u>i</u> es	
Default Report <u>F</u> ormat	
Print Pre <u>v</u> iew	
Print Setup	
Print	+
Print Screen	•
<u>L</u> ock	
0 DefaultSamplePlate	
1 AFLP 06	
E <u>x</u> it	

Option	Description
New	Open an empty sample plate.
Open	Select an existing sample plate to open.
Close	Close the selected sample plate.
Save	Save the current data to the same location with the same file name.
Save As	Save the current data with a newly assigned file name in a newly selected location.
Import	Import a sample plate (tab-delimited ASCII text [*txt] format).
Export	Export a sample plate (tab-delimited ASCII text [*txt] format).
Properties	Open an information window concerning the active sample plate.

Option	Description
Default Report Format	Define the report formats for new and imported sample plates.
Print Preview	Open a print preview of the active document of the active sample plate. A toolbar at the top of the window provides options for navigation, viewing, and printing the display. To restore the default screen, press Esc or click Close .
Print Setup	Define printer properties.
Print	Show a sub-menu with options to print a detailed report of the active sample plate, the contents of the sample plate, the plate summary, or the selected method.
Print Screen	Open a sub-menu with options to print an image of the computer desktop or application window.
Lock	Prevent editing of the currently active plate.
Exit	Close the module.

Edit Menu

 $\label{eq:click-equation} \mbox{Click-equation} \mbox{Edit} \mbox{ to show the following menu.}$

Figure 4-7 Edit Menu, Sample Setup Module

Edit	
Undo	Ctrl+Z
Redo	
Clear All	Ctrl+L
Select All	Ctrl+A
Cut	Ctrl+X
Сору	Ctrl+C
Paste	Ctrl+∀
Find	Alt+F3
Replace	Ctrl+F3
Method	
Selected Method	
Auto-Fill Method Name	Ctrl+F

Option	Description
Undo	Undo the last action performed.

Option	Description
Redo	Reverse the last undo action.
Clear All	Empty all cell properties in the active sample plate.
Select All	Select all cells in the sample plate.
Cut	Copy the selected item to the clipboard and removes it from its current location.
Сору	Copy the selected item to the clipboard without removing it from its current location.
Paste	Insert copied or cut items from the clipboard into the selected location.
Find	Open the Find dialog, which you can use to search for text within the sample name entered in a sample plate.
Replace	Open the Replace dialog, which you can use to search and replace text within the sample names entered in a sample plate.
Method	Select a method to use.
Auto-Fill Method Names	Select the method to apply to selected sample sets (columns) or the entire sample plate.

View Menu

Click **View** to show the following menu.

Note: A check mark next to an option indicates that the option is enabled.

Figure 4-8 View Menu, Sample Setup Module

View
✔ Toolbar
🗸 Status Bar
✓ Cell Coordinates
Sample Property Sets
Summary
View by Subject ID
Working Database

Option	Description
Toolbar	Toggle to show or hide the toolbar.
Status Bar	Toggle to show or hide the status bar.
Cell Coordinates	Toggle to show or hide the coordinates of the plate cells.
Sample Property Sets	Create, delete, or modify the property set.
Summary	Show a summary of the currently selected plate.
View by Subject ID	Toggle the sample plate cell labels between Sample Name and Subject ID .
Working Database	Show the name of the database in use.

Run Menu

Click **Run** to show the following menu.

Figure 4-9 Run Menu, Sample Setup Module



Option	Description
Start	Invoke the Run module and loads the active sample plate.

Window Menu

Click **Window** to show the following menu.

Figure 4-10 Window Menu, Sample Setup Module

Window	
Next	Ctrl+Tab
Close	Ctrl+F4
1 Untitled - Plate 2 Untitled - Plate	

Option	Description
Next	Toggle between open sample plates.

Option	Description
Close	Close the active sample plate or sample plate summary.

Help Menu

Click **Help** to show the following menu.

Figure 4-11 Help Menu

ŀ	elp	
	Help <u>T</u> opics	
	Using Help	
	About GenomeLab System	
	SCIEX Home Page	

Option	Description
Help Topics	Select this option to show the Help topics. By default, the Contents show in the navigation pane on the left and the introductory topic shows in the topic pane on the right. Use the Contents to select and print specific Help topics. In the navigation pane, use the Search and Index tabs to search for information by topic, index entry, and/or keyword.
Using Help	Select this option to open the Contents window on how to use the Windows Help system.
About GenomeLab System	Select this option to access software and system information.
SCIEX Home Page	Select this option to access the SCIEX Home Page on the Internet.

Toolbar

The toolbar for the Sample Setup module has icons that correspond to common menu options.

Figure 4-12 Toolbar, Sample Setup Module



lcon	Description
D	New: Open an empty sample plate.
2	Open: Select an existing sample plate to open.
	Save: Save the current data to the same location with the same file name.Saves the current sample plate to the same directory with the same name and format as was used on the previous save.
4	Print: Print data associated with the active sample plate.
Q	Print Preview: Open a print preview of the active document of the active sample plate. A toolbar at the top of the window provides options for navigation, viewing, and printing the display. To restore the default screen, press Esc or click Close .
ж	Cut: Copy the selected item to the clipboard and removes it from its current location.
B	Copy: Copy the selected item to the clipboard without removing it from its current location.
a	Paste: Insert copied or cut items from the clipboard into the selected location.
Ъ	Unlock: Indicates that the active plate is available for editing. Clicking this icon locks the plate to prevent editing and changes the icon to .
-	Run: Open the Run module and executes the active sample plate.
	Select All: Highlight (select) all cells in the sample plate.
a	Clear All: Remove all cell properties in the sample plate.
	View Summary: Show a summary of a specific sample plate. To use this, open the required sample plate, then click this icon. The Sample Plate Summary spreadsheet opens with a summary of each sample in the plate. This is a read-only spreadsheet and cannot be edited.
ę	Help Topics: Open the Help System. By default, the Contents show in the navigation pane on the left and the introductory topic shows in the topic pane on the right. Use the Contents to select and print specific Help topics. In the navigation pane, use the Search and Index tabs to search for information by topic, index entry, and/or keyword.

lcon	Description
№?	Context-Sensitive Help: Click this icon and then on a menu item to open the Help topic related to the selection.

Using the Sample Setup Module

Open, set up, import, and export sample plates.

To open the Sample Setup module, on the Main Menu, click the SET UP icon



Create a New Sample Plate

- 1. On the Main Menu, click the **SET UP** icon The Sample Plate Selection dialog opens.
- 2. Select the **Create a new sample plate** radio button.

Figure 4-13 Sample Plate Selection Dialog

Sample Plate Selection	X
Create a new sample plate O Open an exist	sting sample plate
Sample Plates	
Database: GEXP MODULE 2006	OK
Filter	Cancel
Stat Date; 02 14 2000	
End Date: 02-14-2006 🗧 Refresh	Help
Project Name:	Find Barcode

3. Click OK.

The Sample Plate window opens.

- 4. Select and highlight the cell or cells where the samples will reside.
- 5. Enter the name of the samples in the Sample Name field, and then press Enter.
- 6. Enter the barcode for the plate in the Barcode field.
- 7. Apply sample specific information in the Note window of the Note tab, and apply property sets and values.
- 8. Assign a method to each sample set.

To edit the method or create a new method, refer to the section: Edit a Method.

- To automatically analyze data after a sample set run, select the **Analysis** tab, then select the **Automatic Analysis** check box. The system will analyze the sample data using the parameter set selected from the list. For details, refer to the section: Define Data Processing Conditions.
- To automatically print a report after a sample set run, refer to the section: Print the Plate Report or Specify Sample Plate Print Options.
- To automatically export data after a sample set run, refer to the section: Specify Sample Plate Export Options.
- 9. Select File > Save As.
 - a. In the Save As dialog, select a Project Name from the list.
 - b. Enter a name for this plate in the name field.
 - c. Click **OK** to save the plate.

Open an Existing Sample Plate

- 1. On the Main Menu, click the SET UP icon
- 2. In the Sample Plate Selection dialog that opens, select the **Open an existing sample plate** radio button.
- 3. Select the appropriate plate from the list.
- 4. To help in the search, filter the plates by the date that shows. Select the **Enable** option.
- 5. Use the **Up** and **Down** scroll arrows to change the **Start Date** and **End Date**.
- 6. Click **OK** to open the selected sample plate.

Find Barcode

Use the **Find Barcode** option to search for a sample plate using its barcode.

- 1. From the list, select the **Project Name** that contains the sample plate barcode.
- 2. Click **Find Barcode** and type the sample plate barcode exactly as it was saved.

If the sample plate barcode exists, it will open the plate. You may also use the wildcards * and **?** in your search. If the search finds more than one match, the Barcode Selection dialog opens, allowing you to select the proper plate.

Figure 4-14 Find Barcode Dialog



Note: To search all projects for the sample name, select **-(none)-** from the **Project Name** list before clicking **Find Barcode**.

Note: If the sample plate does not appear in the list of available items, it may be residing in another project. To select the project where the sample plate resides, select the project from the **Project Name** list.

Note: If the sample plate exists in a different database, you can reset the working database. For details, refer to the section: Set the Working Database.

Other Ways to Open a Sample Plate

You can also open a new or an existing sample plate at any time while using the Sample Setup module.

- To open a new sample plate, select **File > New**.
- To open a new sample plate using the toolbar, click .
- To open an existing sample plate, select File > Open. Use the Sample Plate Selection dialog to select your sample plate.
- To open an existing sample plate using the toolbar, click *Let*. Use the Sample Plate Selection dialog to select your sample plate.

Select Samples

Select individual samples positions. In addition, select the entire plate, row, or column using a single click. Use **Shift** or **Ctrl** to select contiguous and noncontiguous cells.

Select the Entire Sample Plate

Select the entire sample plate by left-clicking the grid box in the upper-left corner of the plate grid. To remove the selection, left-click any sample in the grid.

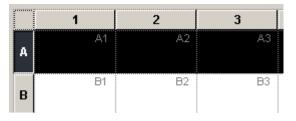
A	A1	A2	A3
			AS
в	B1	B2	B3
с	C1	C2	C3
D	D1	D2	D3

Figure 4-15 Select the Entire Plate

Select the Entire Row

Place the mouse pointer over the row label on the left side of the row to be selected. A small black arrow replaces the mouse pointer. Click once to select the row. To remove the selection, click any sample in the grid.

Figure 4-16 Select an Entire Row



Select the Entire Column

Place the mouse pointer over the column label at the top of the column to be selected. A small black arrow replaces the mouse pointer. Click once to select the column. To remove the selection, click any sample in the grid.

Figure 4-17 Select the Entire Column

		2	3
А	A1	A2	A3
в	B1	B2	B3
с	C1	C2	C3
D	D1	D2	D3

Select Contiguous Cells

- 1. To select contiguous cells, left-click the first cell in the column or row.
- 2. Press and hold **Shift**.
- 3. Left-click the last cell in the column or row to be selected.
- 4. Release Shift.

Figure 4-18 Select Contiguous Cells

	1	2	3	4
A	R1 g1.A01	r9 g1.A02	r8 g2.A03	r7 g3.A04
в	r2 g1.801	r1 g2.802	r9 g2.803	r8 g3.B04
с	r3 g1.C01	r2 g2.C02	r1 g3.C03	r9 g3.C04
D	r4 g1.D01	r3 g2.D02	r2 g3.D03	r1 g4.D04
E	v5 a4 E04	×4 ~2 E02	v2 ~2 E02	v0 ad E0d

Select Noncontiguous Cells

- 1. Click the first cell.
- 2. Use Ctrl + Click to select each additional cell.

Figure 4-19 Selecting Noncontiguous Cells

[1	1 2		4
A	R1 g1.A01	r9 g1.A02	r8 g2.A03	r7 g3.A04
в	r2 g1.801	r1 g2.802	r9 g2.B03	r8 g3.B04
с	r3 g1.C01	r2 g2.C02	r1 g3.C03	r9 g3.C04
D	r4 g1.D01	r3 g2.D02	r2 g3.D03	r1 g4.D04
E	r5 g1.E01	r4 g2.E02	r3 g3.E03	r2 g4.E04

View a Sample Plate by Sample Name/Subject ID

Use the **View > View by Subject ID** to toggle the sample plate view between **Sample Name** and **Subject ID**. This works in conjunction with the options found next to the **Sample Name** and **Subject ID** fields.

Figure 4-20 View by Subject ID / Sample Name Toggle

View		
 ✓ Toolbar ✓ Status Bar ✓ Cell Coordinates 		
Sample Property Sets Summary		
View by Subject ID Working Database		
(View by) Sample Name MySample A01	C (View by) Subject ID:	

Name Samples and Assign Subject IDs

The sample plate window appends the plate location to each sample name entered in a sample cell. For example, enter **MySample** as the name of the cell at position A3 in the plate. The sample plate view identifies the sample as MySample.A3.

Note: Position the mouse pointer over a named cell to show a Tool Tip that identifies both the sample name and subject ID.

If you enter a subject ID before entering a sample name, the system automatically populates the **Sample Name** field, which you can change at any time.

1. To name a single sample, select the cell where the sample resides in the plate.

To name contiguous samples, select the cells where the samples reside in the plate by clicking and dragging the mouse cursor.

- 2. Enter the name of the sample in the **Sample Name** field, and then press **Enter**.
- 3. Enter the subject ID in the **Subject ID** field, and then press **Enter**.

Name All Wells

- 1. To name all wells in the plate:
 - a. Click Select All
 - b. Enter the name of the samples in the **Sample Name** field, and then press **Enter**.
 - c. Enter the subject ID data, and then press Enter.
- 2. To name multiple noncontiguous samples that are not within the same sample set:
 - a. Click the first cell.
 - b. Press and hold **Ctrl** while selecting the additional samples.
 - c. Enter the name of the samples in the **Sample Name** field, and then press **Enter**.
 - d. Enter the subject ID in the **Subject ID** field, and then press **Enter**. The plate editor shows each edited cell with a green background. The Plate Status indicator shows <code>Plate Edited</code>. This indicates that the plate must be saved.
 - e. Save the plate to retain the changes.

Note: If you attempt to close the plate before saving it, a Save Changes dialog opens.

View Cell Coordinates

To view or hide cell coordinates, select View > Cell Coordinates.

Search for Text in the Sample Plate

- 1. Select **Edit > Find**. Use the options in the Find dialog to specify the search criteria.
- 2. Enter the desired text in the **Search what** field.
 - Select **Down** to search down each column, beginning at the highlighted or selected cell.
 - Select **Up** to search up each column, beginning at the highlighted or selected cell.
 - Select **Match whole word only** to locate text that is flanked by spaces. If **Match whole word only** is not selected, the system will find the text, even if it is part of another word.

- Select Match case to find the entered text exactly as it is typed into the field. If Match case is not selected, the system will find all occurrences of the text, regardless of the case.
- Select **Use wildcards** to include wildcard characters in your search. The following table describes the possible wildcards.

Wildcard	Description		
*	The asterisk specifies to search for any letters before or after the entered text.		
? The question mark represents any single letter.			
[!]	The exclamation point within brackets makes the query negative. Enter the letter following the exclamation point within the brackets if you want the system to find anything that is not the entered text. For example, entering [!A] finds any string that does not start with A.		

Table 4-1 Wildcard Characters

Note: The system supports the following wildcard combinations: **[!]***, ***?**, and **?[!]?**. The system does not support the combination ***[!]**.

3. Click **Find Next** to find the next occurrence of the search text.

Search for and Replace Text in the Sample Plate

- 1. Select **Edit** > **Replace**. Use the **Replace** options to specify your Search and Replace settings.
- 2. Enter the desired text in the **Find what** field.
 - Select **Match whole word only** to locate text that is flanked by spaces. If **Match whole word only** is not selected, the system will find the text, even if it is part of another word.
 - Select **Match case** to find the entered text exactly as it is typed into the field. If **Match case** is not selected, the system will find all occurrences of the text, regardless of case.
 - Select **Use wildcards** to include wildcard characters in your search. The follow table describes the possible wildcards.

Table 4-2 Wildcard Characters

Wildcard	Description		
*	The asterisk specifies to search for any letters before or after the entered text.		
?	The question mark represents any single letter.		

Wildcard	Description
[1]	The exclamation point within brackets makes the query negative. Enter the letter following the exclamation point within the brackets if you want the system to find anything that is not the entered text. For example, entering [!A] finds any string that does not start with A.

Table 4-2 Wildcard Characters (continued)

Note: The system supports the following wildcard combinations: **[!]***, ***?**, and **?[!]?**. The system does not support the combination ***[!]**.

- 3. Enter the text you wish to replace with in the **Replace with** field.
- 4. Choose the desired actions:
 - Click Find Next to find the next occurrence of the search text.
 - Click **Replace** to replace the highlighted text with the text in the **Replace with** field.
 - Click **Replace All** to automatically replace all occurrences of the search text with the text in the **Replace with** field.

Use Property Sets

Property sets are groups of information (properties) that apply to a sample.

- 1. To access the sample property sets, select **View > Sample Property Sets** or select a sample cell in the sample plate screen and refer to the Note tab.
- 2. In the property set table, use **Tab** or the arrow keys to move from cell to cell.
 - Press **Tab** to move the focus from the **Property** field to the **Value** field, and then to the next row.
 - Press Enter to move the focus down the table one cell at a time within the same column.
 - · The arrow keys move in the direction selected.

Figure 4-21 Property Set Editor

Note Method Analysis			
		PROPERTY	VALUE
	1	Template Source	No insert
	2	Sample Prep	Alkaline Lysis
	3	Forward Primer	-20, Universal Cycle 🗾 🔽

- 3. To select an entire column for sorting, click its header. The selected column becomes highlighted.
- 4. To select a row, click its number. The selected row becomes highlighted.
- 5. To select consecutive rows, left-click and hold the mouse button while dragging the cursor over table cells.
- 6. To move a selected row up or down one cell at a time, click the toolbar arrow **f** f icons.
- 7. To delete the contents of the rows, select the desired rows and click the **Delete** icon $\boxed{\times}$.

A warning message opens, prompting you to confirm deletion.

- Click No to return to the editor without deleting the property.
- Click **Yes** to delete the contents and remove the row from the property set.

Enter a New Property

- 1. Click . A new row shows in the property table.
- 2. Enter the new property name and value into the cells of the new row.
- 3. To assign a pre-defined property set, click and select from the defined property sets.

Figure 4-22 Sample Property Sets Dialog

Defined Sets Cloning FLP PCR	F	roperties	
Pedigree		PROPERTY	VALUE
	1	Template Source	
	2	Sample Prep	
	3	Forward Primer	
	4	Reverse Primer	
	5	Sequencing Chemistry	
	6	Template Amount	
	7	Pre-Run Cleanup	
- 1	8	DNA Type	
Create Delete			Þ

Create and Store a Custom Property Set

- 1. Click **Create** in the Defined Sets portion of the Sample Property Sets dialog.
- 2. Enter a name in the Create Property Set dialog.
- 3. If desired, use the **Copy settings from** list to choose an existing property set to copy into the new one.

Figure 4-23 Create Property Set Dialog

Create Property Set			X
Set Name:			
Copy settings from:			
ОК	Cancel	Help	

- 4. Click **OK** to save the new property set.
- 5. Click to add properties to the new property set manually.

Use Methods

A method is a program that includes a sequence of events that the system will use to collect the data. The method controls the hardware, temperatures, voltages, and times, which work together to gather data. The system comes with several methods that are optimized for the different software applications.

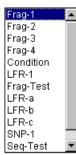
- Use the **Frag** methods to collect data for fragment analysis.
- Use the LFR methods to collect sequence data.
- Use the **SNP** method to collect SNP primer extension data.

After creating a new sample plate, the **Method** selection for each column is left blank.

Assign Methods

- 1. To assign a method, click and select a cell in the column.
- 2. Click the down arrow to open the method selection list and select the method.

Figure 4-24 Method Selection List



3. Edit the new method as desired.

Note: If the 21 CFR Part 11 tools are in use, then the edit function is only available to users with Write access.

Note: You must assign a method to each named row before saving the sample plate. If any named row is not assigned a method and a save is attempted, a warning message opens with a list of each column that needs a method assignment.

Apply Methods to Multiple Sample Sets

- 1. Highlight the desired sample sets as explained in the section: Select Samples.
- 2. Select Edit > Auto-Fill Method Name. The Choose Method dialog opens.
- 3. Select a method from the list.
- 4. In the Auto Fill area of the dialog:
 - To apply a method to multiple sample sets, select **Selected sample sets only**, and then click **OK**.
 - To apply a method to all sample sets, select All sample sets, and then click OK.
- 5. To view the selected method, click the **Method** tab.

Condition the System

It is recommended to purge the manifold with fresh gel followed by a capillary gel fill before each plate run.

If the system has not been used for a long period of time, thoroughly purge the manifold and run the condition method before performing a sample separation. Performing the condition method fills the capillaries with gel six times.

Note: Thoroughly clean the wetting tray with deionized water before every run to remove old or dried gel.

Edit a Method

Note: If the 21 CFR Part 11 tools are in use, then the edit function is only available to users with Write access.

- 1. In the Sample Setup module main window, select **Edit > Method**.
- 2. Highlight the desired method in the Choose Method to Edit dialog, and then click **OK**.
- 3. When the **Method Capillary Temperature** dialog opens, enter a temperature between 35° C and 60° C.
 - a. Select the Wait for Cap Temp option (if desired).
 - b. Continue with step 4 to make additional changes to the method, or click **OK** to exit the Method dialog.
- 4. Select **Denature** from the **Event** list.
 - a. Enter a duration between 0 s and 180 s (if 0 is selected, there will be no denaturation).
 - b. Continue with step 5 to make additional changes to the method, or click **OK** to exit the Method dialog.
- 5. Select **Pause** from the **Event** list.
 - a. Enter a time duration between 0 min and 10 min.
 - b. Continue with step 6 to make additional changes to the method, or click **OK** to exit the Method dialog.
- 6. Select **Inject** from the **Event** list.
 - a. Enter an injection voltage between 0.1 kV and 12.0 kV.
 - b. Enter a time duration in seconds.
 - c. Continue with step 7 to make additional changes to the method, or click **OK** to exit the Method dialog.
- 7. Select **Separate** from the **Event** list.
 - a. Enter the separation voltage between 0.1 kV and 12.0 kV.
 - b. Enter a time duration in minutes.
 - c. Click **Advanced** to specify the two separation phases for the method.
 - d. Click **OK** to return to the Method dialog.

The first phase is to coax the sample into the capillaries. The second phase is to perform the normal separation of the samples.

- 8. Continue to make changes to the method, or click **OK** to exit the Method dialog.
- 9. In the Save As dialog:
 - a. Select a Project Name from the list.
 - b. Enter a name in the name field.
 - c. Click OK.

Define Data Processing Conditions

Analysis parameter sets define the conditions specific to an experiment used in data processing. Modification of the sequence or fragment analysis parameters can only be performed in their respective analysis modules.

Sequence analysis parameter sets define the start and end times for the analysis of raw data, the threshold below which bases are called as *N*s, and the information necessary to detect the start of data to be analyzed, including the delay between the detected start of data and the start of data analysis, the signal-to-noise ratio, the minimum duration, and the threshold above which data are considered peaks.

Fragment analysis parameter sets, including SNP analysis, are used to process the fragment data to estimated fragment sizes and to identify alleles. Fragment sizes are estimated by generating a calibration curve from a set of size standards run with the unknown samples. The fragment analysis parameter set defines the analysis model used to generate the calibration curve, dye label mobility corrections, allele identification criteria (locus tags), quantitation values, and other factors that will affect how your data are analyzed.

Assign Analysis Parameter Sets

- For sequence analysis, select Sequence Analysis as the Parameter Set.
- For fragment analysis, select Fragment Analysis as the Parameter Set.

Perform an Analysis on a Sample

- 1. Select the cell/cells as described in the section: Select Samples.
- 2. Select the **Analysis** tab at the bottom of the window.
- 3. Select Perform Analysis.
- 4. Select the parameter set from the **Parameter Set** list.

Figure 4-25	Analysis	Tab,	Selecting	а	Parameter S	et
-------------	----------	------	-----------	---	--------------------	----

Note Method Analysis		
Analysis	Reports	Export
Perform Analysis	Print Report	Export Data
Parameter Set:		
equenceAnalysisParameters 💌	Edit Print Format For Plate	Edit Export Options For Plate
DefaultSequenceAnalysisParame		
DefaultFragmentAnalysisParamete		
DefaultSNPAnalysisParameters		
DefaultGeXPAnalysisParameters		

Print the Plate Report or Specify Sample Plate Print Options

Define the print options and report format for the currently open sample plate.

- 1. With a cell highlighted, select the **Analysis** tab.
- 2. Select the **Print Report** check box to print a report immediately after completion of the run.
- 3. Click **Edit Print Format For Plate**. The Report Format dialog opens.
- 4. Verify or select the **Printer** and **Page Layout** options.

If desired, click **Colors** to verify or modify the trace colors of the Raw Data and/or Analyzed Data. Click **OK** to accept the changes.

5. In the Sample Elements area of the Report Format dialog, select each element to be printed.

If desired, click **Options** to verify or modify the values for Raw Data, Sequence Analysis Result Data, Fragment Analysis Result Data, Base Sequence Grouping, and Current Trace Options in the Print Options dialog. Click **OK** to accept the changes.

6. Click **OK** in the Report Format dialog to save the changes and close the dialog.

Report Form	at		×
Printer —			
Name:	Microsoft Office I	Document Image Writer 📃 💌	Properties
Status: Type: Where: Comment:)ocument Image Writer Driver ent Imaging Writer Port:	Colors
Page Layou Portra Lands Sample Ele	ait scape ments		
I Head Raw [I Resul I Resul Currer	 Data t Data t Output	Analysis Log Run Log Method Summary Analysis Parameters Quality Parameters	OK Cancel Help
☐ Voltag	je	Trimming Log Alignment Results Alignment Accuracy	Options

Specify Sample Plate Export Options

To export sample results after separation:

- 1. Select **Export Data** in the Export section of the Analysis tab.
- 2. Click Edit Export Options for Plate.
- 3. Select the target folder.
- Select the desired Sample Elements for the export format chosen.
 Refer to the Help System for a description of these parameters and the export formats.
- Select the desired options for the chosen export format.
 For descriptions of the export options used for exporting sample results, refer to the section: Export Options.
- 6. Click OK.

Note: The export formats selected will apply to all samples automatically exported during the plate run.

Export Options Remove the CEQ Tracking Suffix

Select this option to remove the appended sample plate position and time/date stamp from the exported files.

Resolve File Name Conflicts

Select this option to automatically increment the exported files of the same name with a unique sequence number.

Export Options - Sequencing Results

The following options are specific to sequencing results only.

Apply Trimming

Select this option to remove the trimmed data from a sequence before it is exported. The export replaces all internal trims with the letter X.

Export Only If Sequence > X nt

After performing the trimming operation, the resulting sequence may be too small to be useful. Select the **Export Only If Sequence > X nt** option to specify a minimum size for the sequence output, when exporting. This exports only those sequences greater than the set number of nucleotides.

See the Help System for additional export sample elements.

Import Sample Plate Information from txt Files Import a Sample Plate with Default Run Method and Analysis Parameters

Import the **Sample Plate** txt file(s) to the GenomeLab GeXP system. The run default separation method(s) and analysis parameters that were pre-selected in the imported sample plate data sets will be used for running and analyzing the plate.

- 1. In the Data Manager module, select an appropriate working database and project.
- 2. Open the Sample Setup module.
- 3. From the **Menu Bar**, select **File** > **Import**. The Import dialog opens.
- 4. Navigate to the folder containing the Sample Plate txt file.
- 5. Highlight the selected Sample Plate txt file, and then in the Import dialog, click **Open**.

The imported sample plate grid window will open.

- 6. Confirm the values and properties for all **Sample Plate** objects: barcode, sample names, separation method(s), note, analysis parameters, report and export options.
- 7. From the Menu Bar, select File > Save As.
- 8. Save the **Sample Plate** to the desired project with a new name.
- 9. Repeat steps 1 through 8 for the second sample plate, if one is present.

Import a Sample Plate Use Non-Default Run Method or Analysis Parameters

If using non-default methods, the appropriate run method(s) and analysis parameters that were pre-selected in the data sets must be set up in the GenomeLab System software before importing the sample plate txt file(s).

- 1. Launch the Data Manager module.
- 2. Set up an appropriate working database or create a new working database and project.
- 3. Launch the Sample Setup module.
- 4. Cancel the Sample Plate Selection window.
- 5. From the **Menu Bar**, select **Edit** > **Method**. The Choose Method to Edit dialog opens.

Note: If the 21 CFR Part 11 tools are in use, then the edit function is only available to users with Write access.

- 6. Confirm the presence of the appropriate or pre-selected run method(s). If not present, highlight any existing method and click **OK**.
- 7. Select the appropriate **Event Type** and edit the method. Refer to the section: Edit a Method.
- 8. When finished, click **Save As** and save the edited method to an appropriate project with the same method name as specified in the plate Import file.
- 9. After creating the non-default run method and analysis parameters, follow the steps in the section: Import a Sample Plate with Default Run Method and Analysis Parameters.

Use a Sequence Reaction Separation

- 1. Launch the Sequence Analysis module.
- 2. In the Sequence Analysis module, select **Analysis > Working Analysis Parameters**.
- 3. In the Working Parameters dialog, select **Use Stored Parameters**.
- 4. In the Select Analysis Parameters window, confirm the presence of the appropriate/preselected analysis parameters.

If not present:

- a. Cancel the Select Analysis Parameters window.
- b. In the Working Parameters dialog, click Edit.

Note: If the 21 CFR Part 11 tools are in use, then the edit function is only available to users with Write access.

- 5. In the Sequence Analysis Parameters Editor dialog, select the desired tab and edit the parameters as appropriate. Refer to the section: Edit Sequence Analysis Parameters.
- 6. When finished, click **Save As** and save the edited parameters to an appropriate project with the same analysis parameters name as specified in the plate Import file.

Perform a Fragment Analysis Separation

- 1. Launch the Fragment Analysis module.
- 2. In the Fragment Analysis module, select **Analysis > Analysis Parameters**. The Analysis Parameters dialog
- 3. Select the appropriate analysis parameters in the **Select Analysis Parameters Set** field. If not present:
 - a. Click Edit.
 - b. In the Edit Fragment Analysis Parameters dialog, modify the appropriate analysis parameters. Refer to the section: Define Fragment Analysis Parameters.
- 4. When finished, click **Save As** and save the edited parameters to an appropriate project with the same analysis parameters name as specified in the plate Import file.

Export Sample Plate Data

Export the sample plate data to use in compatible equipment or to edit the plate information manually.

IMPORTANT If a sample plate file is already open, make sure it has been saved before proceeding.

To export the sample plate:

- 1. Open the sample plate file.
- 2. Select File > Export.
- 3. Select **Resolve Filename Conflicts** to prevent the file from overwriting a previous file with the same name. If another file with the same name exists, the export function increments the new file with a unique sequence number.

Note: If you do not select **Resolve Filename Conflicts** and a file with the same name exists, the system shows a warning message, asking if you want to replace the old file with the new one. Select **Yes** to overwrite the existing file or **No** to cancel the export.

- 4. Select a location to save the file.
- 5. Enter a file name or leave the name as it is.
- 6. Click OK.

Lock a Sample Plate

To prevent editing of the currently shown plate, select **File > Lock**. The Notes, Method, and Analysis tabs are disabled when the sample plate file is locked.

View the Summary

To open a read-only window that shows the attributes of the currently selected plate, select **View** > **View Summary**.

This chapter provides an overview of the Run module, including its menu options, toolbars, and dialogs. It also shows how to run sample plates and set the display options using this module.

Run Module Overview

Use the Run module to launch pre-programmed sample plates and control individual functions of the GenomeLab GeXP system.

Use the Run module to do the following:

- Run a sample plate
- Perform control and operational functions such as:
 - Denature samples
 - Inject and separate samples
 - Load and unload plates
 - Control the capillary chamber temperature
 - Fill the capillaries with gel

Main Window

The main window for the Run module has the following sections.

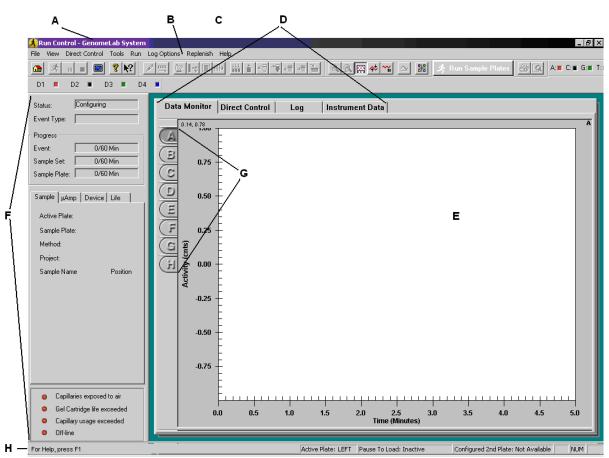


Figure 5-1 Main Window, Run Module

ltem	Description
A	Title Bar: Shows the name of the module (Run Control) and the user- defined system name.
В	Menu Bar: Refer to the section: Menu Bar.
С	Toolbars: Refer to the section: Toolbars.
D	Window Selection Tabs: Toggle between windows. Refer to the section: Window Selection Tabs.
E	Display Area: Graphically shows the raw data of the selected capillary or capillaries.
F	Status Monitor: Shows the current state of the run, capillary and gel cartridge status, and system on-line/off-line status.

ltem	Description
G	Capillary Buttons: Letters A through H represent the eight capillaries in the array. Selecting one of these buttons shows an associated pane in the display area.
н	Status Bar: • Shows messages such as:
	Currently active database: Shows the name of the active database
	 Active Plate: Shows the side of the active plate (left or right).
	 Pause To Load: If you selected Pause to Load, this area shows the wait time.
	 Configured 2nd Plate: If you loaded a second plate, this area shows the name of the plate.
	 Fields on the right of the status bar indicate which of the following keys are latched down:
	CAP: Caps Lock is enabled.
	NUM: Num Lock is enabled.
	SCRL: Scroll Lock is enabled.

Menu Bar

The following example shows the menu bar for the Run module.

Figure 5-2 Run Module Menu Bar

File View Direct Control Tools Run Log Options Replenish Help

The following topics describe each of these menus and their options.

File Menu

Click **File** to show the following menu.

Figure 5-3 File Menu, Run Module

Eile		
R	estore Data Monitor Defaults	
S	ave Log	
2	ystem Preferences	
P	rint Set <u>u</u> p	
P	rint Pre <u>v</u> iew	
E	rint Log,	
P	ri <u>n</u> t Selected Pane Ctrl+S	
P	rint Screen	۲
E	<u>x</u> it	

Option	Description
Restore Data Monitor Defaults	Restore the colors of the data monitor to the factory default.
Save Log	Save the system log in a selected folder. The log is identified by the date and a unique identifier.
System Preferences	View and modify the System Name, Operator Name, Project Name, and Dye Names for fragment analysis and/or to activate or deactivate the alarms. Refer to the section: Define System Preferences.
Print Setup	Define printer properties.
Print Preview	Open a print preview of the active document of the active sample plate. A toolbar at the top of the window provides options for navigation, viewing, and printing the display. To restore the default screen, press Esc or click Close .
Print Log	Print the system log.
Print Selected Pane	Print the selected pane.
Print Screen	Open a sub-menu with options to print an image of the computer desktop or application window.
Exit	Close the module.

View Menu

Click **View** to show the following menu.

Note: A check mark next to an option indicates that the option is enabled.

Figure 5-4 View Menu, Run Module

View
<u>T</u> oolbars
✓ Status Bar
✓ Status Monitor
Working Database

Option	Description
Toolbars	Select which toolbars to show. Refer to the section: Toolbars.
Status Bar	Toggle to show or hide the status bar.
Status Monitor	Activate or deactivate the Status Monitor window. The default setting is activated. Refer to the section: Status Monitor.
Working Database	Show the name of the database in use.

Direct Control Menu

Click **Direct Control** to show the following menu.

Figure 5-5 Direct Control Menu, Run Module

Direct Control	
Access Plates.	
Plate Position.	
Capillary Temp	erature
Denature Sam	ples
Optical Alignme	ent
Inject	
Separate	
Gel Capillary Fi	II
Manifold Purge	h

Option	Description
Access Plates	Move the plate holder to the position at the front of the GenomeLab GeXP system to load or unload plates. Refer to the section: Load the Sample Plate - Dual.
Plate Position	Graphically select the well location where the capillaries will be immersed.
Capillary Temperature	Set the capillary holding temperature. This temperature will be over-ridden at the end of each sample plate or when cancelled. Refer to the section: Set the Capillary Temperature.

Option	Description
Denature Samples	Initiate the denaturing of samples. Refer to the section: Denature Samples.
Optical Alignment	Align the lasers with the detection windows of the eight capillaries. Save and view the data in the Sequence Analysis module. Refer to the section: Perform an Optical Alignment.
Inject	Initiate the injection of the sample prior to separation. Refer to the section: Inject Samples.
Separate	Initiate the separation of injected samples. The data generated is not saved. Refer to the section: Perform a Separation.
Gel Capillary Fill	Fill all capillaries with fresh gel. (This forces any gel present in the capillary out to the specified waste position in the buffer plate or wetting tray.) Refer to the section: Replenish the Capillaries with Gel.
Manifold Purge	Clear the manifold of air bubbles and/or remaining DNA fragments before the next separation process. Refer to the section: Purge the Manifold.

Tools Menu

Click **Tools** to show the following menu.

Note: A check mark next to an option indicates that the option is enabled.

Figure 5-6 Tools Menu, Run Module



Option	Description
Autoscroll	Toggle to enable or disable autoscrolling. When enabled, all data is scaled to the last 10 min of the run on the X-axis and confined to the pane on the Y-axis.

Option	Description
Autoscale	Toggle to enable or disable autoscaling. When enabled, data in the window is automatically scaled to fit in the pane. When disabled, data is scaled to its true values.
Pause Data Display	Pause or resume data display. When enabled, the data display pauses. (Pausing data does not stop the stream of data, only the display of data.)
Unzoom	Undo one zoom level.
Unzoom All	Undo all zoom levels.
Display Options	Open the Display Options dialog to modify the parameters for currently shown data, including its title, X-axis, Y-axis, dye traces, current traces, and colors. For details, refer to the section: Set or Change Display Options.
View Last Analysis	Open the Sequence Analysis or Fragment Analysis module and show the most recently analyzed sample set of a running sample plate. For details, refer to the section: View the Last Analysis Performed.

Run Menu

Click **Run** to show the following menu.

Figure 5-7 Run Menu, Run Module



Option	Description
Start Sample Plate	Open the Sample Plate Run Confirmation dialog.
Pause	Pause a running sample plate.
Pause to Load	Load a second plate while a plate is running. For details, refer to the section: Pause to Load.
Stop System	Stop a running sample plate or Direct Control process. For details, refer to the section: Stop a Sample Plate Run.

Option	Description
Monitor Baseline	Set the desired parameters. For details, refer to the section: Monitor the Baseline.
Diagnostics	View the instrument version, laser power, PC settings, and monitor status. Home the plates and/or the gel pump. For details, refer to the section: Diagnostics.
Reset	Resynchronize system hardware with the firmware and resets the system log.

Log Options Menu

When the Log tab is selected, the **Log Options** menu is available. Click **Log Options** to show the following menu.

Note: A check mark next to an option indicates that the option is enabled.

Figure 5-8 Log Options Menu, Run Module

Log Options
Errors Only
🗸 All Detail
Sample Plate Only
Freeze Log

Option	Description
Errors Only	Toggle to show or hide errors only in the Log window.
All Detail	Toggle to show or hide all messages and system activity in the Log window.
Sample Plate Only	Toggle to show or hide only the items related to a running sample plate in the Log window.
Freeze Log	Toggle to freeze or resume the display in the Log window. (Freezing the log data does not stop the run, only the display of log data.)

Replenish Menu

Click **Replenish** to show the following menu.

Figure 5-9 Replenish Menu, Run Module

_	
R	eplenish
	Capillary Information
	Install Capillary Array
	Release Capillary Array
	Gel Cartridge/Buffer Information
	Install Gel Cartridge
	Release Gel Cartridge
	Replace Wetting Tray
	Empty Waste Bottle

Option	Description
Capillary Information	Open the Capillary Information dialog to view or enter information about the capillary array. For details, refer to the section: View Capillary Information.
Install Capillary Array	Open the Install Capillary Array dialog to verify or change information, then install the capillary array.
Release Capillary Array	Show the first in a series of dialogs to remove the currently installed capillary array. For details, refer to the section: Remove and Replace the Capillary Array.
Gel Cartridge/ Buffer Information	Open the Gel Cartridge/Buffer Information dialog to view information about the gel cartridge and buffer. For details, refer to the section: View Gel Information.
Install Gel Cartridge	Open the Install Gel Cartridge dialog.
Release Gel Cartridge	Remove the gel cartridge, or if you are not going to install a working gel cartridge, insert a clean empty cartridge or gel pump plug into the cartridge chamber. For details, refer to the section: Remove and Replace a Gel Cartridge/Gel Pump Plug for Dual-Rail System.
Replace Wetting Tray	Show the first in a series of dialogs to replace the wetting tray. For details, refer to the section: Replace the Wetting Tray.
Empty Waste Bottle	Show the first in a series of dialogs to empty the waste bottle. Refer to the section: Replace the Gel Waste Bottle for Dual-Rail System or Replace the Gel Waste Bottle for Single-Rail System.

Help Menu

Click **Help** to show the following menu.

Figure 5-10 Help Menu

lel	р
	Help <u>T</u> opics
	<u>U</u> sing Help
	About GenomeLab System
	SCIEX Home Page
_	

Option	Description
Help Topics	Select this option to show the Help topics. By default, the Contents show in the navigation pane on the left and the introductory topic shows in the topic pane on the right. Use the Contents to select and print specific Help topics. In the navigation pane, use the Search and Index tabs to search for information by topic, index entry, and/or keyword.
Using Help	Select this option to open the Contents window on how to use the Windows Help system.
About GenomeLab System	Select this option to access software and system information.
SCIEX Home Page	Select this option to access the SCIEX Home Page on the Internet.

Toolbars

The Run module provides six toolbars: Standard, Direct Control, Data Monitor, Log, Sequence Dye Colors, and Fragment Dye Colors. Each icon of the various toolbars corresponds to a commonly used menu item.

The following tables describe the function of each of the toolbar icons.

Standard Toolbar

The following example shows the Run module's Standard toolbar.

Figure 5-11 Standard Toolbar, Run Module

|--|

lcon	Description
	Restore Data Monitor Defaults: Show the default color selections at the time of installation.

lcon	Description
×	Run Sample Plate: Run a sample plate.
н	Pause: Pause the currently running sample plate.
	Stop: Stop the currently running sample plate or Direct Control process.
	View Last Results: Execute the Sequence Analysis or Fragment Analysis module with the analysis of the last sample set run.
8	Help Topics: Open the Help System. By default, the Contents show in the navigation pane on the left and the introductory topic shows in the topic pane on the right. Use the Contents to select and print specific Help topics. In the navigation pane, use the Search and Index tabs to search for information by topic, index entry, and/or keyword.
№?	Context-Sensitive Help: Click this icon and then on a menu item to open the Help topic related to the selection.

Direct Control Toolbar

The following example shows the Run module's Direct Control toolbar.

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lcon	Description
1	Inject: Inject sample prior to its separation and detection. Refer to the section: Inject Samples.
	Separate: Initiate the separation of injected samples. Refer to the section: Perform a Separation.
<u>m</u>	Fill Capillary with Gel: Fill all eight of the capillaries with fresh gel. Refer to the section: Replenish the Capillaries with Gel.
	Capillary Temperature: Specify the desired temperature for the capillaries. Refer to the section: Set the Capillary Temperature.
	Denature: Denatures the samples residing in a specific sample set. Refer to the section: Denature Samples.
## >	Optical Alignment : Perform the alignment of the lasers with the capillary windows. Refer to the section: Perform an Optical Alignment.

Icon	Description
tit	Plate Position: Select where to position and immerse the capillaries.
*	Load/Unload Plates: Select the position where the capillaries are immersed in the plate or wetting tray and moves the plates to the Load position at the front of the GenomeLab GeXP system for loading/unloading plates.
۳Ð	Release Gel Cartridge: Remove the gel cartridge. Refer to the section: Remove and Replace a Gel Cartridge/Gel Pump Plug for Dual-Rail System.
*₽	Install Gel Cartridge: Install a gel cartridge.
÷∰	Release Capillary Array: Remove the current capillary array. Refer to the section: Remove and Replace the Capillary Array.
→iiii	Install Capillary Array: Install the capillary array.
	Replace Wetting Station: Replace the wetting tray. Refer to the section: Replace the Wetting Tray.

Data Monitor Toolbar

The following example shows the Run module's Data Monitor toolbar.

9	9	☆	~~	\sim	#

lcon	Description
2	Unzoom: Undo one zoom level.
2	Unzoom All: Undo all zoom levels.
	Autoscroll: Scale all data to the last 10 min of the run on the X-axis and confine the display of data to the pane on the Y-axis.
<u>ŕ</u> *	Autoscale On/Off: By default, data showing on the screen is scaled automatically to the confines of the screen (Autoscale is on). If Autoscale is off, the data is scaled to its true values.
~	Pause Data Update: Pause the display of data. (Pausing data does not stop the stream of data, just the display of data.)

lcon	Description
\sim	Monitor Baseline: Show the baseline data trace. For details, refer to the section: Monitor the Baseline.
	Display Options: Modify any or all display parameters. Refer to the section: Set or Change Display Options.

Log Toolbar

The following example shows the Run module's Log toolbar.

Figure 5-14 Log Toolbar, Run Module



lcon	Description
a	Print: Print the system log at any time the program is active. System Logs contain all of the messages and activity from the time the program is opened to the time the program is closed.
Q	Print Preview: Show a print preview of the System Log.

Run Sample Plates Button

The following example shows the Run module's **Run Sample Plates** button.

Figure 5-15 Run Sample Plates Button

🖈 Run Sample Plates

Click **Run Sample Plates** to open the Sample Plate Run Confirmation dialog.

Sequence Dye Colors Toolbar

The following example shows the Run module's Sequence Dye Colors toolbar.

Figure 5-16 Sequence Dye Colors Toolbar, Run Module

A: 🗉 C: 🗉 G: 🗖 T: 🗧 🛛

lcon	Description
A (red)	Red is the default color assigned to the adenine nucleotide bases in the analyzed data pane.
C (black)	Black is the default indicator assigned to the cytosine nucleotide bases in the analyzed data pane.
G (green)	Green is the default color assigned to the guanine nucleotide bases in the analyzed data pane.
T (blue)	Blue is the default color assigned to the thymine nucleotide bases in the analyzed data pane.

Fragment Dye Colors Toolbar

The following shows the Run module's Fragment Dye Colors toolbar.

Figure 5-17 Fragment Dye Colors Toolbar

D1 D2 D3 D4 D4

Icon	Description
D1 (red)	Red is the default color assigned to Dye #1 in the fragment data pane and D1 is the default name.
D2 (black)	Black is the default color assigned to Dye #2 in the fragment data pane and D2 is the default name.
D3 (green)	Green is the default color assigned to Dye #3 in the fragment data pane and D3 is the default name.
D4 (blue)	Blue is the default color assigned to Dye #4 in the fragment data pane and D4 is the default name.

Status Monitor

The following example shows the Status Monitor, which shows the status of the current run.

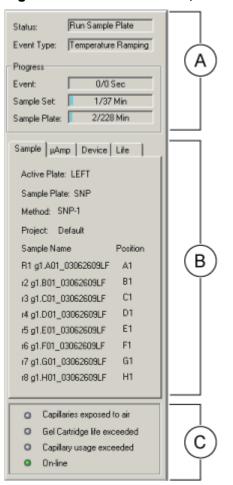


Figure 5-18 Status Monitor, Run Module

ltem	Description
A	Progress Indicator: Shows the Status, Event Type, and Progress of a running sample plate or process.
	Note: Times shown are approximations.

ltem	Description
В	Information Tabs: Select any of four tabs to show the corresponding status details:
	• Sample: Lists the sample plate, sample names, and method being used.
	 Device: Shows the condition or value of devices or parameters.
	 μAmp: Shows the approximate overall microamperes (μA) and voltage used during the separation.
	Life: Shows the remaining gel, capillary usage, and laser hours.
С	Capillary Status: Indicates certain capillary usage, gel cartridge life, and on-line status. When the life of the gel or capillary array is exceeded, the associated indicator turns red. When the system is on-line, the indicator is green.

Window Selection Tabs

View data on the Data Monitor, Direct Control, Log, and Instrument Data tabs. The following topics describe the windows that open.

Data Monitor Tab

The following example shows the Data Monitor window. To open this window, select the **Data Monitor** tab.

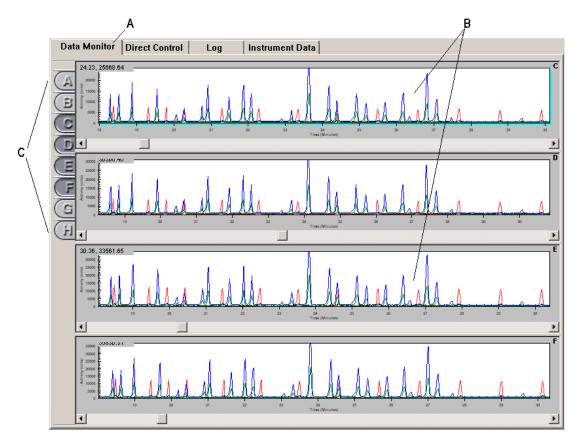


Figure 5-19 Data Monitor Window, Run Module

ltem	Description
A	Window Selection Tab: Shows the Data Monitor window.
В	Data Monitor Window: Shows the data for the running sample plate for the selected capillary buttons.
С	Capillary Buttons: These buttons (A through H) represent the eight capillaries of the array.

Direct Control Window

This window contains selectable areas and buttons that perform some of the distinct tasks available on the **Direct Control** and **Replenish** menus. For details, refer to the sections: **Direct Control** Menu and **Replenish** Menu. When you click a selectable area, the system initiates a unique Direct Control task.

For procedures about using the Direct Control window to replenish the system, refer to the section: Direct Control and Replenishment.

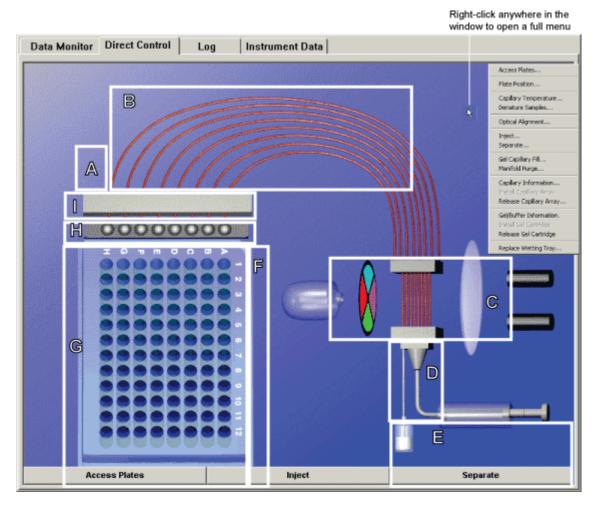


Figure 5-20 Direct Control Window, Run Module

Item	Description
A	Capillary Temperature: Change the capillary temperature.
В	Gel Capillary Fill: Fill all capillaries with fresh gel. (Any gel present in the capillary is forced out to the specified waste position in the buffer plate or wetting tray.)
С	Optical Alignment: Align the lasers with the detection windows of the eight capillaries. Data can be saved and viewed in the Sequence Analysis module.
D	Manifold Purge: Clear the manifold of air bubbles and/or remaining DNA fragments before the next separation process.
E	Install/Release Gel Cartridge: Release and/or installs a gel cartridge.

ltem	Description
F	Denature Samples: Initiate the denaturing of samples.
G	Plate Position: Select the plate or tray position where the capillaries will be immersed.
н	Wetting Tray: Replace or refill the wetting tray.
I	Install/Release Capillary: Install the capillary array. Verify or change the information requested, and then install the array.
Access Plates	Load/Unload Plates: Shows the Unload Plates dialog and gives options to:
	Select the position for capillary immersion in the plate or wetting tray.
	 Move the plates to the Load position at the front of the GenomeLab GeXP system to load or unload plates.
Inject	Inject: Initiates injection of the sample prior to separation.
Separate	Separate: Initiate the separation of injected samples.

Log Window

View all of the messages and activity for a sample plate run. The following example shows the Log window. To open this window, select the **Log** tab.



A B (c
Data Monitor Direct Control Log Instrument Data	
Date/Time Logged Events Freeze Log	
10/12/01 12:20:27System configured, instrument detected10/12/01 12:30:20Gel life, Capillary Array life exceeded.10/12/01 12:38:26Capillary Array life exceeded.10/12/01 12:38:37Direct Control stage [Load Capillaries] starts10/12/01 12:38:35Direct Control stage [Detecting Array] starts10/12/01 12:38:36Direct Control stage [Detecting Array] starts10/12/01 12:39:35Direct Control stage [Detecting Array] starts10/12/01 12:39:35Sample Plate "DefaultSamplePlate" begins10/12/01 12:39:35Sample Set Startup10/12/01 12:39:35Sample Set "DefaultStartupMethod" begins10/12/01 12:39:35Sample Set "DefaultStartupMethod" ends10/12/01 12:39:35Sample Set "DefaultStartupMethod" ends10/12/01 12:39:35Sample Set "DefaultStartupMethod" ends10/12/01 12:39:35Sample Set "DefaultStartupMethod" ends10/12/01 12:39:36Begin Sample Set 110/12/01 12:40:10Method stage [Temperature Ramping] starts10/12/01 12:40:15Method stage [Denature Sample] starts10/12/01 12:40:15Method stage [Denature Sample] starts10/12/01 12:40:51Current capillary temperature is 34 degree C10/12/01 12:42:19Method stage [Sample Set Pause] starts10/12/01 12:42:19Method stage [Sample Set Pause] starts10/12/01 12:42:30Method stage [Sample Set Pause] starts10/12/01 12:42:39Method stage [Sample Set Pause] starts10/12/01 12:43:30Method stage [Sample Set Pause] starts10/12/01 12:43:30Method stage [Samp	

ltem	Description
A	Window Selection Tab: Select this tab to access the Log window.
В	Freeze Log: Freeze the display in the Log window. (Freezing the log data does not stop the collection of data, just the display of data.)
С	Display Area: Shows a list of logged events.

Instrument Data Window

View the current for the eight capillaries and the voltage level of the GenomeLab GeXP system for the current run. To open this window, select the **Instrument Data** tab.

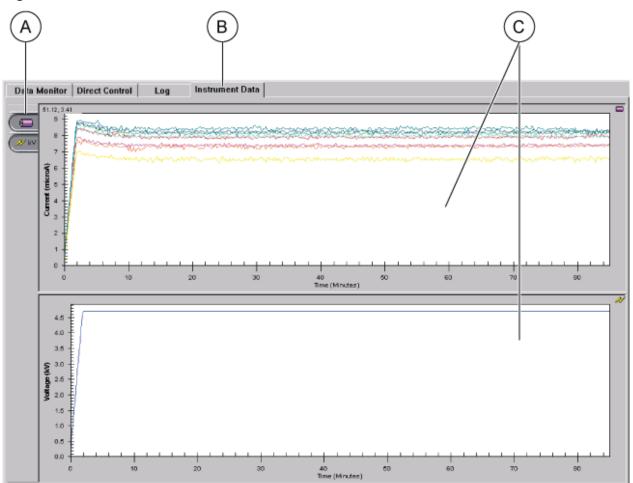


Figure 5-22 Instrument Data Window, Run Module

ltem	Description
A	Current and Voltage buttons: Select panes for display.
В	Window Selection Tab: Select this tab to access this window.
С	Display Area: Shows the current and voltage levels of selected current run.

Using the Run Module

Run sample plates and set the display options.

To open the Run module, on the Main Menu, click the **RUN** icon

Gel Plug Warning

When you open the Run module, the system initializes and checks the gel cartridge condition. If the system detects an installed gel plug, the following Device Warning Message opens.

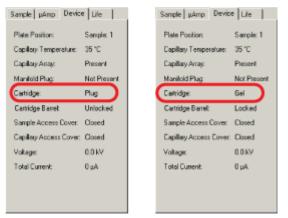
Figure 5-23 Device Warning Message, Gel Plug Installed

Device Warning Message	×
Instrument status: Gel Plug Installed, Loading Gel Cartridge Please install a Gel Cartridge for proper operation.	
ОК	

In addition to this warning message, the Device tab indicates the state of the cartridge. When a gel plug is present, the Device tab shows <code>Plug</code> in the **Cartridge** field. Refer to the left side of the figure: Figure 5-24.

The plug must be removed and replaced with a gel cartridge before the next run. When a cartridge is present, the Device tab shows Gel in the **Cartridge** field. Refer to the right side of the figure: Figure 5-24.

Figure 5-24 Device Tab



Define System Preferences

1. To define the system preferences, select **File > System Preferences**.

The System Preferences dialog opens.

Figure 5-25 System Preferences Dialog

System Preferences		×
Defaults		ОК
System Name: Operator Name: Project Name:	GenomeLab System Test Operator Default	Cancel
Dye Names D1 D2 D3 D4		
Alarms C Active C Inact	ive	

- 2. Enter the System Name and Operator Name.
- 3. Select the **Project Name**.
- 4. Define **Dye Names** for the Fragment Analysis module, if desired.
- 5. Enable or disable the alarms.
- 6. Click OK.

Run Sample Plates

To run two sample plates (once plate information has been created and saved for both plates):

- Select Run > Start Sample Plate. The Sample Plate Run Configuration dialog opens.
- 2. Select the name of the sample plate from the **Sample Plate Name** list for the Left and Right Plates.
- Click Load Plates. The Access Plates dialog opens.
- 4. Click **Start**. The Capillaries Exposed dialog opens.
- 5. Follow the screen instructions in Capillaries Exposed dialogs to load the wetting tray, sample plate, and buffer plate by following the instructions.
- 6. Click Load to finish loading plates.
- 7. Verify the sample position in the plate.
- 8. Click **Start** to initiate the run.

Note: If the working database exceeds 500 MB, an error message appears. If this happens, follow the troubleshooting procedures described in the section: Database Size, then return to these steps.

Database Size

If you select a database that is larger than 1700 MB, the following warning message opens when you create and run a sample plate.

Figure 5-26 Run Control Message, Database Size Warning



To resolve this problem:

- 1. Click **OK** to dismiss the warning dialog.
- 2. Exit the Run module and the Sample Setup module.
- 3. Make sure all of the other modules are closed.
- 4. Open the Data Manager module.
- 5. Create a new database. For details, refer to the section: Create a Database.
- 6. Copy the sample plate from the previous working database to the new database.
- 7. Click **OK All** to copy any objects referenced by the sample plate to the new database.
- 8. Exit the Data Manager module and launch the Run module.
- 9. Select **Run > Start Sample Plate**.
- 10. Select the new sample plate, and then click **OK**.

The system processes the plate and stores it in the new database.

Note: It is recommended that you archive databases that are larger than 1700 MB. For details, refer to the section: Check the Database Size.

Gel Re-estimation

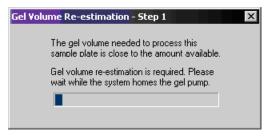
The system determines if the gel cartridge volume is sufficient to safely process the plate.

Run Module

- If the gel volume is greater that 1.5 mL more than the minimum amount needed to process the plate, the plate runs and the system does not re-estimate the gel volume.
- If the system determines that the gel cartridge volume is less than the volume needed to safely process the plate, the system opens two re-estimation messages before running the sample plate.

In the first step, the system homes the gel pump and shows the Gel Volume Re-estimation - Step 1 message.

Figure 5-27 Gel Volume Re-estimation - Step 1 Message



In the second step, the system engages the gel pump plunger and accurately determines the gel volume The Gel Volume Re-estimation - Step 2 message opens.

Figure 5-28 Gel Volume Re-estimation - Step 2 Message

Gel Volume Re-estimation - Step 2
Re-positioning the gel cartridge plunger for gel volume re-estimation.
Please wait.

If the actual volume (re-estimated by the system) is less than 1.0 mL more than the total volume to run the plate, the Sample Plate Gel Usage Confirmation dialog opens.

Figure 5-29 Sample Plate Gel Usage Confirmation Dialog

Sample Plate Gel Usage Confirmatio	n X
The total available gel is sufficient	torun 3 sample sets
Left Plate	Right Plate
Total sample sets: 4	Total sample sets: 4
Run the first 0 sample sets	Run the first 3 sample sets
Accept Replace (Gel Cartridge Cancel

- 1. Click Accept to proceed with the run.
- 2. Specify the sample sets to run.
- 3. Click **Replace Gel Cartridge** to replace the gel cartridge before the run (if necessary).

Gel Volume Notification

If you attempt any operation that requires gel and there is not enough gel in the cartridge to perform the operation, a warning message shows.

For example, if you attempt to perform a capillary gel fill when the cartridge does not have enough gel, the system shows the following message.

Figure 5-30 Run Control Message, Gel Warning

Run Cont	rol
8	There is not enough gel to perform capillary fill. The gel cartridge reserves 1.0 ml of gel for system operation margin.
	ОК

If you attempt to run a sample plate and the gel cartridge does not have enough gel to process any sample sets in that plate, the system shows the following message.

Figure 5-31 Sample Plate Gel Usage Confirmation Dialog



To end the run and return to the Run module main window, click Abort.

Pause a Sample Plate Run

To pause the currently executing sample plate, click the **Pause** icon . The system pauses at the next executable step of the sample plate.

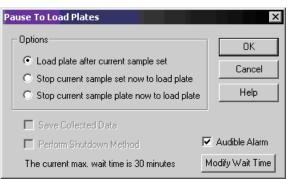
To resume the run, click the **Pause** icon **u** again.

Pause to Load

To pause a current run so you can load another plate:

1. Select **Run > Pause to Load** to pause the system and load another plate. The Pause to Load Plates dialog opens.

Figure 5-32 Pause to Load Plates Dialog



- 2. Select one of the following load options:
 - Load plate after the current sample set: This option delays loading of plates until after immediate sample run. After completion of sample separation, the system enters the Access Plates condition. After loading the new plate, the system runs the next sample set from the original sample plate.

Note: If the plate has not been loaded by the time set in **Modify Wait Time** (max. 30 min), the run resumes with the next sample set.

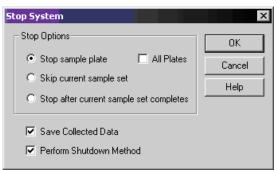
- Stop current sample set now to load plate: This option immediately stops the run to allow for plate loading. If selected, the Save Collected Data option becomes available, allowing the previously collected data to be saved.
- Stop current sample plate now to load plate: This option breaks the plate run before the next sample. If selected, the Save Collected Data option becomes available, allowing the previously collected data to be saved.
- 3. Click **OK**.

Stop a Sample Plate Run

To stop the currently executing sample plate:

Select Run > Stop System or click the Stop System icon .
 The Stop System dialog opens.

Figure 5-33 Stop System Dialog



- 2. Select the desired Stop Options:
 - Stop sample plate: Stops the currently running sample plate immediately.
 - All Plates: Check this box to make the Stop sample plate option apply to both sample plates.
 - Skip current sample set: Skips the currently running sample set.
 - Stop after current sample set completes: Stops the run after the currently running sample set has been completed.
 - Save Collected Data: Saves data already collected for the currently executing sample set.

- **Perform Shutdown Method**: Purges the system of any remaining DNA fragments and refills the capillaries with fresh gel after the sample plate has been cancelled. (The capillary temperature will be set to 35 °C.)
- 3. Click OK.

Set or Change Display Options

To set or change the graph data display options:

- 1. Open the Display Options dialog using one of these methods:
 - Select Tools > Display Options.
 - While viewing a graph display of sequence data, right-click the graph and select **Display Options**.

Figure 5-34 Display Options Dialog

Display O	ptions					×
Colo Title	ors]	Dye Trac X Axis Opti			rrent Traces Axis Options	
_ Title	Property					
Title	e:					
Title	e Color: 🛛					
Title	e Font: 🛛	Arial		•		
🔽 Save	e Customiz	ed Setting	□ S	ave Setti	ing Now	
OK		Cancel	A	oply	<u>H</u> elp	,

2. Select the tab that identifies the type of display options you want to change. For details, refer to the sections:

- Title Tab
- X Axis Options Tab
- Y Axis Options Tab
- Colors Tab
- Dye Traces Tab
- Current Traces Tab

Note: For detailed descriptions while using this dialog, click Help.

Title Tab

Set or change the title of the active data panel:

- 1. In the Display Options dialog, select the **Title** tab.
- 2. Change any item as necessary.
- 3. Click Apply to continue or OK to close the Display Options dialog.

X Axis Options Tab

To define the X-axis properties of the active data panels:

- 1. In the Display Options dialog, select the **X Axis Options** tab.
- 2. Change any item as necessary.
- 3. Click **Apply** to continue or **OK** to close the dialog.

Y Axis Options Tab

To define the Y-axis properties of the active data panels:

- 1. In the Display Options dialog, select the **Y** Axis Options tab.
- 2. Change any item as necessary.
- 3. Click **Apply** to continue or **OK** to close the dialog.

Colors Tab

Define the color options of the window, current traces, voltage trace, or optical scan trace:

- 1. In the Display Options dialog, select the **Colors** tab.
- 2. Change any item as necessary.
- 3. Click Apply to continue or OK to close the Display Options dialog.

Dye Traces Tab

To set or change the dye trace properties:

- 1. In the Display Options dialog, select the **Dye Traces** tab.
- 2. Select the traces to show or not show under Show Dye Traces.
- 3. To change a color of any dye trace, click the appropriate **Dye Colors** dye, select a color from the Color dialog, then click **OK**.
- 4. When finished, click **Apply** to continue or **OK** to close the dialog.

Current Traces Tab

To set or change the current trace options:

- 1. In the Display Options dialog, select the **Current Traces** tab.
- 2. Select the radio button for the current type to show under Data.
- 3. Click OK.

View the Last Analysis Performed

View the last analysis performed on the GenomeLab GeXP system:

 With a sample plate running (and with Automatic Analysis selected), select Tools > View > Last Analysis.

The Sequence Analysis Module dialog opens and shows the last row of sample analyzed, along with the Sample Plate toolbar.

2. To view additional samples, select the appropriate sample positions in the Sample Plate toolbar, and then click **Open**.

If the last analysis was a sequence analysis result, the Sequence Analysis module opens, showing the analyzed sequence data. If a fragment analysis separation was performed, the raw data will be shown in the Fragment Analysis module.

Using Direct Control

Click the **Direct Control** icon.

Note: Use the following illustration to locate hardware components referenced in this section.

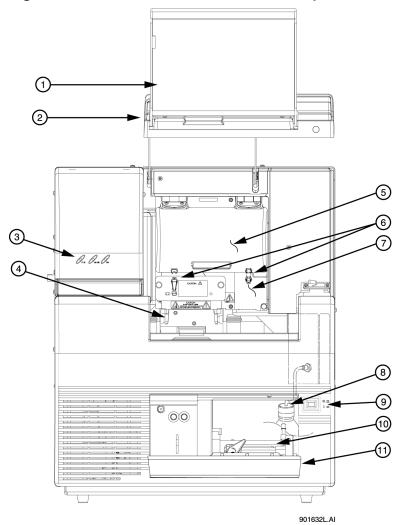


Figure 5-35 User Accessible Hardware Components

Item	Description	
1	Sample access cover (extended)	
2	Capillary access cover (extended)	
3	Status indicators	
4	Plate holders/sample transport	
5	Capillary temperature control cover	
6	Rubber latches	
7	Manifold access cover	

Item	Description
8	Gel waste bottle
9	Power switch
10	Gel pump
11	Gel pump/gel cartridge access cover

Load the Sample Plate and Buffer Plate for Dual-Rail System

1. From the Run module menu bar, select **Direct Control > Access Plates**.

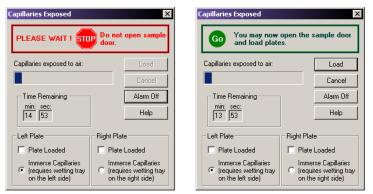
Figure 5-36 Access Plates Dialog

Access Plates		
If you are going to load new plates, please prepare your plates before clicking on	Start	
Start. You have 15 minutes to change plates once you click on Start.	Cancel	
This time limit is critical so that the capillaries are not adversely affected by prolonged exposure to air.	Help	

2. Click Start.

The Capillaries Exposed dialog opens.

Figure 5-37 Capillaries Exposed Dialogs

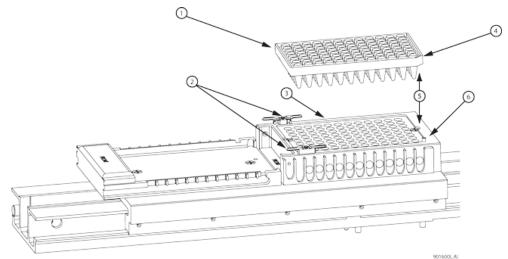


- 3. Wait until the dialog warning turns green.
- 4. Open the sample access cover (refer to the figure: Figure 5-35) and lift it to the vertical locking position.

Load the Sample Plate - Dual

- 1. Make sure the wetting tray is installed. Refer to the section: Install the Wetting Tray.
- 2. Align the sample plate guide pin with the notched corner of the sample plate and gently lower the plate into position.

Figure 5-38 Loading the Sample Plate

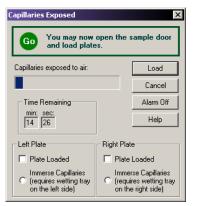


Item	Description
1	Sample plate
2	Wetting tray retainers
3	Sample plate holder
4	Sample plate notched edge
5	ALIGN together
6	Sample plate guide pin

- 3. When finished positioning the sample and buffer plates, close the sample access cover and then select the position from which the plates were loaded (left or right).
- 4. In the Capillaries Exposed dialog, click **Load**.

IMPORTANT The separation gel within the capillaries will dry out if the capillaries are left exposed to the air for more than 15 min.

Figure 5-39 Capillaries Exposed Dialog



Load the Buffer Plate and Evaporation Cover - Dual

- 1. Align the notched corner of the buffer plate with the alignment line on the buffer plate holder. Refer to the figure: Figure 5-40.
- 2. Gently push the buffer plate towards the front of the GenomeLab GeXP system and then set the plate into the transport.
- 3. At the rear of the buffer plate holder, align the buffer evaporation cover guide pin with the buffer evaporation cover alignment notch and then gently lower the cover over the buffer plate.

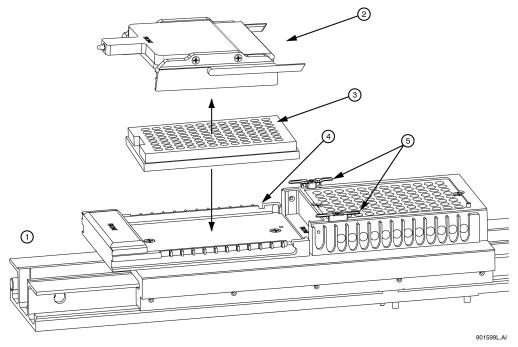


Figure 5-40 Loading the Buffer Plate and Buffer Evaporation Cover

Item	Description
1	Front location
2	Buffer evaporation cover
3	Buffer plate
4	Buffer evaporation cover alignment notch
5	Wetting tray retainers

- 4. When finished positioning the sample and buffer plates, close the sample access cover, and then select the position from which the plates were loaded (left or right). The Capillaries Exposed dialog opens.
- 5. Click Load.

IMPORTANT The separation gel within the capillaries **will dry out** if the capillaries are left exposed to the air for more than 15 min.

Load the Sample Plate and Buffer Plate for Single-Rail System

1. From the Run module menu bar, select **Direct Control > Unload Plates**.

Figure 5-41 Unload Plates Dialog



2. Click Unload.

The Capillaries Exposed dialog opens.

Figure 5-42 Capillaries Exposed Dialog

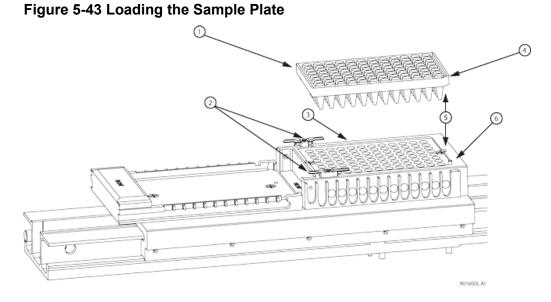
Capil	aries Exposed		×
	ou may now open the sa ad plates.	mple acce:	ss cover to
Сар	illaries exposed to air:		Load
			Cancel
1	ime Remaining		<u>A</u> larm Off
	min: sec: 14 47		<u>H</u> elp

- 3. Open the sample access cover (refer to the figure: Figure 5-35) and lift it to the vertical locking position.
 - If Sample Plate was selected, refer to the section: Load the Sample Plate Single.
 - If **Buffer Plate** was selected, refer to the section: Load the Buffer Plate and Evaporation Cover Single.

Load the Sample Plate - Single

1. Make sure the wetting station is installed or perform the procedure in the section: Install the Wetting Tray, and then return here.

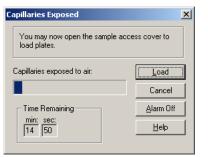
2. Align the sample plate guide pin with the notched corner of the sample plate and gently lower the plate into position. Refer to the figure: Figure 5-43.



ltem	Description
1	Sample plate
2	Wetting tray retainers
3	Sample plate holder
4	Sample plate notched edge
5	ALIGN together
6	Sample plate guide pin

- 3. When finished positioning the plate, close the sample access cover.
- 4. In the Capillaries Exposed dialog, click **Load**.

Figure 5-44 Capillaries Exposed Dialog



IMPORTANT The separation gel within the capillaries **will dry out** if the capillaries are left exposed to the air for more than 15 min.

Load the Buffer Plate and Evaporation Cover - Single

- 1. Align the notched corner of the buffer plate with the alignment line on the buffer plate holder (refer to the figure: Figure 5-40).
- 2. Gently push the buffer plate towards the front of the GenomeLab GeXP system and then set the plate into the transport.
- 3. At the rear of the buffer plate holder, align the buffer evaporation cover guide pin with the buffer evaporation cover alignment notch and then gently lower the cover over the buffer plate.

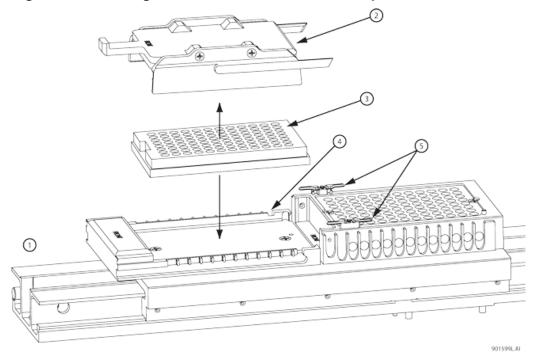
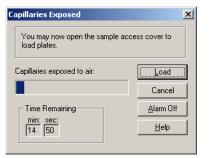


Figure 5-45 Loading the Buffer Plate and Buffer Evaporation Cover

ltem	Description
1	Front location
2	Buffer evaporation cover
3	Buffer plate
4	Buffer evaporation cover alignment notch
5	Wetting tray retainers

- 4. When finished positioning the plate, close the sample access cover.
- 5. In the Capillaries Exposed dialog, click **Load**.

Figure 5-46 Capillaries Exposed Dialog



Set the Capillary Temperature

- 1. From the Run module menu bar, select **Direct Control** > **Capillary Temperature**. The Capillary Temperature dialog opens.
- 2. Enter a capillary holding temperature value in degrees Centigrade.
- 3. Select Wait for temperature to be reached and then click Start.

Denature Samples

- 1. From the Run module menu bar, select **Direct Control > Denature**. The Denature Samples dialog opens.
- 2. Enter a time duration in seconds.
- 3. Identify the position of the **Sample Set** and click **Denature**.

Inject Samples

- From the Run module menu bar, select Direct Control > Inject. The Inject dialog opens.
- 2. Enter the Voltage in kV and the Time Duration in seconds.
- 3. Use the Sample Set option to set the position of the samples, and then click Inject.

Perform a Separation

- 1. From the Run module menu bar, select **Direct Control > Separate**.
- 2. In the **Separate** dialog:
 - a. Enter a value for the voltage in kV.
 - b. Enter a time duration in minutes.

c. Identify the position of the buffer using the **Buffer Set** spin controls and then select **Separate**.

Note: Data will not be saved.

Replenish the Capillaries with Gel

- 1. From the Run module menu bar, select **Direct Control** > **Gel Capillary**. The Gel Capillary Fill dialog opens.
- 2. Select **Buffer Plate** or **Wetting Tray** to identify the position where waste will be expelled from the capillaries.
 - If **Buffer Plate** was selected, use the **Buffer Plate** spin controls to identify the waste position in the buffer plate and then select **Fill**.
 - If Wetting Tray was selected, click Fill.

Purge the Manifold

1. From the Run module menu bar, select **Direct Control > Manifold Purge**. The Manifold Purge dialog opens.

Figure 5-47 Manifold Purge Dialog

Manifold Purge	X
Purge	Purge
Volume: 0.40 📑 ml Cycles: 1 📑	Cancel
	Help

- 2. Enter a volume in milliliters (mL).
- 3. Enter the number of cycles and then select **Purge**.

Perform an Optical Alignment

To align the lasers with the detection windows of the eight capillaries:

- 1. From the Run module menu bar, select **Direct Control > Optical Alignment**.
- 2. To save the alignment data, select the Autosave check box.
- 3. Enter a name in the Name field.
- 4. Select a **Work Folder**.
- 5. Select Align.

IMPORTANT Prior to performing an optical alignment, it is advisable to purge the array manifold and fill the capillaries with fresh gel (in that order). Refer to the sections: Purge the Manifold and Replenish the Capillaries with Gel.

Monitor the Baseline

To ensure that the optics are working correctly and that the capillaries are clean, view the baseline after installing a new capillary array, filling with gel for the first time, or performing an optical alignment. The baseline trace should be low and relatively flat.

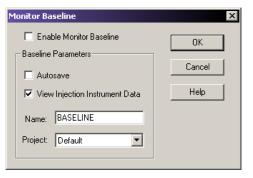
Note: You must perform the optical alignment procedures prior to monitoring the baseline. Refer to the section: Perform an Optical Alignment.

To monitor the baseline:

1. From the Run module menu bar, select **Run > Monitor Baseline**.

The Monitor Baseline dialog opens.

Figure 5-48	8 Monitor	Baseline	Dialog
-------------	-----------	----------	--------



Note: If you select the **Autosave** check box, the system stores baseline data under the Sample Data node (for the selected project) in the Data Manager module. If you do not manually end the Monitor Baseline function, it collects baseline data until you run a sample plate or 6 hours have elapsed from the start of the baseline monitoring process.

- 2. Select the **Enable Monitor Baseline** check box.
- 3. Click OK.
- 4. To view the baseline trace, select the **Data Monitor** tab.
 - a. Access the desired analysis module.
 - b. Select File > Open.
 - c. Select the Sample Data tab.

- d. Select the desired baseline data.
- 5. After reviewing the baseline trace, disable **Monitor Baseline**.
 - a. Select **Run > Monitor Baseline**. The Monitor Baseline dialog opens.
 - b. Select the Enable Monitor Baseline check box to clear the check mark.
 - c. Click **OK**.

View Capillary Information

1. From the Run module menu bar, select **Replenish** > **Capillary Information**. The Capillary Information dialog opens.

Figure 5-49 Capillary Information Dialog

C	apillary Information		×
	Capillary Part Number: Serial Number: Date Installed: Time Installed:	608087 0401050128 04/27/2006 17:01:47	OK Cancel Print Advanced
	Number of Runs: Days on instrument:	66 13.1	Help

2. Use the Capillary Information dialog to view the following information:

Option	Description
Part Number	Shows the part number of the installed capillary array.
Serial Number	Shows the serial number of the installed capillary array. (The serial number is used to track the capillary array along with the samples that have used the array.)
Date Installed	Shows the date the capillary array was installed.
Time Installed	Shows the time the capillary array was installed.
Number of Runs	Specify the desired number using the spin-control. (Set more than the recommended number of separations to be performed before the system alerts you that the recommended number of runs has been exceeded.)

Option	Description
Days on instrument	Specify the number of days on the GenomeLab GeXP system using the spin-control. (This allows the capillary array to reside on the GenomeLab GeXP system longer than the recommended length of time before the system alerts you that the recommended number of days has been exceeded.)
Advanced	Opens the Capillary Advanced Information dialog. This dialog shows the length and diameter of the installed capillary array based on the part number entered in the New Capillary Array dialog. The fields are read-only and cannot be edited. Click OK to enter the information and close the dialog.

3. When finished, click **OK** to close the dialog.

View Gel Information

1. From the Run module menu bar, select **Replenish** > **Gel Cartridge/Buffer**. The Gel Cartridge/Buffer Information dialog opens.

Gel Cartridge/Buffer Ir	formation	×
Gel		
Part Number:	391438	ОК
Lot Number:	, S602066	Cancel
Gel Name:	, LPA1	Print
Date Installed:	04/27/2006	Help
Time Installed:	17:00:59	
Hours on Instrument:		
Buffer		
Part Number:	608012	
Lot Number:	CEQ Sequencing Separation Buffer	

Figure 5-50 Gel Cartridge/Buffer Information Dialog

2. Use the Gel Cartridge/Buffer Information dialog to view the following information:

Option	Description
Part Number	Shows the part number of the installed gel cartridge.

Option	Description	
Lot Number	Shows the lot number of the installed gel cartridge. (The lot number is used to track the gel lot along with the separations that have used the gel.)	
Gel Name	Shows the name of the gel.	
Date Installed	Shows the date the gel cartridge was installed.	
Time Installed	Shows the time the gel cartridge was installed.	
Hours on Instrument	Shows the number of hours that the gel cartridge has remained on the GenomeLab GeXP system.	

3. When finished, select **OK** to close the dialog.

View or Change Buffer Information

- 1. From the Run module menu bar, select **Replenish** > **Gel Cartridge/Buffer**. The Gel Cartridge/Buffer Information dialog opens.
- 2. To change the value in the Lot Number field, enter a new value, and then click OK.

Note: The buffer lot number can only be changed when the GenomeLab GeXP system is not performing a separation.

Remove and Replace the Capillary Array

CAUTION: Potential System Damage. Take care when installing or removing the capillary array to prevent damage and maintain low background signals. Clean all gel residues thoroughly.

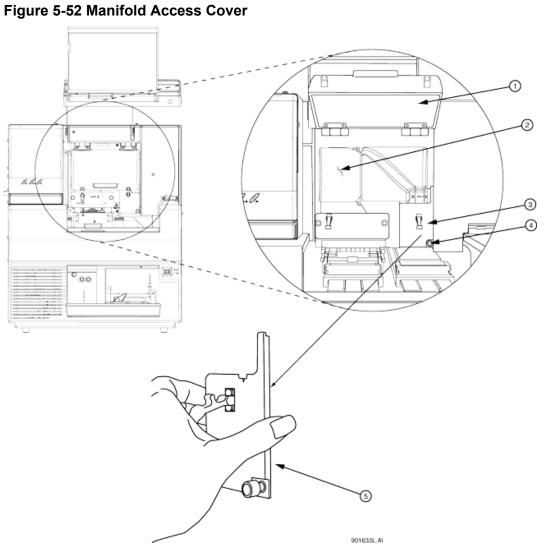
Note: This procedure assumes that an expended capillary array is being replaced with a new capillary array.

 From the Run module menu bar, select Replenish > Release Capillary Array. After the system prepares for release of the capillary array, the Remove Capillary Array dialog opens.

nove Capillary Array	×
Go You may now open sample/c access doors to change cap	
Fo replace the capillary array immediately, select "Replace capillary array."	OK
Fo install a manifold plug, select "Install nanifold plug."	Cancel
To clean the capillary windows, select "Clean capillaries."	Help
Remove Capillary Array Options Replace capillary array Install manifold plug Clean capillaries	
Capillaries exposed to air:	
Time Remaining min: sec: 14 14	

Figure 5-51 Remove Capillary Array Dialog

- 2. Open the sample access cover (refer to the figure: Figure 5-52) and lift it to the vertical locking position.
- 3. Open the capillary access cover and lift it to the vertical locking position.
- 4. Disconnect the two rubber latches holding the capillary temperature control cover and lift the cover to the vertical locking position.
- 5. Loosen the manifold access cover captive screw (refer to the figure: Figure 5-52), then remove the cover and set it aside.



ltem	Description	
1	Capillary temperature control cover (open)	
2	Plenum assembly	
3	Manifold access cover	
4	Captive screw	
5	Manifold access cover (removed)	

Run Module

6. Loosen the two plenum assembly captive screws (refer to the figure: Figure 5-53), then pull the plenum assembly straight back and away from the GenomeLab GeXP system and set it aside.

IMPORTANT Slowly remove the plenum assembly, as the electrode block may disengage from its mounting posts and become damaged.

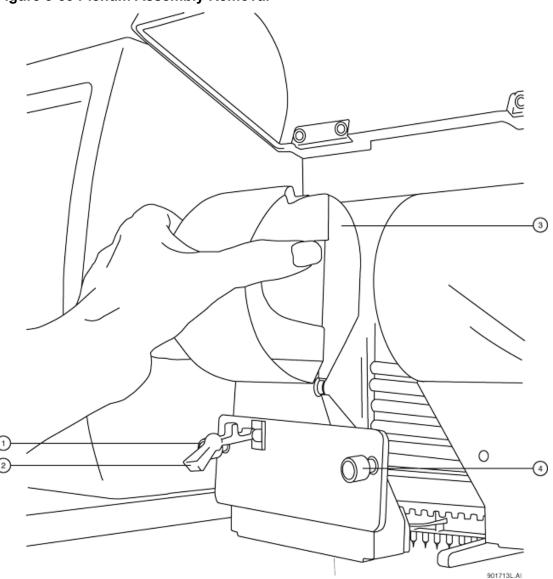
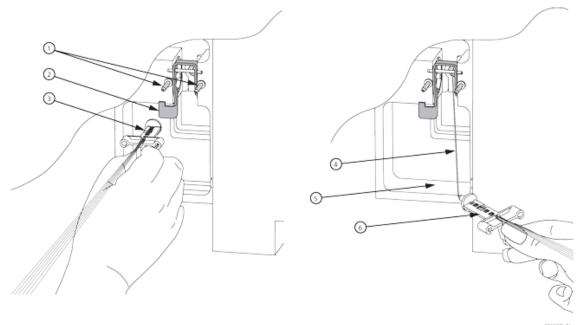


Figure 5-53 Plenum Assembly Removal

Item	Description
1	Captive screw
2	Rubber latch
3	Plenum assembly
4	Captive screw

- 7. Lift the eject lever to release the array fitting. Refer to the figure: Figure 5-54.
- 8. Grasp the array fitting tab. Refer to the figure: Figure 5-54.
 - a. Pull the fitting out of the manifold approximately 1 in..
 - b. Touch the tip of the fitting to the bottom of the optics base plate.
 - c. Hold and wait 5 s for the gel strand to dry.
 - d. Pull the fitting away from the GenomeLab GeXP system.
 - e. Wipe gel strands off of the GenomeLab GeXP system using a damp tissue.

Figure 5-54 Removing/Replacing the Array Fitting



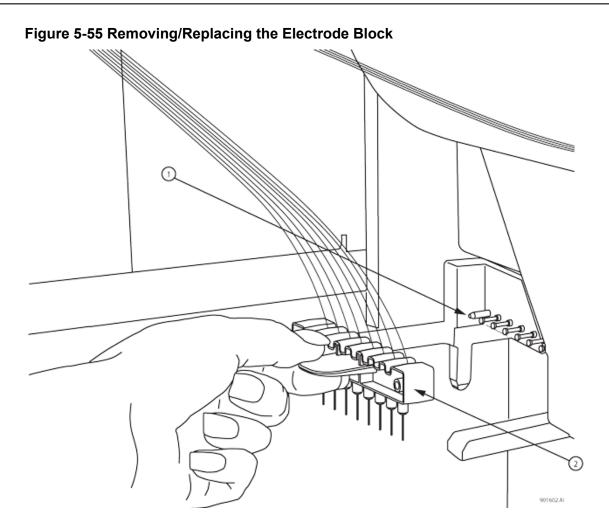
ltem	Description
1	Guide pins

Run Module

ltem	Description
2	Eject lever
3	Array fitting
4	Gel strand
5	Optics base plate (bottom)
6	Array fitting

Note: Note the number of runs and days on the GenomeLab GeXP system for this capillary array for reference. If this capillary array is to be re-installed, this information is necessary. A label has been provided on the back of the array packaging container so that this information may be recorded.

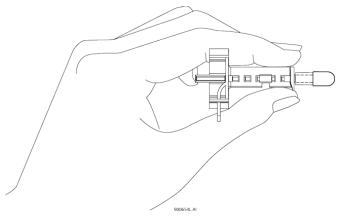
- 9. Grasp the electrode block tab (refer to the figure: Figure 5-55), and then pull the block out and away from the GenomeLab GeXP system.
- 10. In the Remove Capillary Array dialog (refer to the figure: Figure 5-51), select **Replace Capillary Array** and click **OK**.



ltem	Description
1	Guide block pins
2	Electrode block

Note: Always grip the capillary array fitting near the end during removal or installation of the tip cap to prevent flexing and possible breakage of the capillary array. Refer to the figure: Figure 5-56.





- 11. While holding the array fitting tab of the new capillary array, align the array fitting with the manifold opening and guide pins. Refer to the figure: Figure 5-54. Push the fitting into the manifold until it is completely seated against the bases of the guide pins. Make sure that the fitting tab is placed downward when inserting the capillary array into its slot.
- While holding the electrode block tab, align the electrode block with the guide block pins and gently push it in until resistance is met. Refer to the figure: Figure 5-55.
 The resistance is from the spring-loaded contacts.
- 13. When installing the capillary array, carefully route the capillaries through the hole in the plenum assembly (refer to the figure: Figure 5-57) and straight across the guide block pins (refer to the figure: Figure 5-55).

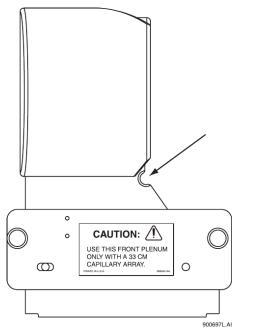


Figure 5-57 Plenum Assembly - Capillary Routing Hole

Note: When reinstalling the plenum assembly, gently gather the capillaries between your thumb and forefinger to make sure they pass through the hole in the plenum assembly without any constriction.

- 14. Replace the plenum assembly and tighten the two captive screws. Refer to the figure: Figure 5-53.
- 15. Replace the manifold access cover and tighten the captive screw. Refer to the figure: Figure 5-52.
- 16. Lower the capillary temperature control cover and secure the two rubber latches.
- 17. Lower the capillary access cover and sample access cover to their locking positions.
 - If you are installing a new capillary array, in the Install Capillary Array dialog (refer to the figure: Figure 5-58), select the correct part number, enter the serial number, click Set to New, then click Done. The number of runs and days on the GenomeLab GeXP system will revert to 0.
 - If you are installing the previous capillary array, do not change the serial number, number of runs, or number of days on the GenomeLab GeXP system, as they will be correct.
 - If you are installing a capillary array that was previously used, but not the last capillary array on the GenomeLab GeXP system, enter its part number (if applicable) and serial number, and adjust the number of runs and the previous cumulative days on the GenomeLab GeXP system. Click **Done**.

Note: Note the number of runs and days on the GenomeLab GeXP system for this capillary array for reference.

Figure 5-58 Install Capillary Array Dialog

Please enter the serial number for the capillary array. If you wish to reset the number of runs for the capillary or number of days it has been on the instrument, enter the new values. Done Click on Done when you have installed the capillary array and have closed both the capillary access cover and the sample access cover. Set to New Capillaries exposed to air: Time Remaining min: 14 sec: 11 Help Capillary Array min: 14 sec: 11 Capillary Array Total Length: 33.00 cm Part Number: 608087 Total Length: 30.00 cm Date Installed: 04/27/2006 Internal Diameter: 75.00 µm Time Installed: 16:45:20 Number of Runs: Q Number of Runs: Q x Length to Detector: Days on Instrument: 13.1 x	Install Capillary Array					×
Click on Done when you have installed the capillary array and have closed both the capillary access cover and the sample access cover. Capillaries exposed to air: Time Remaining min: 14 sec: 11 Capillary Array Part Number: Capillary Array Capillary Array Part Number: Capillary Array Capillary Array Part Number: Capillary Array Capillary Arr	the number of runs for the capillary or number of days it has been on the					
closed both the capillary access cover and the sample access cover. Capillaries exposed to air: Time Remaining min: 14 sec: 11 Capillary Array Part Number: 608087 Total Length: 33.00 cm Serial Number: Date Installed: 04/27/2006 Internal Diameter: 75.00 µm Time Installed: 16:45:20 Number of Runs: 0	Click on Dono when yo	u have installed the	oppilloru orrou	and have	Canc	
Capillaries exposed to air: Time Remaining min: 14 sec: 11 min: 14 sec: 11 Capillary Array Part Number: 608087 Serial Number: 608087 Date Installed: 04/27/2006 Internal Diameter: 75.00 µm Time Installed: 16:45:20 Number of Runs: 0					Set to New	
Part Number: 608087 Total Length: 33.00 cm Serial Number: Length to Detector: 30.00 cm Date Installed: 04/27/2006 Internal Diameter: 75.00 μm Time Installed: 16:45:20 Internal Diameter: 75.00 μm	Capillaries exposed to a	air:		· · · · · · · · · · · · · · · · · · ·	Help	
Serial Number: Length to Detector: 30.00 cm Date Installed: 04/27/2006 Internal Diameter: 75.00 μm Time Installed: 16:45:20 Internal Diameter: 75.00 μm	Capillary Array					
Date Installed: 04/27/2006 Internal Diameter: 75.00 μm Time Installed: 16:45:20	Part Number:	608087	•	Total Length:	33.00	cm
Time Installed: 16:45:20 Number of Runs: 0	Serial Number:			Length to Detector:	30.00	cm
Number of Runs:	Date Installed:	04/27/2006		Internal Diameter:	75.00	μm
	Time Installed:	16:45:20]		,	
Days on Instrument: 13.1	Number of Runs:	Q	÷			
	Days on Instrument:	13.1	÷			

The Confirm Capillary Array Selection dialog opens.

18. Read it and make the selection **Yes** to continue, or **No** to abort the capillary installation.

Remove and Replace a Gel Cartridge/Gel Pump Plug for Dual-Rail System

CAUTION: Potential System Damage. Be careful when installing/removing a gel cartridge due to the viscosity of the gel mixture.

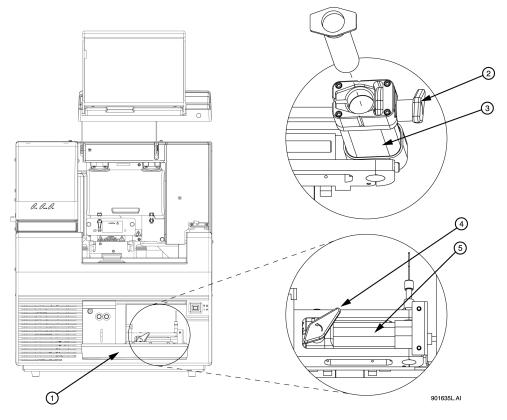
Note: This procedure assumes that an expended gel cartridge is being replaced with a fresh gel cartridge or that a used gel cartridge is being removed (for storage purposes) and being replaced with the gel pump plug.

1. From the Run module menu bar, select **Replenish > Release Gel Cartridge**.

When the system is ready to release the gel cartridge, the Release Gel Cartridge dialog opens. Wait until the lead screw is completely disengaged.

- Open the gel pump/gel cartridge access cover by gently pushing in on the top of the cover. Refer to the figure: Figure 5-59. The cover is spring-loaded and will pop open.
- 3. Pull on the cartridge locking lever. The barrel will swing outwards to approximately a 90-degree angle from its locked position.

Figure 5-59 Removing/Replacing the Gel Cartridge



ltem	Description	
1	Gel pump/gel cartridge access cover	
2	Cartridge locking lever	
3	Cartridge barrel	
4	Cartridge locking lever	
5	Cartridge barrel	

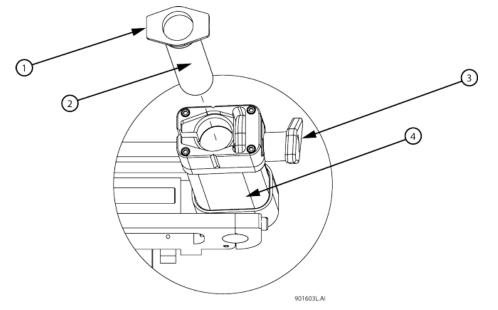
Run Module

4. Grasp the wings of the gel cartridge/gel pump plug and pull it out of the barrel. Refer to the figure: Figure 5-60.

Note: Always note the lot number and **Hours on Instrument** for a gel cartridge if you are planning to use it for more than one session. If the gel cartridge has been on the GenomeLab GeXP system for more than 72 hr, it is likely that the gel will produce undesirable results.

5. If necessary, use a tissue to wipe gel strands off of the GenomeLab GeXP system.

Figure 5-60 Removing/Replacing the Gel Cartridge - Continued



ltem	Description
1	Cartridge wings
2	Gel cartridge or gel pump plug
3	Cartridge locking lever (open)
4	Cartridge barrel

- 6. Remove any air pockets from the gel cartridge by grasping the cartridge wings between your first two fingers and pressing the plunger with your thumb until a small amount of gel is pushed out of the cartridge tip.
- 7. Insert the new cartridge or the gel pump plug into the barrel and lock it into position by aligning the cartridge wings with the cartridge holder and pushing in.

- 8. Push the cartridge locking lever towards the back of the GenomeLab GeXP system (approximately a 90-degree angle) into its locked position.
- 9. Close the gel pump/gel cartridge access cover.
- 10. In the Remove Gel Cartridge dialog (refer to the figure: Figure 5-61), do one of the following:
 - Click **Install Cartridge** to indicate that a gel cartridge was installed.
 - Click Install Plug to indicate that the gel pump plug (or empty cartridge) was installed.

Figure 5-61 Remove Gel Cartridge Dialog

Remove Gel Cartridge X	Remove Gel Cartridge
PLEASE WAIT ! STOP Do not open gel access door.	Go You may now open gel access door and change gel cartridge.
Releasing gel cartridge:	If you are not going to install a working gel cartridge, please insert the gel pump plug or clean empty cartridge to prevent any remaining gel from dying in the system. Then click on Install Plug. Please check the waste bottle to ensure that the bottle will not overflow in future runs.

11. If **Install Plug** was selected, refer to the section: Remove and Replace a Gel Cartridge/Gel Pump Plug for Dual-Rail System.

If **Install Cartridge** was selected, use the Install Gel Cartridge dialog (refer to the figure: Figure 5-62) to enter the following information:

- If you are installing a new gel cartridge, select **New**. The system will automatically update the date and time installed and set the **Hours on Instrument** to **0**. Enter the lot number.
- If you are installing a used gel cartridge, select **Used**.
 - If you are installing the last previously used gel cartridge, do not change the lot number or **Hours on Instrument**, as they will be correct.
 - If you are installing a previously used gel cartridge, but it was not the last one on the GenomeLab GeXP system, enter its part number and/or lot number, and adjust the hours to properly reflect the hours this cartridge has been on the GenomeLab GeXP system.

Note: Always note the lot number and **Hours on Instrument** for a gel cartridge if you are planning to use it for more than one session. If the gel cartridge has been on the GenomeLab GeXP system for more than 72 hr, it is likely that the gel will produce undesirable results.

12. In the Install Gel Cartridge dialog, click Done.

Figure 5-62 Install Gel Cartridge Dialog

Install Gel Cartri	dge	X
Gel Cartridge —		Done
Part Number:	391438	
Lot Number:		Cancel
Gel Name:	LPA1	Help
Date Installed:	04/27/2006	
Time Installed:	17:15:00	
C New		
O Used	Hours on Instrument: 27	

13. Dispose of the used tissue and spent gel cartridge. Refer to the section: Biological Waste Disposal.

Remove and Replace a Gel Cartridge/Gel Pump Plug for Single-Rail System

CAUTION: Potential System Damage. Be careful when installing/removing a gel cartridge due to the viscosity of the gel mixture.

Note: This procedure assumes that an expended gel cartridge is being replaced with a fresh gel cartridge or that a used gel cartridge is being removed (for storage purposes) and being replaced with the gel pump plug.

- From the Run module menu bar, select **Replenish** > **Release Gel Cartridge**. When the system is ready to release the gel cartridge, the Release Gel Cartridge dialog opens. Wait until the lead screw is completely disengaged.
- Open the gel pump/gel cartridge access cover by gently pushing in on the top of the cover. Refer to the figure: Figure 5-63. The cover is spring-loaded and will pop open.
- Pull on the cartridge locking lever.
 The barrel will swing outwards to approximately a 90-degree angle from its locked position

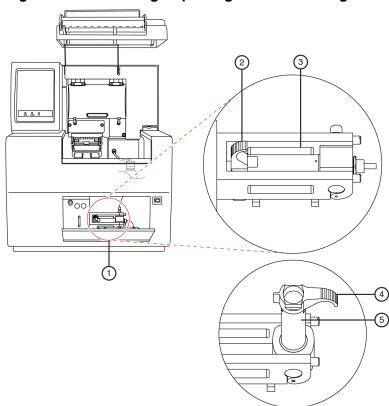


Figure 5-63 Removing/Replacing the Gel Cartridge

ltem	Description
1	Gel pump/gel cartridge access cover
2	Cartridge locking lever (closed)
3	Cartridge barrel
4	Cartridge locking lever (opened)
5	Cartridge barrel

4. Grasp the wings of the gel cartridge/gel pump plug and pull it out of the barrel. Refer to the figure: Figure 5-64.

Note: Always note the lot number and **Hours on Instrument** for a gel cartridge if you are planning to use it for more than one session. If the gel cartridge has been on the GenomeLab GeXP system for more than 72 hr, it is likely that the gel will produce undesirable results.

5. If necessary, use a tissue to wipe gel strands off of the GenomeLab GeXP system.

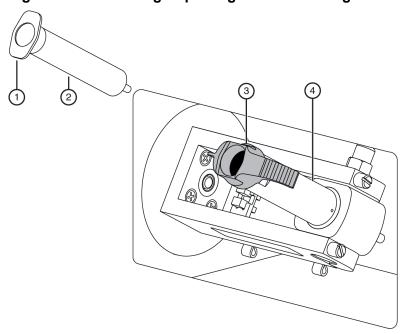
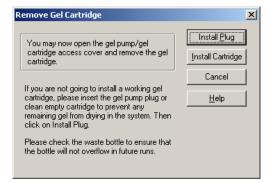


Figure 5-64 Removing/Replacing the Gel Cartridge - Continued

ltem	Description
1	Cartridge wings
2	Gel cartridge or gel pump plug
3	Cartridge locking lever (open)
4	Cartridge barrel

- 6. Remove any air pockets from the gel cartridge by grasping the cartridge wings between your first two fingers, and pressing the plunger with your thumb until a small amount of gel is pushed out of the cartridge tip.
- 7. Push the cartridge locking lever towards the back of the GenomeLab GeXP system (approximately a 90-degree angle) into its locked position.
- 8. Insert the new cartridge or the gel pump plug into the barrel and lock it into position by aligning the cartridge wings with the cartridge holder and pushing in.
- 9. Close the gel pump/gel cartridge access cover.
- 10. In the Remove Gel Cartridge dialog (refer to the figure: Figure 5-65), do one of the following:
 - Click **Install Cartridge** to indicate that a gel cartridge was installed.
 - Click Install Plug to indicate that the gel pump plug (or empty cartridge) was installed.

Figure 5-65 Remove Gel Cartridge Dialog



11. If **Install Plug** was selected, refer to the section: Remove and Replace a Gel Cartridge/Gel Pump Plug for Dual-Rail System.

If **Install Cartridge** was selected, use the Install Gel Cartridge dialog (refer to the figure: Figure 5-66) to enter the following information:

- If you are installing a new gel cartridge, click **Set to New**, select the part number from the menu, enter the lot number, and then click **OK**. The system will automatically update the date and time installed and set the **Hours on Instrument** to **0**.
- If you are installing the last previously used gel cartridge, do not change the lot number or **Hours on Instrument**, as they will be correct.
- If you are installing a previously used gel cartridge, but it was not the last one on the GenomeLab GeXP system, enter its part number and/or lot number, and adjust the hours to properly reflect the hours this cartridge has been on the GenomeLab GeXP system.

Note: Always note the lot number and **Hours on Instrument** for a gel cartridge if you are planning to use it for more than one session. If the gel cartridge has been on the GenomeLab GeXP system for more than 72 hr, it is likely that the gel will produce undesirable results.

12. In the Install Gel Cartridge dialog, click **Done**.

Figure 5-66 Install Gel Cartridge Dialog

Ir	stall Gel Cartridge		×
	-Gel Cartridge Part Number:	608010 •	 Set to <u>N</u> ew
	Lot Number: Gel Name: Date Installed:	CEQ Sequencing Gel	Cancel
	Time Installed:	13:16:49	<u>H</u> elp
	Hours on Instrument:	0	

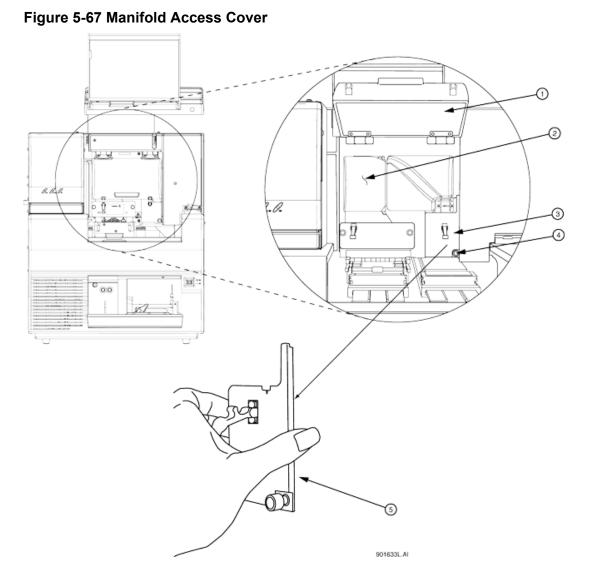
13. Dispose of the used tissue and spent gel cartridge. Refer to the section: Maintenance and Diagnostics.

Remove the Manifold Plug

The manifold plug is installed during shipping and when the GenomeLab GeXP system is not in use to prevent the gel from drying.

Note: This procedure assumes that the manifold plug is being removed in preparation for capillary array installation.

- 1. From the Run module menu bar, select **Replenish > Release Capillary Array**.
- 2. Wait for the Remove Manifold Plug dialog to open.
- 3. Open the sample access cover and lift it to the vertical locking position. Refer to the figure: Figure 12-1.
- 4. Open the capillary access cover and lift it to the vertical locking position.
- 5. Unlatch the two rubber latches holding the capillary temperature control cover and lift it to the vertical locking position. Refer to the figure: Figure 5-67.
- 6. Loosen the manifold access cover captive screw, remove the cover and set it aside.



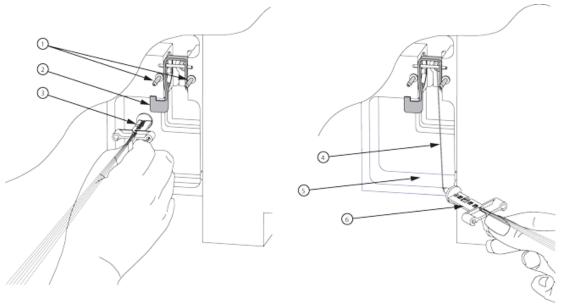
ltem	Description
1	Capillary temperature control cover
2	Plenum assembly
3	Manifold access cover
4	Captive screw
5	Manifold access cover (removed)

7. Lift the eject lever to release the manifold plug.

Run Module

- 8. Grasp the manifold plug tab. Refer to the figure: Figure 5-68.
 - a. Pull the plug out of the manifold approximately 1 in.
 - b. Touch the tip of the plug to the bottom of the optics base plate.
 - c. Hold and wait 5 s for the gel strand to dry.
 - d. Pull the plug out and set it aside for future use.
 - e. Wipe gel strands off of the GenomeLab GeXP system using a damp tissue.

Figure 5-68 Removing the Manifold Plug



11	59	17	L	A)	

ltem	Description
1	Guide pins
2	Eject lever
3	Array fitting
4	Gel strand
5	Optics base plate
6	Array fitting

- 9. In the Remove Manifold Plug dialog, click **OK**.
- 10. To install the capillary array, refer to the section: Remove and Replace the Capillary Array.

This chapter provides an overview of the Sequence Analysis module, including its menu options, toolbars, and dialogs. It shows you how to use this module to run a sequence analysis and to set up its parameters, result properties, and display options. It also describes how to work with, report, and export sequence analysis results.

Overview

Use the Sequence Analysis module to view, analyze, compare, edit, and print data of the following types:

Note: Only analyzed data and base sequences can be edited.

- Raw data
- Current data
- Voltage data
- Analyzed data
- Base sequences
- Optical scan data
- Baseline data
- Quality parameters

This module accepts raw data and analyzed data. Editing and reanalysis functions enable you to verify the accuracy of the base calls. You can also export these data to third-party packages for further analysis.

Main Window

The main window for the Sequence Analysis module has the following sections.

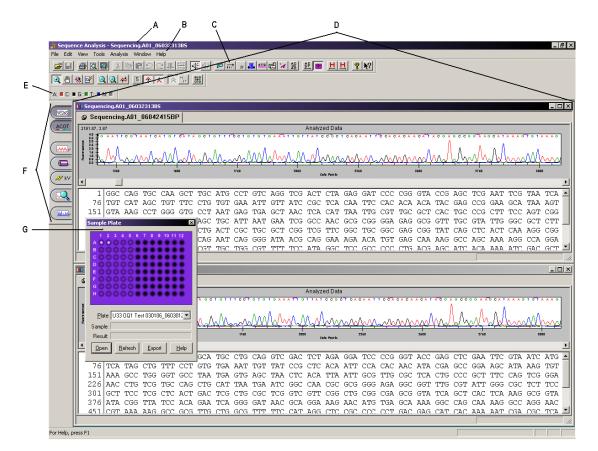


Figure 6-1 Main Window, Sequence Analysis Module

ltem	Description	
A	Title Bar: Shows the module name (Sequence Analysis) and the currently active sample.	
В	Menu Bar: Refer to the section: Menu Bar.	
С	Toolbar: Refer to the section: Toolbars.	
D	Display Area: Graphically shows the opened data.	
E	Double-clicking on a base sequence text letter (A , C , G , T) in the Dye Colors toolbar opens the Color dialog, which you can use to change the dye color that shows for the selected dye.	

Item	Description	
F	Analyzed Data: Shows the data that has been analyzed for the active sample.	
	Base Sequence: Shows a text view of the bases from the analyzed data for the active sample.	
	Raw Data: Shows the raw data for the active sample.	
	Current: Shows the current data for the active sample.	
	Voltage: Shows voltage data for the active sample.	
	Compare Data: Compare the active analyzed data set against another analyzed data set.	
	Quality Parameters: Shows a graphical representation of the quality values for the active sample.	
G	Sample Plate - Enable the Sample Plate toolbar using View > Toolbars (shown as in Undocked mode). Use this dialog to select the samples to open or export.	

Menu Bar

The following example shows the menu bar for the Sequence Analysis module.

Figure 6-2 Sequence Analysis Module Menu Bar

<u>File Edit View Tools Analysis Window Help</u>

The following topics describe each of these menus and their options.

File Menu

Click **File** to show the following menu.

-	
Open	Ctrl+O
⊆lose	
Close Ta <u>b</u>	
Save	Ctrl+S
Save <u>A</u> s	
Import	
<u>E</u> xport	
Export from Plate	
Prefere <u>n</u> ces	
Properties	
Report <u>F</u> ormat	
Print Pre <u>v</u> iew	
Print <u>R</u> eport	Ctrl+R
Print Selected <u>P</u> ane	Ctrl+P
Print <u>D</u> esktop	
0 DATS 100-D2.F03_010605226D	
1 139424sprot.C06_99121315XJ	
2 MySample.D1_010924106B	
3 MySample.A1_010924106B	
4 DATS 100-D2.F03_010605226D	
5 139424sprot.C06_000526143X	
6 MySample.A1_010924106B	
7 MySample.B1_010924106B	
E <u>x</u> it	

Figure 6-3 File Menu, Sequence Analysis Module

Option	Description	
Open	Open data files, including: Sample Data, Sequence Analysis Parameters, Optical Scan Data, Sequence Results, and Sample Plate Results.	
Close	Close the open sample.	
Close Tab	Close the active tab.	
Save	Save the current data to the same location with the same file name.	
Save As	Save the current data with a newly assigned file name in a newly selected location.	
Import	Locate and select data to import. The system can import data in standard chromatogram format (*.scf) or electropherogram sample data format (*.esd).	

Option	Description
Export	Export data in the following formats: • Standard chromatogram format versions 2.10 and 3.00 (*.scf)
	Tab-delimited ASCII text (*.txt)
	SEQ sequence text (*.seq)
	FASTA and Quality (*.fasta)
	PHRED and SCF (*.scf.phd.1)
	Electropherogram sample data format (*.esd)
	• CEQ (*.cq*)
	For more information about export file types in the Sequence Analysis module, refer to the section: Sequence Analysis Module Export File Types.
Export from Plate	Open or export samples from a specific sample plate. To select multiple samples, press Shift while selecting samples. (The Sample Plate toolbar must be open to use this menu option.)
Preferences	Used to:View or change the panes that show when you first open a sample.
	 Show the Report Format dialog selecting File > Print Report.
	 Open all associated results that belong to a sample when a samples is opened.
	Open/export the most recent results from a sample plate.
	Show the analysis log during analysis.
	Print the entire desktop.
	Print the application window, including all toolbars.
	Print only the panes that show.
	Select audio playback speed.
Properties	View the general properties of the currently selected item. You can view the item type, database, and project where the item resides, as well as the date and time the item was last modified. You may also view the sample name, sample plate, sample position in the sample plate, and operator name.
	You can also view the property set, run method, analysis, alignment, consumables, and notes for the currently selected sample.

Sequence Analysis Module

Option	Description
Report Format	Specify the format of the report to be generated for the active sample.
Print Preview	Open a print preview of the active document of the active sample plate. A toolbar at the top of the window provides options for navigation, viewing, and printing the display. To restore the default screen, press Esc or click Close .
Print Report	Print the report.
Print Selected Pane	Print the selected pane.
Print Desktop	Print the desktop, application, or main window as defined in the Preferences dialog.
Recent Objects	Show a list of the most recently opened objects. The first set of four is sample data and the second set of four is sequence results.
Exit	Close the module.

Edit Menu

Click **Edit** to show the following menu.

Figure 6-4 Edit Menu, Sequence Analysis Module

Edit	
Undo Ctrl+Z	
Redo	Ctrl+Y
Cut	Ctrl+X
Сору	Ctrl+C
Paste	Ctrl+V
Apply Quality-based Trimming	
Apply Sequence-based Trimmin]
 Audio Enable Audio Playback 	

Option	Description
Undo	Undo the last action performed.
Redo	Reverse the last undo action.
Cut	Copy the selected item to the clipboard and removes it from its current location.

Option	Description
Сору	Copy the selected item to the clipboard without removing it from its current location.
Paste	Insert copied or cut items from the clipboard into the selected location.
Apply Quality- based Trimming	Commit to quality-based trimming.
Apply Sequence- based Trimming	Commit to sequence-based trimming.
Audio Enable	Toggle to enable or disable audio. If enabled while in Edit mode, each base letter is audibly announced as it is typed.
Audio Playback	Audibly announce a series of selected base sequence text.

View Menu

Click **View** to show the following menu.

Note: A check mark next to an option indicates that the option is enabled.

Figure 6-5 View Menu, Sequence Analysis Module

View	
Toolbars	
✔ Status Bar	
✓ Analyzed Data	
✓ Base Sequence	
Raw Data	
Current	
Voltage	
Alignment	
Alignment Accuracy	
Quality Parameters	
Trimming Report	
Analysis Log	
Parameters Used to Compute Sequence	
Run Log	
Working Database	

Option	Description
Toolbars	Select which toolbars to show. Refer to the section: Toolbars.

Option	Description
Status Bar	Toggle to show or hide the status bar.
Analyzed Data	Show the data that has been analyzed for the active sample.
Base Sequence	Show a text view of the bases from the analyzed data for the active sample.
Raw Data	Show the raw data for the active sample.
Current	Show the current data for the active sample.
Voltage	Show voltage data for the active sample.
Alignment	Show the alignment data for the active sample.
Alignment Accuracy	Show the alignment accuracy data for the active sample.
Quality Parameters	Show a graphical representation of the quality values for the active sample.
Trimming Report	Toggle to show or hide the trimming report pane.
Parameters Used to Compute Sequence	Show the sequence analysis parameters used to produce the analyzed data. This dialog provides the same information that shows when editing these parameters; however, the fields in this dialog are read-only and are not editable. This item is not available for data that has not yet been analyzed. For parameter descriptions, refer to the section: Edit Sequence Analysis Parameters, or click Help when using the dialog.
Run Log	Show the messages received from the GenomeLab GeXP system during the sample run.
Working Database	Show the name of the database in use.

Tools Menu

Click **Tools** to show the following menu.

Note: A check mark next to an option indicates that the option is enabled.

Figure 6-6 Tools Menu, Sequence Analysis Module

Tools	
✓ Zoom	
Pan	
Align	
Edit	
Unzoom	
Unzoom All	
Autoscale	
Base Spacing	
✓ Base Synch	
✓ Bases on Top	
Compare	
Compare Synch	
Align Compare Views	
Display Options	
Heterozygotes Display Color	

Option	Description
Zoom	Magnify a specific area of data in the display.
Pan	Move analyzed data in the X and Y direction.
Align	Visually align bases and peaks in the analyzed data pane.
Edit	Insert, change, and/or delete bases.
Unzoom	Undo one zoom level.
Unzoom All	Undo all zoom levels.
Autoscale	Scale data to the confines of the pane. If autoscaling is disabled, the system scales the data to their true values. Use this item to turn autoscaling on or off.
Base Spacing	Set the space between base sequence text in groups of 0, 3, 5, or 10.
Base Synch	With both the analyzed data and base sequence panes open, synchronize the analyzed data display with the highlighted base sequence text, showing the corresponding peak or peaks between two hairlines for the selected base.
Bases on Top	Toggle the base sequence text between showing on the top or bottom in the analyzed data pane.
Compare	Select an analyzed data set to compare to the current analyzed data set.
Compare Synch	Synchronize scaling, zooming, and panning of two analyzed data sets while in the Compare mode.

Option	Description
Align Compare Views	Align selected points of each of the analyzed data sets that show while in the Compare mode.
Display Options	Open the Display Options dialog, which provides tabs to modify any or all of the following parameters: Title , X Axis Options , Y Axis Options , Dye Traces , Current Traces , Colors , and Quality Parameters . For details, refer to the section: Set or Change Display Options , or click Help when using the dialog.
Heterozygote Display Color	Open the Colors dialog to change the color of the heterozygotes in the base sequence pane. For details, refer to the section: Change the Display Colors.

Analysis Menu

Click **Analysis** to show the following menu.

Figure 6-7 Analysis Menu, Sequence Analysis Module



Option	Description
Analyze	Analyze active sample data or reanalyze existing sequence results. Selecting this option opens the current Working Parameters dialog. Click OK to start the analysis.
Stop	Terminate the analysis currently in progress.
Working Analysis Parameters	View and change the analysis parameters to be used for subsequent analyses. For details, refer to the section: Edit Sequence Analysis Parameters.
Restore Original Base Sequence	Return the analyzed data displays and base sequence text to their original states (losing all edits).
Batch Analysis	Analyze multiple sample data or sample plate results sequentially. Selecting this option opens the following dialog. For details, refer to the section: Perform a Batch Analysis.
Reanalyze Batch	After performing a batch analysis, perform the analysis again. For details, refer to the section: Reanalyze a Batch.
View Selected Batch Sample Result	After performing a batch analysis, view the results of a selected sample in the upper pane of the Batch Analysis window.
Skip Current Sample	While performing a batch analysis, pass over the sample currently being analyzed. This option is not available if Sample Plate Results was selected in the Batch Analysis Selection dialog.
Skip Current Sample Set	While performing a batch analysis, pass over the sample set currently being analyzed. This option is not available if Sample Data was selected in the Batch Analysis Selection dialog.
Skip Current Sample Plate	While performing a batch analysis, pass over the sample plate currently being analyzed. This option is not available if Sample Data was selected in the Batch Analysis Selection dialog.
Pause After Current Sample	Momentarily stop a batch analysis after the system analyzes the currently running sample.
Resume Batch Processing	Resume a batch analysis after it has been paused.
Third Party Analysis	Launch the third-party analysis package specified in the Third Party Analysis Setup dialog.
Third Party Analysis Setup	Specify the path, third-party analysis package (loaded on your PC), and export format of the data.

Option	Description
Service Alignment	Align the sequence against puc18dG, print the alignment report, and export the alignment data as a text file.
Align with Template	Perform an alignment of the sequence against the current alignment settings.
Batch Alignment	Perform a batch alignment.
Trim Based on Quality	Open the Current Working Parameters dialog. Click Edit to open the Quality-based Trimming tab.
	Note: If the 21 CFR Part 11 tools are in use, then the edit function is only available to users with Write access.
Trim Based on Sequence	Open the Current Working Parameters dialog. Click Edit to open the Sequence-based Trimming tab in the Sequence Analysis Parameters Editor dialog.
	Note: If the 21 CFR Part 11 tools are in use, then the edit function is only available to users with Write access.
Batch Trim	Open the Batch Trimming dialog.

Window Menu

Click **Window** to show the following menu.

Figure 6-8 Window Menu

Window	
Cascade	
Tile Horizontally	
Tile Vertically	
Close All	
Arrange Icons	
✓ 1 <untitled></untitled>	

Option	Description
Cascade	Cascade the open windows.
Tile Horizontally	Tile the windows in a horizontal orientation.
Tile Vertically	Tile the windows in a vertical orientation.

Option	Description
Close All	Close any currently active windows.
Arrange Icons	Automatically arrange the icons.

Help Menu

Click **Help** to show the following menu.

Figure 6-9 Help Menu

F	telp
	Help <u>T</u> opics
	Using Help
	About GenomeLab System
	SCIEX Home Page

Option	Description
Help Topics	Select this option to show the Help topics. By default, the Contents show in the navigation pane on the left and the introductory topic shows in the topic pane on the right. Use the Contents to select and print specific Help topics. In the navigation pane, use the Search and Index tabs to search for information by topic, index entry, and/or keyword.
Using Help	Select this option to open the Contents window on how to use the Windows Help system.
About GenomeLab System	Select this option to access software and system information.
SCIEX Home Page	Select this option to access the SCIEX Home Page on the Internet.

Toolbars

The Sequence Analysis module provides seven toolbars: Standard, Data, Sample View, Dye Colors, Batch Control, Sample Plate, and Base Sequence. Each icon on the various toolbars corresponds to a commonly used menu item. By default, all toolbars (except Sample Plate and Base Sequence) show when you first open the Sequence Analysis module.

The following tables describe the function of each of the toolbar icons.

Standard Toolbar

The following example shows the Sequence Analysis module's Standard toolbar.

Figure 6-10 Standard Toolbar, Sequence Analysis Module

Icon	Description
3	Open: Open data files, including: Sample Data, Sequence Analysis Parameters, Optical Scan Data, Sequence Results, and Sample Plate Results.
	Save: Save the current data to the same location with the same file name.
a	Print: Print the report.
Q	Print Preview: Open a print preview of the active document of the active sample plate. A toolbar at the top of the window provides options for navigation, viewing, and printing the display. To restore the default screen, press Esc or click Close .
9	Print Desktop: Print the desktop, application, or main window as defined in the Preferences dialog.
×	Cut: Copy the selected item to the clipboard and removes it from its current location.
	Copy: Copy the selected item to the clipboard without removing it from its current location.
æ	Paste: Insert copied or cut items from the clipboard into the selected location.
S	Undo: Undo the last action performed.
2	Redo: Reverse the last undo action.
-111-	Apply Quality-based Trimming: Commit to quality-based trimming.
ATTE DTTR	Apply Sequence-based Trimming: Commit to sequence-based trimming.
*	Audio Enable: Toggle to enable or disable audio. If enabled while in Edit mode, each base letter is audibly announced as it is typed.

lcon	Description
₽	Audio Playback: Audibly announce a series of selected base sequence text.
	Working Analysis Parameters: View and/or change the analysis parameters to be used for subsequent analyses.
RRIE	Analyze: Analyze active sample data or reanalyze existing sequence results. Selecting this option opens the current Working Parameters dialog. Click OK to start the analysis.
	Stop: Terminates the analysis currently in progress.
+II+	Trim Based on Quality: Perform a trim based on quality.
DITE	Trim Based on Sequence: Perform a trim based on a sequence.
£₽	Restore Original Base Sequence: Return the analyzed data display and base sequence text back to its original state (losing all edits).
'A	Third Party Analysis: Open the third-party analysis package specified in the Third Party Analysis Setup dialog.
	Align: Perform an alignment of the sequence against the current alignment settings.
Å₽	Base Sequence Toolbar: Show the base sequence toolbar to search for text and ambiguity codes.
	View Sample Plate Toolbar: Show the sample plate toolbar to select the samples to open or export.
Ħ	Heterozygote Backward Search: Search backward for heterozygotes in the base sequence text pane.
H	Heterozygote Forward Search: Search forward for heterozygotes in the base sequence text pane.
8	Help Topics: Open the Help System. By default, the Contents show in the navigation pane on the left and the introductory topic shows in the topic pane on the right. Use the Contents to select and print specific Help topics. In the navigation pane, use the Search and Index tabs to search for information by topic, index entry, and/or keyword.
▶?	Context-Sensitive Help: Click this icon and then on a menu item to open the Help topic related to the selection.

Data Toolbar

The following example shows the Sequence Analysis module's Data toolbar.

Figure 6-11 Data Toolbar, Sequence Analysis Module



lcon	Description
A	Zoom Mode: Magnify a specific area of data in the display.
٩	Pan Mode: Move analyzed data in the X and Y direction.
AR	Align Mode: Visually align bases and peaks in the analyzed data pane.
	Edit Mode: Insert, change, and/or delete bases.
	Note: If the 21 CFR Part 11 tools are in use, then the edit function is only available to users with Write access.
2	Unzoom: Undo one zoom level.
Q	Unzoom All: Undo all zoom levels.
* *	Autoscale On/Off: By default, data showing on the screen is scaled automatically to the confines of the screen (Autoscale is on). If Autoscale is off, the data is scaled to its true values.
3	Base Spacing: Set the space between base sequence text in groups of 0, 3, 5, or 10.
<u>↑</u>	Base Synch: With both the analyzed data and base sequence panes open, synchronize the analyzed data display with the highlighted base sequence text showing the corresponding peak or peaks between two hairlines for the selected base.
*	Bases on Top: Toggle to show the base sequence text on the top or bottom in the analyzed data pane.
*	Compare Synch: Synchronize scaling, zooming, and panning of two analyzed data sets while in Compare mode.
+1 1+	Align: Align selected points of each of the analyzed data sets that show while in Compare mode.

lcon	Description
##	Display Options: Open the Display Options dialog.

Sample View Toolbar

The following example shows the Sequence Analysis module's Sample View toolbar.

Figure 6-12 Sample View Toolbar, Sequence Analysis Module



lcon	Description
	Analyzed Data: Show the data that has been analyzed for the active sample.
ACGT	Base Sequence: Show a text view of the bases from the analyzed data for the active sample.
	Raw Data: Show the raw data for the active sample.
	Current: Show the current data from the capillary arrays for the active sample.
Ar k∨	Voltage: Show the voltage data from the GenomeLab GeXP system for the active sample.
	Compare Data: Compare the active analyzed data set against another analyzed data set.

lcon	Description
	Quality Parameters: Show a graphical representation of the quality values for the active sample.

Dye Colors Toolbar

The following example shows the Sequence Analysis module's Dye Colors toolbar.

Figure 6-13 Dye Colors Toolbar, Sequence Analysis Module

A: 🔳 C: 🔳 G: 🔳 T: 🔳 N: 🔳

lcon	Description
A (red)	Red is the default color assigned to the adenine nucleotide bases in the analyzed data pane.
C (black)	Black is the default indicator assigned to the cytosine nucleotide bases in the analyzed data pane.
G (green)	Green is the default color assigned to the guanine nucleotide bases in the analyzed data pane.
T (blue)	Blue is the default color assigned to the thymine nucleotide bases in the analyzed data pane.
N (gray)	Gray is the default color assigned to ambiguous nucleotide bases in the analyzed data pane.

Batch Control Toolbar

The following example shows the Sequence Analysis module's Batch Control toolbar.

Figure 6-14 Batch ControlToolbar, Sequence Analysis Module

		0 c	P	~ G	
--	--	-----	----------	-----	--

lcon	Description
	Batch Analysis: Analyze multiple sample data or sample plate results sequentially.
111	Batch Alignment: Select sequence results or sample plate results to align.

lcon	Description
ACTC 1111	Batch Trimming: Trim multiple sample data or sample plate results sequentially.
►	Reanalyze Batch: After performing a batch analysis, use this option to perform a new analysis on the same data set.
	Stop Analysis: Terminate the analysis currently in progress.
1	Pause: Momentarily stop a batch analysis after the currently running sample has been analyzed.
E]	Resume: Resume a batch analysis after it has been paused.
Ġ	Skip Sample: While performing a batch analysis, click this icon to pass over the sample currently being analyzed. (This item is not available if Sample Plate Results was selected in the Batch Analysis Selection dialog.)
Ċ	Skip Set: While performing a batch analysis, click this icon to pass over the sample set currently being analyzed. (This item is not available if Sample Data was selected in the Batch Analysis Selection dialog.)
Cii	Skip Plate: While performing a batch analysis, click this icon to pass over the sample plate currently being analyzed. (This item is not available if Sample Data was selected in the Batch Analysis Selection dialog.)
	View Results: After performing a batch analysis, click this icon to view the results of a selected sample in the upper pane of the batch analysis window.

Sample Plate Toolbar

Select **View > Toolbars**, then select the **Sample Plate** check box to open the Sample Plate toolbar.

Toolbars	Sample Plate 🗙
Image: Standard Close Image: Data Help Image: Sample View Image: Sample Plate Image: Sample Plate Image: Sampl	1 2 3 4 5 6 7 8 9 10 11 12 A 0
	Plate: U33 0Q1 Test 030106_0603012 ▼ Sample: Result: <u>Open R</u> efresh <u>Export H</u> elp

Figure 6-15 Sample Plate Toolbar, Sequence Analysis Module

Use this toolbar to specify the samples to open or export. Select the desired plate from the **Plate** list. Valid samples show as filled wells. Wells not specified as samples show dark or empty. Click the samples to open or export.

- To view the name of a sample and associated result, position the cursor over the sample. The sample and result names show in the **Sample** and **Result** fields.
- To select contiguous samples, click the first sample in the series, hold down the **Shift** key, then click the last sample in the series. The selected samples are highlighted.

Base Sequence Toolbar

Select View > Toolbars, then select Base Sequence to open the Base Sequence toolbar.

Toolbars × I Standard Qlose I Data Help I Sample View Help I Sample Plate Base Sequence	Base Sequence Search S	× ≥ Search <u>H</u> ighlighted Range
Dye Colors Batch Control	Selected Bases Base: 1 G Migration: 22.44 min Edited Inserted	Quality Scores Call: 1.00 A: 0 C: 0 G: 253 T: 0

Figure 6-16 Base Sequence Toolbar, Sequence Analysis Module

Use this toolbar to search for specific base sequences and to view quality parameters. Enter the desired string of bases and click the arrow buttons to search backwards and forwards through the base sequence text.

To use regular expressions to locate the desired text, use the characters described in the following table.

 Table 6-1 Characters Used in Expressions

Character	Description
I	The pipe allows either expression on its side to match the target string. The expression $A B$ will match A as well as B .
	The dot will match any character.
*	The asterisk indicates that the character to the left of the asterisk in the expression should match 0 or more times.
+	The plus is similar to the asterisk but there should be at least one match of the character to the left of the plus in the expression.
?	The question mark matches the character to its left 0 or 1 times.
[]	Brackets enclosing a set of characters indicates that any of the enclosed characters may match the target character.

- To search for the text regardless of case, select the **Ignore Case** check box.
- To search for the exact match for text (not the IUB codes), select the **Exact Match** check box.

Sequence Analysis Module

• To search for text within a range, highlight the range of interest in the base sequence pane and select the **Search Highlighted Range** check box.

The following table identifies the IUB codes you can enter to find the corresponding bases when the **Exact Match** check box is cleared.

IUB	Bases
N	G, A, T, or C
V	G, A, or C
В	G, T, or C
Н	A, T, or C
D	G, A, or T
К	G or T
S	G or C
W	A or T
М	A or C
Y	C or T
R	A or G

Table 6-2 IUB Codes

Using the Sequence Analysis Module

Run a sequence analysis, and set up its parameters, result properties, and display options, then work with, report, and export sequence analysis results.

To open the Sequence Analysis module, on the Main Menu, click the SEQUENCING icon

View Sample Data

To view sample data:

 Click Sequence Analysis, then select File > Open. The Open dialog opens.

Figure 6-17 Open Dialog

en Sequence Analysis Parameter:		cal Scan Data
Sample Data Sequence R	esults Sam	ple Plate Results
Database: 032206 CEQ 1	ROUBLESHOO	1
		. ок
Filter		
0	Enable	Cancel
Start Date: 07-21-2005	Endblo	
End Date: 07-21-2005	Refresh	Help
End Date: 07-21-2005	<u>H</u> erresn	
Name	Date-Time	- Canala - All
Sequence Test.A03 0603012	03/02/06 0	Sample 🔺
Sequence Test.A05_0603012	03/02/06 0	Sequence
Sequence Test.A05_0003020	03/02/06 0	Sequence
Sequence Test.B03 0603012	03/02/06 0	Sequence
Sequence Test.B04_0603020	03/02/06 0	Sequence
Sequence Test.B05_0603020	03/02/06 0	Sequence 1
Sequence Test.C03_0603012	03/02/06 0	Sequence
Sequence Test.C04_0603020	03/02/06 0	Sequence 1
Sequence Test.C05_0603020	03/02/06 0	Sequence 1
Sequence Test.D03_0603012	03/02/06 0	Sequence 1
Sequence Test.D04_0603020	03/02/06 0	Sequence 💳
Sequence Test.D05_0603020	03/02/06 0	Sequence]
Sequence Test.E03_0603012	03/02/06 0	Sequence]
Sequence Test.E04_0603020	03/02/06 0	Sequence]
Sequence Test.E05_0603020	03/02/06 0	Sequence]
Sequence Test.F03_0603012	03/02/06 0	Sequence
Sequence Test.F04_0603020	03/02/06 0	Sequence 🔽
Desired Manage		
Project Name: Default	•	Search

- 2. Select the appropriate tab (Sample Data, Sample Plate Results, Sequence Results, Sequence Analysis Parameters, or Optical Scan Data).
- 3. Select the desired item from the list, then click **OK**.
- 4. Use the Sample View toolbar icons to show the desired data. For more information about the Sample View toolbar, refer to the section: Sample View Toolbar.

The following table describes each tab in the Open dialog.

Table 6-3 Open Dialog Tabs, Sequence Analysis Module

Tab	Description
Sample Data	Shows the list of raw data and baseline data stored in the selected project. On the right side of the list you may view the date and time the sample was run. You may want to open a raw data set to analyze it.

Tab	Description	
Sequence Results	Shows the list of analyzed data that resides in the selected project. Of the right side of the list you may view the date and time the sample wa run as well as the raw data from which the sequence result was derive Sequence data consists of analyzed data and its base sequence text. When you open sequence data you may also view the associated raw data, current, and voltage. This option is available for sequence analy only.	
	To filter the list, select the Enable check box and enter the Start and End dates for the items you wish to view. Click Refresh to view the filtered results.	
Sample Plate Results	Shows the list of sample plates run in the selected project. Selecting a sample plate result shows the Sample Plate toolbar. To open samples, click the desired samples, then click Open .	
Sequence Analysis Parameters	Shows the sequence analysis parameters saved in the project. The sequence analysis parameter set determines the start and end times for the analysis of raw data, threshold above which data are considered peaks, data start criteria, heterozygote detection parameters, and alignment template and parameters. You may also edit sequence analysis parameters on the Analysis tab in the Sample Setup module.	
Optical Scan Data	Lists the alignment optical scan data available in the selected project. Optical scan data are the result of the two lasers scanning the capillaries to determine the position of the capillary windows where the lasers focus when collecting data. If the scan is successful, eight peaks appear for each laser, denoting that all eight capillary windows were established.	

Table 6-3 Open Dialog Tabs, Sequence Analysis Module (continued)

Edit Sequence Analysis Parameters

Note: If the 21 CFR Part 11 tools are in use, then the edit function is only available to users with Write access.

The Sequence Analysis Parameters Editor dialog has six tabs: General, Initial Data Detection, Heterozygote Detection, Alignment Reference, Quality-based Trimming, and Sequence-based Trimming.

To edit the sequence analysis parameters:

1. From the Sequence Analysis module menu bar, select **Analysis > Working Analysis Parameters**.

The Working Parameters dialog opens.

2. Click Edit.

The Sequence Analysis Parameters Editor dialog opens.

Figure 6-18 Sequence Analysis Parameters Editor Dialog

Sequence Analys	is Parameters Editor - DefaultSequenceAnalysisPar 🗙			
Quality-based Tri General	mming Sequence-based Trimming Alignment Reference Initial Data Detection Heterozygote Detection			
N <u>T</u> hreshold:	0.60			
Analysis <u>S</u> tart:	0.0 min. 🗖 Use Default Mobility Calib.			
Analysis St <u>o</u> p:	0.0 min. 🗖 Pre-peak <u>R</u> eduction			
<u>C</u> olor	DefaultColorCalibration			
Heterozygote Detection: No				
Quality-based Trimming: None Sequence-based Trimming: No				
Automatic Alignment: No				
ОК	Save <u>A</u> s Print Cancel Help			

- 3. Use the tabs in this dialog to make appropriate changes. For parameter descriptions, refer to the sections:
 - General Tab
 - Initial Data Detection Tab
 - Heterozygote Detection Tab
 - Alignment Reference Tab
 - Quality-based Trimming Tab
 - Sequence-based Trimming Tab
- 4. Click **Save As** to create a new **Sequence Analysis Parameter** name, or click **OK** to save the changes and exit the editor.

General Tab

Use the General tab of the Sequence Analysis Parameters Editor dialog to specify the parameters used to perform the base-calling operation on the desired samples, whether or not you are performing an alignment.

Note: If you opened this dialog by selecting **View > Parameters Used to Compute Sequence**, this dialog shows the analysis values, but disables the fields described below.

The following table describes the options available on the Sequence Analysis Parameters Editor dialog's General tab.

Option	Description			
N Threshold	Specifies the value for the threshold below which <i>N</i> s are substituted for called bases. Enter the desired value in the N Threshold field. The range is 0.00 to 1.00. For example, entering:			
	• 1.00 calls all bases as indeterminate (<i>N</i> s).			
	• 0.50 calls all bases with an estimated error of probability greater than 50% as <i>N</i> .			
	• 0.00 does not call any <i>N</i> s.			
	• 0.60 is recommended (called bases with an estimated error probability greater than 40% will be called <i>N</i> s).			
Analysis Start/ Stop	 Enter the time in minutes from the start of the raw data where the analysis is to begin, and the time in minutes from the start of the raw data where the analysis is to end. Start: The default value of 0 for the start time uses the system algorithm to find the start of valid data and the values entered on the Initial Data Detection tab. Stop: The default value of 0 for the stop time analyzes to the end of collected raw data. 			
PCR Product	Selecting this check box causes analysis to end when a sudden drop off of signal lasting at least 2 min is encountered. This prevents the analysis of baseline data.			
<200 Bases	Signifies that the sequences generated from PCR are less than 200 nucleotides in length. Selecting this check box changes the default values for the Delay and Minimum Duration on the Initial Data Detection Initial Data Detection tab. The default value for Delay is 0.20 min and the default value for Minimum Duration is 2.00 min . Note: This option is available only when PCR Product is selected.			

Table 6-4 General Tab, Sequence Analysis Parameters Editor Dialog

Option	Description
Use Default Mobility Calib.	Select this check box to optimize the analysis results of most short PCR products. This stabilizes the data adjustment that accounts for the difference in mobility of fragments due to their respective dye labels. This differential mobility has the greatest effect on the shorter fragments and has to be accounted for in the analysis of data that includes short fragments.
	Note: This option is automatically selected when <200 Bases is selected, which is recommended.
Pre-peak Reduction	Select this check box if you want the software to identify and reduce the inclusion of pre-peaks in the analyzed data. Pre-peaks are artifacts that may appear one base prior to the significant peak. They are usually caused by including primers that are not full-length in the sequencing reaction. Sometimes they are caused by slippage of the enzyme during the extension reaction in which case the pre-peaks are often found starting near the end of a series of the same base.
Color	Click this button to view the color calibration for the analysis parameters. The Color Calibration dialog opens. The values in the matrix show the cross talk between the filters and the emissions for A , C , G , and T . If you want to select another color calibration, click Select Stored Color Calibration , select the new color calibration, then click OK . The name of the selected color calibration shows in the field.
Informational Display	The lower section of this dialog shows specific features that are enabled/ disabled from other tabs. To change these settings, select the appropriate tab and edit the feature.

Table 6-4 General Tab, Sequence Analysis Parameters Editor Dialog (continued)

Initial Data Detection Tab

Use the Initial Data Detection tab of the Sequence Analysis Parameters Editor dialog to enable the system to detect the start of data to be analyzed. The system attempts to detect valid data using the system algorithm and values entered on this tab only if the **Analysis Start** time on the General tab is set to **0.0**.

Sequence Analysis Parameters Editor - DefaultSequenceAnalysisPar 🗙					
Quality-based T	Quality-based Trimming Sequence-based Trimming Alignment Reference				
General	Initial Dat	a Detection	Het	erozygote	Detection
	Thresho <u>l</u> d: 🚺	•	%		
	Delay: 0.	75 •	min.		
Signa	al to <u>N</u> oise: 7.	0 🛃			
Minim <u>u</u> r	n Duration: 5.	00 •	min.		
OK	Save <u>A</u> s	P <u>r</u> int	Ca	ncel	Help

Figure 6-19 Initial Data Detection Tab, Sequence Analysis Parameters Editor Dialog

Note: If you opened this dialog by selecting **View > Parameters Used to Compute Sequence**, this dialog shows the analysis values, but disables the fields described below.

Note: Set the Initial Data Detection parameters based on the sample cleanup method used. If the cleanup method leaves large peaks due to unincorporated dye at the beginning of data, set the parameters to avoid these blobs. (This makes it difficult to read to the end of the primer; however, the base-call accuracy improves since dye peaks do not interfere with early mobility correction.) If the cleanup method tends to leave dye peaks at the beginning of data, set the **Threshold** to **30** or **35** and set the **Delay** to **0.75**. (If there are still fat peaks at the start of analyzed data, make this a larger delay.)

Note: If the cleanup eliminates dye peaks, you can read closer to the end of the primer by setting the **Threshold** to **20**, and the **Delay** to **0.6**.

The following table describes the options available on the Sequence Analysis Parameters Editor dialog's Initial Data Detection tab.

Option	Description
Threshold	Enter the maximum ratio value of all the data in the energy profile above which the signal must rise before the analysis starts detecting data. The data must stay above this threshold for the minimum duration without gaps for more than 21 s. The range is 0 to 100%. The default is 40%.

Table 6-5 Initial Data Detection Tab, Sequence Analysis Parameters Editor Dialog

Table 6-5 Initial Data Detection Tab, Sequence Analysis Parameters Editor Dialog
(continued)

Option	Description				
Delay	Enter a value for the delay between the detected start of data when the data that will be analyzed. The range is 0.00 min to 180.00 min.				
	 If <200 Bases is not checked on the General tab, the default value is 0.75 min. 				
	 If <200 Bases is checked, the default value is 0.20 min. 				
Signal to Noise	Enter the Signal to Noise value to specify the signal to noise ratio for significant data. The range is 3.00 to 1000.00. The default value is 7.00 .				
Minimum Duration	Enter the value at which the signal-to-noise threshold must exceed with no gaps greater than 21 s. The range is 2.00 min to 50.00 min.				
	 If <200 Bases is not checked on the General tab, the default value is 5.00 min. 				
	 If <200 Bases is checked, the default value is 2.00 min. 				

Heterozygote Detection Tab

Use the Heterozygote Detection tab of the Sequence Analysis Parameters Editor dialog to enable heterozygote detection and to specify the parameters for detecting heterozygotes. If you enable this feature, the next analysis run detects heterozygotes and shows ambiguity codes where appropriate. Figure 6-20 Heterozygote Detection Tab, Sequence Analysis Parameters Editor Dialog

Sequence Analysis Parameters Editor - DefaultSequenceAnalysisPar 🗙							
Quality-based Trimming		Sequence-based Trimming		Alignment Reference	1		
General	Initial D) ata Detection	Het	Heterozygote Detection			
	Detect Heterozygotes						
5	% of Average	Peak Spacing: 7	0	- % -			
	Height Ratio: 30 📃 %						
		Sensiti <u>v</u> ity: 0	.25				
Range: <u>F</u> ro	m: 50	nt <u>t</u> o 5	50	.≍ nt			
or	to 10	nt befor	e the last	called base			
ОК	Save <u>A</u> s	Print	Ca	incel Help			

Note: If you opened this dialog by selecting **View > Parameters Used to Compute Sequence**, this dialog shows the analysis values, but disables the fields described below.

The following table describes the options available on the Sequence Analysis Parameters Editor dialog's Heterozygote Detection tab.

Option	Description		
Detect Heterozygotes	Select this check box if you want heterozygotes detected. When selected, the N Threshold (on the General tab) is disabled, and <i>N</i> s will not be called		
	The system detects heterozygotes after it determines the "final base call". It detects peaks that meet the sensitivity criterion and examines each called base to determine if another peak nearby meets the Spacing and Height Ratio criteria specified. If one or more nearby peaks meet the criteria, the system merges their identities with the called base to produce an ambiguity code.		
	Note: If you specify the Detect Heterozygote option, <i>N</i> s are not called. The N Threshold field is grayed out to disable calling <i>N</i> s.		
% of Average Peak Spacing	Enter a value to determine how far to look on either side of each called base to look for other peaks. This value is centered over the peak for the called base; for instance, a value of 50 looks 25% on either side of the peak for secondary peaks. The range is 0 to 100%.		

Table 6-6 Heterozygote Detection Tab, Sequence Analysis Parameters Editor Dialog(continued)

Option	Description
Height Ratio	Enter the height ratio of the secondary peak relative to called base peak. The range is 0 to 100%.
Sensitivity	Enter the value that specifies the minimum sharpness of detected secondary peaks. The higher the value, the higher the sensitivity used to detect smaller, less visually defined peaks. The range is 0.00 to 1.00.
Range	Enter the range in the called sequence where the system looks for heterozygotes within a called sequence. Also provide the number of bases prior to the last called base you want heterozygote detection to end in the case of short PCR samples that may vary in length. The range is 0-length of the sequence.

If a value is outside of an expected range, a dialog opens to request that you to enter a valid value. The system uses the IUB ambiguity codes shown in the following table to represent heterozygous bases.

Table 6-7 IUB Ambiguity Codes

Code	Definition	Mnemonic
A	Adenine	A
С	Cytosine	С
G	Guanine	G
Т	Thymine	Т
R	AG	puRine
Y	СТ	pYrimidine
K	GT	Keto
М	AC	aMino
S	GC	Strong 3 H bonds
W	AT	Weak 2 H bonds
В	CGT	Not A
D	AGT	Not C
Н	ACT	Not G

Code	Definition	Mnemonic
V	ACG	Not T
N	AGCT	aNything

Table 6-	7 IUB A	mbiauity	Codes	(continued))
		mangany	00000	(oonunaca)	,

The system reports the heterozygous bases in the analyzed data and in the base sequence data.

Alignment Reference Tab

Use the Alignment Reference tab of the Sequence Analysis Parameters Editor dialog to enable automatic alignment and to specify the template against which you want to align the active sequence.

Figure 6-21 Alignment Reference Tab, Sequence Analysis Parameters Editor Dialog

Sequence Analysis Parameters Editor - DefaultSequenceAnalysisPar 🗙
General Initial Data Detection Heterozygote Detection Quality-based Trimming Sequence-based Trimming Alignment Reference
✓ Perform Alignment
Reference
⊙ puc18 <u>d</u> G ⊂ <u>p</u> uc18 ⊂ <u>m</u> 13 ⊂ <u>R</u> eference File
Eile: Browse
Alignment Accuracy
Cutoff Accuracy %: 98.5 Allowed 'N' %: 2.0
Advanced Alignment Parameters
OK Save <u>A</u> s P <u>r</u> int Cancel Help

Note: If you opened this dialog by selecting **View > Parameters Used to Compute Sequence**, this dialog shows the analysis values, but disables the fields described below.

The following table describes the options available on the Sequence Analysis Parameters Editor dialog's Alignment Reference tab.

Option	Description
Perform Alignment	Select this check box to perform an alignment automatically when running an analysis. The alignment uses the alignment template and parameters (specified below) and in the Advanced Alignment Parameters dialog.
Reference	Select the desired alignment template by clicking on its corresponding radio button (puc18dG , puc18 , or m13).
	To select a different template, click Reference File . Click Browse and navigate to the folder that contains the reference file, then select it and click OK . The field shows the name of the selected reference file.
Cutoff Accuracy	Enter the desired Cutoff Accuracy . The alignment detects the base number in the called sequence at which the accuracy falls below this Cutoff Accuracy value. The range is 0.0 to 100.0%.
Allowed N	Enter the percentage of <i>N</i> s allowed to occur before they are counted as errors when determining the alignment accuracy. The range is 0.0 to 100.0%.
Advanced Alignment Parameters	Click this button to open the Advanced Alignment Parameters dialog.

Table 6-8 Alignment Reference Tab, Sequence Analysis Parameters Editor Dialog

Advanced Alignment Parameters Dialog

Use the Advanced Alignment Parameters dialog to define the advanced alignment parameters used to perform alignments with the active sequence.

Figure 6-22 Advanced Alignment Parameters Dialog, Sequence Analysis Module

Advanced Alignment Parameters	×
-Alignment scoring	
Match: 1.0 - Insert start: -4.0 - Insert extend: -3.0	-
Mismatch: -2.0 Delete start: -4.0 Delete extend: -3.0	
Alignment controls	
✓ Find matching substrings ✓ Left Edge Free Minimum	
□ Find Local alignment ☑ Right Edge Free substring length: 30	•
OK Cancel <u>H</u> elp	

Note: If you opened this dialog by selecting **View > Parameters Used to Compute Sequence**, this dialog shows the analysis values, but disables the fields described below.

The system uses a scoring matrix to perform the alignment and shows the alignment that produces the highest total score. In general, matches should be given higher scores than non-matches.

The following table describes the options available in the Advanced Alignment Parameters dialog.

Option	Description
Alignment Scoring	Match : Sets the value of a match between a base position of the sequence and the reference template. The range is -1000.0 to 1000.0. The default value is 1 .
	MisMatch : Sets the value of a mismatch between a base position of the sequence and the alignment sequence. The range is -1000.0 to 1000.0. The default value is -2.0 .
	Insert start : Sets the value of an insertion of a base in the sequence that does not exist in the alignment sequence. The range is -1000.0 to 1000.0. The default value is -4.0 .
	Insert extend : Sets the value of a consecutive insertion of a base in the sequence that does not exist in the alignment sequence. The range is -1000.0 to 1000.0. The default value is -3.0 .
	Delete start : Sets the value of a deletion of a base in the sequence that does not correspond to the alignment sequence. The range is -1000.0 to 1000.0. The default value is -4.0 .
	Delete extend : Sets the value of a consecutive deletion of a base in the sequence that does not exist in the alignment sequence. The range is -1000.0 to 1000.0. The default value is -3.0 .

Table 6-9 Advanced Alignment Parameters Dialog, Sequence Analysis Module

Option	Description
Alignment Controls	To optimize the alignment to find matching substrings first, then run the alignment algorithm only on the bases remaining outside the substrings, reducing execution time and storage space, select Find matching substrings . This check box is selected by default.
	To use only substrings with the highest score, and ignore the rest, as opposed to using every character from both strings to compute the similarity score, select Find Local alignment .
	To specify that overhanging bases on the template or sequence will not be counted in the alignment score, select Left Edge Free and/or Right Edge Free .
	To specify a minimum length for detected substrings, enter the number of bases needed to constitute a substring in the Minimum substring length field. The range is 5 to 2,000. The default value is 30 .

Table 6-9 Advanced Alignment Parameters Dialog, Sequence Analysis Module (continued)

Quality-based Trimming Tab

Use the Quality-based Trimming tab of the Sequence Analysis Parameters Editor dialog to trim poor quality data from the ends of the sequence. The resulting base sequence and trace views show the base letters of the poor quality data as strike-through.

Figure 6-23 Quality-based	I Trimming Tab	, Sequence Analysis	Parameters Editor Dialog
---------------------------	----------------	---------------------	--------------------------

Sequence Analysis Param	neters Editor - DefaultSe	equenceAnalysisPar 🗙
		Heterozygote Detection
Quality-based Trimming	Sequence-based Trimming) Alignment Reference
Ends To Be Trimmed—		
⊙ <u>N</u> one O <u>B</u> ot	h Ends 💦 🔿 Left (5') End	C <u>R</u> ight (3') End
Trimming Stringency		
Low	Medium	High
I	· <u> </u>	· ·
<u> </u>		
OK Save As	Print	Cancel Help
OK Save <u>A</u> s.		Cancel Help

The following table describes the options available on the Sequence Analysis Parameters Editor dialog's Quality-based Trimming tab.

Option	Description
Ends To Be Trimmed	 Click the radio button to select the ends to be trimmed: Select Both Ends to trim outside the 5' and 3' coding region.
	Select Left (5') End to trim to the left of the 5' end.
	Select Right (3') End to trim to the right of the 3' end.
	• Select None if you do not want to perform quality-based trimming.
Trimming Stringency	Click and drag the slider control to set the quality values from low to high. There are five possible selections ranging from Low to High that correspond to error rate of 0.07, 0.06, 0.05, 0.03, and 0.01. The default value is Medium (5%).

Table 6-10 Quality-based Trimming Tab, Sequence Analysis Parameters Editor Dialog

Sequence-based Trimming Tab

Use the Sequence-based Trimming tab of the Sequence Analysis Parameters Editor dialog to apply contaminant (sequence-based) trimming. This can be done in batch analysis mode or in sequence analysis mode. The resulting base sequence and trace views show the base letters of the vector and other subsequences as underlined. If multiple vector or subsequences matched, they show as multiple underlined in the trace view.

Figure 6-24 Sequence-based Trimming Tab, Sequence Analysis Parameters Editor Dialog

Sequence Analysis Parar	neters Editor - DefaultSe	equenceAnalysisPar 🗙
Quality-based Trimming	al Data Detection H Sequence-based Trimming erform Sequence-based Trim	
Vector/Other Sequence		<u>× 1</u>
Max. # of ⊻ector/Other Sequences: 2 Min. Substring Length: 5 Enable Internal Matches		
OK Save As	s P <u>r</u> int	Cancel Help

Contaminant trimming can be based on any combination of the following:

- Vector sequences such as plasmid, BAC, PAC, and cosmids
- Primer sequences
- Restriction enzyme sites

Note: If you opened this dialog by selecting **View > Parameters Used to Compute Sequence**, this dialog shows the analysis values, but disables the fields described below.

The following table describes the options available on the Sequence Analysis Parameters Editor dialog's Sequence-based Trimming tab.

Table 6-11 Sequence-based Trimming Tab, Sequence Analysis Parameters Editor Dialog

Option	Description
Perform Sequence- based Trimming	Select this check box to enable sequence-based trimming.
Vector/Other Sequence Files	 Click to select a reference sequence. It must be in the .fasta format and stored in an accessible folder. Click to remove a previously selected reference sequence.
	Note: There is no practical limit on the number of .fasta reference sequences used; however, larger files (greater than 200 Kbase) increase processing time.
Max. # of Vector/ Other Sequences	Set the number of sequences to show in the result. This number is determined by the goodness of alignment by scoring the found subsequences and showing only the top number selected. The trimming log details all vectors/other sequences found regardless of the score.
Min. Substring Length	Set the minimum substring length. Only the subsequences that meet or exceed this length will be trimmed.
Distance from End	Set the distance from the end of the sequence and contaminant for automatic removal. You may set the Left (5') independently from the Right (3') end.
Enable Internal Matches	Select this check box to allow trimming within the sequence.

Perform Quality-based Trimming Set Up Quality-based Trimming

To set up quality-based trimming:

- 1. Open the Sequence Analysis module.
- 2. Open a result and select Analysis > Working Analysis Parameters.
- Click Edit. The Sequence Analysis Parameters Editor dialog opens.
- 4. Select the Quality-based Trimming tab.
- 5. Select Ends To Be Trimmed and Trimming Stringency.
- 6. Click **Save As** and save the new parameters.
- 7. Click OK.
- Select Analysis > Trim Based on Quality. The analysis parameters, created in the steps above, should be listed. If not, click Use Stored Parameters and select the appropriate analysis parameters.
- 9. Click OK.

The resulting base sequence and trace views show the base letters, if poor quality, as strike-through after performing a quality-based trim.

Note: When performing quality-based and sequence-based trimming, quality-based trimming is always performed first.

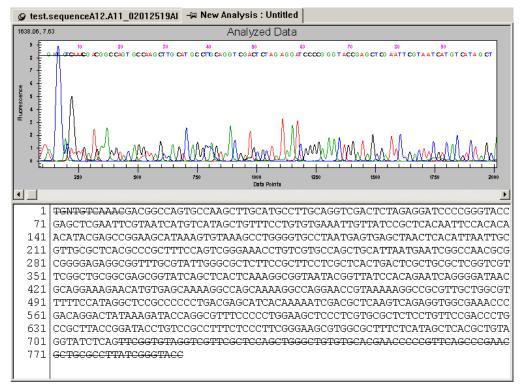


Figure 6-25 Base Sequence and Trace Views after Quality-based Trimming

Apply Quality-based Trimming

If the trimming is acceptable, select **Edit** > **Apply Quality-based Trimming** to apply the resulting base sequence and trace view.

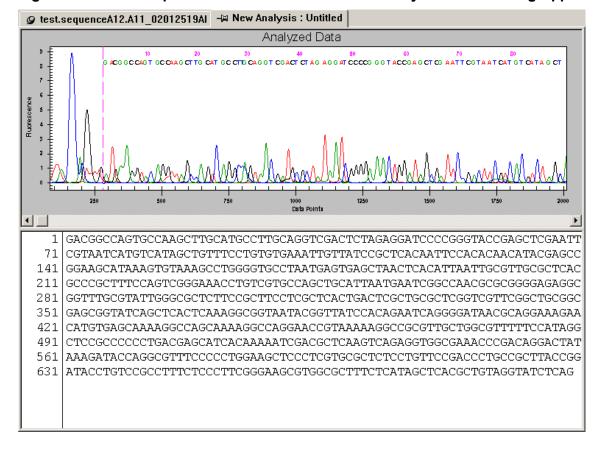


Figure 6-26 Base Sequence and Trace Views After Quality-based Trimming Applied

To recover the original sequence after applying quality-based trimming, select Edit > Undo.

Perform Sequence-based Trimming Set Up Sequence-based Trimming

To set up sequence-based trimming:

- 1. Open the Sequence Analysis module.
- 2. Open a result and select Analysis > Working Analysis Parameters.
- Click Edit. The Sequence Analysis Parameters Editor dialog opens.
- 4. Select the Sequence-based Trimming tab.
- 5. Select the **Perform Sequence-based Trimming** check box and select the desired options.
- 6. Click **Save As** and save the new parameters.
- 7. Click OK.

Select Edit > Trim Based on Sequence. The analysis parameters, created in the steps above, should be listed. If not, click Use Stored Parameters and select the appropriate analysis parameters.

9. Click OK.

Note: When performing quality-based and sequence-based trimming, quality-based trimming is always performed first.

The following example shows the resulting base sequence and trace view after performing a sequence-based trim.

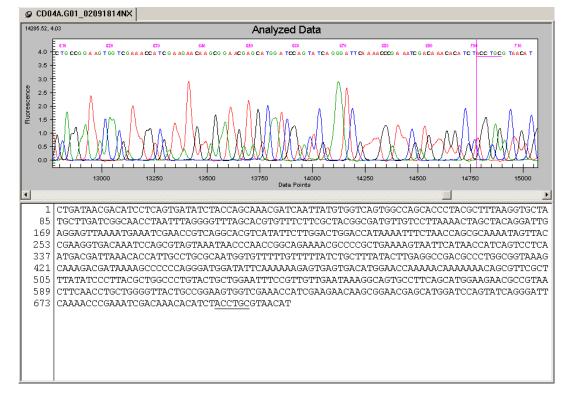


Figure 6-27 Base Sequence and Trace Views After Sequence-based Trimming

Apply Sequence-based Trimming

If the trimming is acceptable, select **Edit** > **Apply Sequence-based Trimming** to remove the trims from the sequence. The following example shows the resulting base sequence and trace view after applying sequence-based trimming.

Note: When applying sequence-based trimming, quality-based trimming is automatically applied first if quality-based trimming was performed.

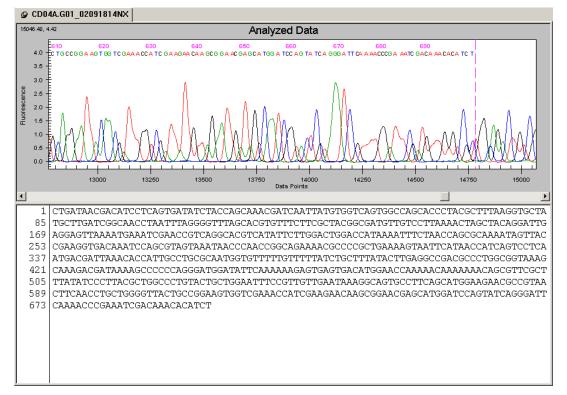


Figure 6-28 Base Sequence and Trace Views after Sequence-based Trimming

To recover the original sequence after applying sequence-based trimming, select **Edit** > **Undo**.

Internal Trimming

Select the **Enable Internal Matches** check box on the Sequence-based Trimming tab to trim subsequences from within the sequence. To exclude a subsequence from final removal, right-click the subsequence and select **Vector/Other Subsequences** > *subsequence* where *subsequence* is the subsequence name.

Note: The system removes only the selected subsequences from the sequence when you apply trimming.

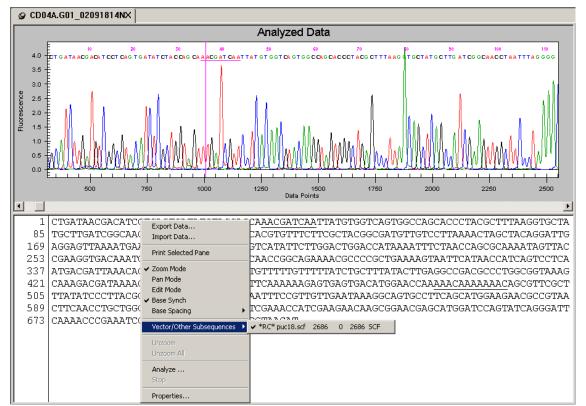


Figure 6-29 Base Sequence and Trace Views after Sequence-based Trimming – Enable Internal Matches

After you apply sequence-based trimming, the system replaces the internal matches in the resulting base sequence and trace view with the letter *X*.

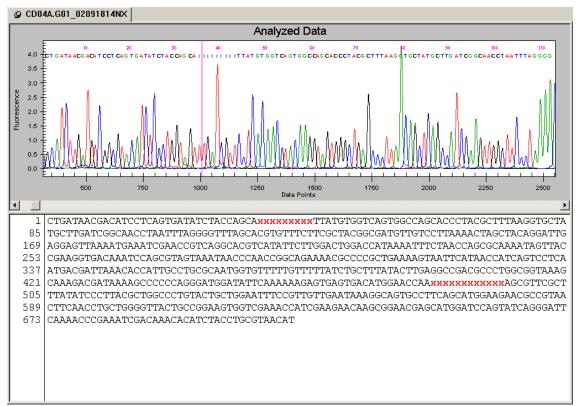


Figure 6-30 Base Sequence and Trace Views after Sequence-based Trimming – Internal Matches

Batch Trimming

Enable automatic trimming of a large number of results. To perform batch trimming:

- 1. Open the Sequence Analysis module.
- 2. Select Analysis > Batch Trimming.
- 3. Select the Sequence Results or Sample Plate Results tab.
- 4. Select the results to be trimmed.
- 5. Click the right-arrow to move the selected results to the "selected" list.

Batch Trimming Selection	X
Sequence Results Sample Plate Results	
Database: 032206 CEQ TROUBLESH001	ОК
Start Date: 07-21-2005 Enable	Cancel
End Date: 07-21-2005 <u>R</u> efresh	<u>H</u> elp
Name Date-Time Sequence Test.A05 03/02/06 03:52:51AM Sequence Test.B05 03/02/06 03:52:56AM Sequence Test.D05 03/02/06 03:53:02AM Sequence Test.D05 03/02/06 03:53:02AM Sequence Test.D05 03/02/06 03:53:08AM Sequence Test.E05 03/02/06 03:53:13AM Sequence Test.E05 03/02/06 03:53:13AM Sequence Test.E05 03/02/06 03:53:13AM Sequence Test.E05 03/02/06 03:53:13AM Sequence Test.B05 03/02/06 03:53:30AM Sequence Test.B05 03/02/06 03:53:30AM Sequence Test.B05 03/02/06 03:53:30AM Sequence Test.B04.06030200LZ Sequence Test.B04.06030200LZ Sequence Test.D04.06030200LZ Sequence Test.E04.06030200LZ Sequence Test.E04.0615:19:30PM Sequence Test.E04.06030200LZ Sequence Test.E04.06030200LZ Sequence Test.E04.06030200LZ Sequence Test.E04.06030200LZ Sequence Test.E04.06030200LZ Sequence Test.E04.06030200LZ Sequence Test.E04.06030200LZ Sequence Test.E04.06030200LZ Sequence Test.E04.06030200LZ Sequence Test.E04.06030200LZ	
Project Name: Default 💌 Search Load List Sa	ave List

Figure 6-31 Batch Trimming Result Selection

- 6. Click **OK**.
- 7. Verify that the required analysis parameters are selected in the Working Parameters dialog. (If not, click **Use Stored Parameters** and select the appropriate analysis parameters).
- If necessary, select the Export Results check box to export the results to files. The Edit Export Options button becomes available. For details, refer to the section: Export Dialog Options.
- 9. Click OK.

Trimming Report

Exporting results creates a Trimming Report, which you can view and print.

To view the Trimming Report, select **View > Trimming Report**. The new pane opens at the bottom of the screen showing the following details:

- Result Name
- Original Sequence Length

Sequence Analysis Module

- Length after Quality-based Trimming
- Vector/Other Subsequence Name
- First Base Trimmed
- Last Base Trimmed
- Enabled
- Length after Sequence-based Trimming
- Exported

From the Trimming Report pane, you can format the grid, print the grid data, and export the data as comma separated (.csv) or tab delimited (.txt) text files.

To access these options, right-click over the Trimming Report, then select the desired option from the menu.

The following table describes these options.

Option	Description
Print Grid Data	Select this option to print the entire grid as it is represented on the screen.
Export Grid Data	Select this option to save the grid data as a comma separated (*.csv) or tab delimited (*.txt) text file.
Format Grid	Select this option to open a cascading menu with formatting options. Print Grid Data Format Grid Freeze Rows Freeze Columns Unfreeze Columns Resize Rows Resize Columns Hide Cols Unhide All Styles Use this menu to customize the look of the data grid. If you hide rows or columns using this menu, the Print Grid Data option omits them; however, this does not affect the Export Grid Data (this exports all data).

Table 6-12 Trimming Report Right-Click Menu

Export Dialog Options

The Export dialog provides two options you can choose when exporting sequence data.

Figure 6-32 Export Dialog

Export		? ×
Sample Elements	Save jn: 🗀 Export	✓ ← € ▲ Ⅲ
Result Data Result Output Quality Parameters Alignment Results Alignment Accuracy	Sequence Test.A04_06030200LZ.fasta Sequence Test.B03_060301225Z.fasta Sequence Test.B04_06030200LZ.fasta Sequence Test.C03_060301225Z.fasta Sequence Test.C04_06030200LZ.fasta	Sequence Test.D04_06030200L2.ft Sequence Test.E03_0603012252.ft Sequence Test.E04_06030200L2.ft Sequence Test.F03_0603012252.ft Sequence Test.F03_0603012252.ft Sequence Test.F04_06030200L2.ft
Options Remove CEQ Tracking Suffix Resolve Filename Conflicts Apply Trimming	File name: DEFAULT Save as type: FASTA (FASTA and QUAL)	([*] .fasta) Cancel

The following table describes the Export dialog options.

Table 6-13 Export Dialog Options

Option	Description
Apply Trimming	If you selected Export during sample setup or batch analysis, selecting Apply Trimming removes the trims from the sequence before it is exported. The exported file replaces all internal trims with the letter <i>X</i> .
Export Only If Sequence > X nt	The resulting sequence, after trimming, may be too small to be useful. Select Export Only If Sequence > X nt to specify a minimum size that the sequence must be when exporting. This exports only those sequences greater than the set number of nucleotides.

Edit Bases

Note: If the 21 CFR Part 11 tools are in use, then the edit function is only available to users with Write access.

You can edit bases that show in the analyzed and bases data panes using the Sequence Analysis module's **Tools** menu. The system identifies edited bases by showing them in lower-case letters.

Insert Bases in the Analyzed Data Pane

To insert bases in the analyzed data pane:

1. Select Tools > Edit.

Sequence Analysis Module

- 2. Click an area between two bases. The Insert Base dialog opens.
- 3. Select the desired base.
- 4. Click **OK**.

The screen inserts the base as a lower-case letter in both the analyzed data and the base sequence panes.

Insert Bases in the Base Sequence Pane

To insert bases in the base sequence pane:

- 1. Select **Tools** > **Edit**.
- 2. Place the mouse cursor between the two bases where you want to insert the base.
- 3. Type in the new base.

The screen inserts the base as a lower-case letter in both the analyzed data and the base sequence panes.

Change Bases in the Analyzed Data Pane

To change bases in the analyzed data pane:

- 1. Select **Tools > Edit**.
- 2. Click a base in the analyzed data pane. The Change Base dialog opens.
- 3. Select the desired base.
- 4. Click OK.

The screen shows the changed base as a lower-case letter in both the analyzed data and the base sequence panes.

Change Bases in the Base Sequence Pane

To change bases in the base sequence pane:

- 1. Select **Tools** > **Edit**.
- 2. Highlight the bases to change in the base sequence text.
- 3. Type in the new bases.

The screen shows the changed base as a lower-case letter in both the analyzed data and the base sequence panes

Note: The left and right arrow keys move the cursor through the data in the base sequence pane. To select bases, hold **Shift** down while using the left and right arrow keys to select the desired bases.

Delete Bases in the Base Sequence Pane

To delete bases in the base sequence pane:

- 1. Select **Tools > Edit**.
- 2. Highlight the bases in the base sequence text to delete.
- 3. Press **Delete** or use the **Cut** icon

The screen removes the selected bases from both the base sequence and analyzed data panes.

Specify Base Grouping

To specify base grouping in the base sequence pane:

- 1. Select **Tools > Base Spacing** to open the cascading menu.
- 2. Select the desired group spacing.

View the Analysis Log

The system logs all the steps used in performing the base-calling routine on the raw data in the analysis log. For example, the system logs a message when it is unable to find the end of PCR product data.

You may view the analysis log to see if the system was unable to find the end of PCR data. The following message shows in the analysis log.

Analyzed Data: Cannot compute stop time. Using data stop time of xxx.xx minutes

Figure 6-33 Analysis Log

09/28/00 10:28:34	Analyzed Data: Estimate Noise Variance
09/28/00 10:28:34	Analyzed Data: Warning: Excessive noise in channel 4 at time 2228.392857
09/28/00 10:28:34	Analyzed Data: DeSpike
09/28/00 10:28:34	Analyzed Data: Deadtime Correct
09/28/00 10:28:34	Analyzed Data: Correct for Data Truncation
09/28/00 10:28:34	Analyzed Data: Align Time Base
09/28/00 10:28:34	Analyzed Data: Find Start
09/28/00 10:28:34	Analyzed Data: Find End
09/28/00 10:28:34	Analyzed Data: Cannot compute stop time. Using data stop time of 103.99 minute
09/28/00 10:28:34	Analyzed Data: Spike Analysis
09/28/00 10:28:34	Analyzed Data: Smooth
09/28/00 10:28:35	Analyzed Data: Compute Color Calibration

Occasionally, the system cannot find the end of PCR product data, even if the **PCR Product** check box is selected. To determine if this is the case, review the analysis log and use this information in future analysis of the same sample.

To view the analysis log:

1. Open the sequence results you would like to review.

Note: If the sample data has not yet been analyzed, analyze the sample data set using the desired analysis parameters. Select the **PCR Product** check box and enter **0.0** for both the **Analysis Start** and **Stop** times.

2. Select View > Analysis Log to view the analysis log for the sequence result.

The analysis log opens in a pane at the bottom of the window.

Sequence Result Properties

View method, analysis, alignment, consumable, general, notes, and property set information. To view these properties:

 Select File > Properties when a sequence result is open. The Properties dialog opens.

Note: The Alignment tab shows in this dialog only if you have performed an alignment on the open sequence result.

2. Select the tab that shows the type of properties you want to view.

For details, refer to the sections:

- General Tab
- Note Tab
- Property Set Tab

- Method Tab
- Analysis Tab
- Alignment Tab
- Consumables Tab

Note: To view detailed descriptions while using this dialog, click Help.

3. When finished reviewing the properties, click **Close**.

General Tab

Use the General tab to view the general properties of the currently selected sequence result.

Analysis General	Alignment Consumables Note Property Set Method			
Sequenci	e Test.A03_060301225Z			
Type: 9	Sequence Result			
Database: (CEQ DATABASE			
Project: [Default			
Modified: N	Monday, April 24, 2006 16:08:36			
Sample Name:	Sequence Test.A03_06030122SZ			
Sample Plate:	U33 0Q1 Test 030106			
Sample Position:	A:3			
Operator Name:	DC			
CEQ System Nam	e: CEQ 8000			
Firmware Version:	6.0.2			
Instrument S/N:	3067033			
Data Collection Software Version:	8.0.52			
Collection Date:	Thursday, March 02, 2006 00:32:34			
Sample Subject II):			
	<u>C</u> lose <u>H</u> elp			

Figure 6-34 General Tab, Properties Dialog

View the item type, database, and project where the item resides, as well as the date and time the item was last modified. You may also view the sample name, sample plate, sample position in the sample plate, instrument, and operator name.

Note Tab

Use the Note tab to view the note that was entered in the Sample Setup module when the sample ID was defined.

Select File > Properties > Notes.

Figure 6-35 Note Tab, Properties Dialog

Properties				×
Analysis	Alignmer		Consun	
General	Note	Property Set		Method
Note:				
	<u>C</u> lose			<u>H</u> elp

Property Set Tab

Use the Property Set tab to view the selected property set that was entered in the Sample Setup module when the sample ID was defined.

Select File > Properties > Property Set.

Figure 6-36 Property Set Tab, Properties Dialog

Properties	×
Analysis Alignment Cons General Note Property Set	umables Method
Property Set: Static	
Property Value	
Close	<u>H</u> elp

Method Tab

View the method associated with the result, and the steps used to perform the separation, as well as their associated values.

In addition, view the parameters for the first part and the second part of the separation, as well as the total separation time.

Select File > Properties > Method.

Analysis Ali General Note	gnment Property Se	Consumables Method
Method: Seq-Test	1	· • • •
Capillary Temperature:	52.0 °C	
Wait for Temperature:	Yes	
Denature Temperature:	90.0 °C	
Denature Duration:	120 sec	
Pause Duration:	1 sec	
Inject Voltage:	2.0 kV	
Inject Duration:	15 sec	
Separate:		
Stage 1 Primary Voltage:	4.2 kV	
Stage 1 Ramp Duration:	5.0 min	
Stage 2 Separation Voltage:	4.5 kV	
Stage 2 Start Time:	5.0 min	
Stage 2 Ramp Duration:	0.0 min	
Total Separation Duration:	85.0 min	
	llose	Help

Figure 6-37 Method Tab, Properties Dialog

Analysis Tab

View the analysis parameters used to produce the open sequence result. View the name of the parameter set used as well as all of the parameters and color calibration used to perform the analysis.

Select File > Properties > Analysis.

Figure 6-38 Analysis Tab, Properties Dialog

operties	
General Note Analysis	- I control control
Analysis	Alignment Consumables
Analysis Parameter Set	QC Method for Sequence Analysis
N Threshold:	0.00
Analysis Start Time:	0.0 minutes
Analysis End Time:	0.0 minutes
PCR Product:	No
Color Calibration:	DefaultColorCalibration
% Threshold:	40.00
Delay:	0.8 minutes
Signal to Noise:	7.00
Minimum Duration:	5.0 minutes
Pre-peak Reduction:	No
Detect Heterozygotes:	No
Base Range:	
Before Last Called Base:	
Average Peak Spacing:	
Height Ratio:	
Sensitivity:	
Automatic Alignment:	Yes
	Close Help
	<u>C</u> lose <u>H</u> elp

Alignment Tab

View the alignment template information and the alignment parameters used to create the alignment of the currently open result.

Select File > Properties > Alignment.

Figure 6-39 Alignment Tab, Properties Dialog

operties		
General Not	e Property Set	Method
Analysis	Alignment	Consumables
Alignment Template		
Template Name:	puc18dG	
Cutoff Accuracy:	98.0 %	
Allowed 'N':	2.0 %	
Alignment Parameters		
Match Score:	1.0	
Mismatch Score:	-2.0	
Insert Start:	-4.0	
Insert Extend:	-3.0	
Delete Start:	-4.0	
Delete Extend:	-3.0	
Find Match Substrings:	Yes	
Find Local Alignment:	No	
Left Edge Free:	Yes	
Right Edge Free:	Yes	
Min. Substring Length:	30	
	<u>C</u> lose	<u>H</u> elp

Note: This tab is visible only if an alignment has been performed on the sequence result.

Consumables Tab

View the status and identity of the capillary array, gel, and buffer information.

Select File > Properties > Consumables.

Figure 6-40 Consumables Tab, Properties Dialog

	Aligr Serial Number:	ment	Co	nsumables
apillary Array	Serial Number:	44000500		
		11020500	78	
otal Length	Part Number:	608087		
	of Capillary:	33.0 cm		
ength of Cap	illary to Detector:	30.0 cm		
nternal Diame	eter of Capillary:	75.0 µm		
lumber of Ru	ins:	2		
ays on Instru	ument:	0.1 days		
iel Part Num	ber:	608010		
iel Lot Numb	er:	S601091		
iel Algorithm	Туре:	0		
lours on Inst	rument:	2 hours		
uffer Part Nu	imber:	608012		
uffer Lot Nu	mber:	CEQ Sequ	encing Sep	aration Buffer

Set or Change Display Options

To set or change the graph data display options:

- 1. Open the Display Options dialog using one of these methods:
 - Select Tools > Display Options.
 - While viewing a graph display of sequence data, right-click the graph and select **Display Options**.

Figure 6-41 Display Options Dialog

Display ()pti <mark>ons</mark>		×
Cole Title	ors Dye Tra	12	
_ Title	e Property-		
Titl	le:		
Titl	le Color: 🔳		
Titl	le Font: Arial	•	
🔽 Savi	e Customized Setting	Save Setting Now	
	_		1
			1

- 2. Select the tab that identifies the type of display options you want to change. For details, refer to the sections:
 - Title Tab
 - X Axis Options Tab
 - Y Axis Options Tab
 - Colors Tab
 - Dye Traces Tab
 - Current Traces Tab

Note: For detailed descriptions while using this dialog, click Help.

Change the Display Colors

In addition to using the Colors tab in the Display Options dialog to set display colors, you can change colors used in raw data and heterozygote base displays, as described in the following topics.

Raw Data Colors Toolbar

To show the Raw Data Colors toolbar:

- 1. Select View > Toolbars.
- 2. Select the desired toolbar options.

Raw Data Traces

To change the colors of the raw data traces:

1. On the Raw Data Colors toolbar, click the trace color to change (**A**, **C**, **G**, or **T**). The Color dialog opens.

Figure 6-42 Color Dialog



- 2. Select the new color.
- 3. Click OK.

Heterozygote Display Color

The heterozygous bases are bold and red, by default. To change the color of the heterozygotes in the base sequence pane:

1. Select Tools > Heterozygotes Display Color.

The Color dialog opens.

- 2. Select a new color.
- 3. Click **OK**. The color of the ambiguity codes changes in the base sequence text.

Note: To search for heterozygotes, use the Heterozygote Backward Search icon 💾 and
the Heterozygote Forward Search icon 📕 in the Standard toolbar.

Pin Results

Several analyses (single or batch) may be necessary before a given result is satisfactory. Overwrite a previous result or produce additional result tabs using functions in the GenomeLab GeXP system.

- To overwrite a previous result, click the **Unpin** icon in the left corner of the result tab to unpin the result. A reanalysis of that sample overwrites the existing result.
- To produce a new results tab, click the **Pin** icon **Pin** to pin the result. Upon reanalysis, the system creates a new tab containing the reanalyzed results.

Perform a Batch Analysis

To analyze more than one sample simultaneously:

 Select Analysis > Batch Analysis. The Batch Analysis Selection dialog opens.

ample Data Sample Plate Resul	ts			
Atabase: CEQ DATABA Filter Start Date: 07-21-2005	NSE Enable <u>R</u> efresh			OK Cancel <u>H</u> elp
Name Sequence Test H04_0603020 Sequence Test G04_0603020 Sequence Test F04_0603020 Sequence Test D04_0603020 Sequence Test A04_0603020 Sequence Test A04_0603020 Sequence Test A03_0603012 Sequence Test A03_0603012 Sequence Test E03_0603012 Sequence Test E03_0603012 Sequence Test E03_0603012 Sequence Test B03_0603012 FA Test H02_060301210	03/02/06 02:10:26AM 03/02/06 02:10:24AM		Sequence Test A05_06030202 Sequence Test.B05_06030202 Sequence Test.D05_06030202 Sequence Test.D05_06030202 Sequence Test.E05_06030202 Sequence Test.F05_06030202 Sequence Test.G05_06030202	8P 8P 8P 8P 3P 8P

Figure 6-43 Batch Analysis Selection Dialog

- 2. Select a project name from the **Project** list at the bottom of the dialog.
- 3. Select the appropriate tab: **Sample Data** or **Sample Plate Results**.
- 4. Select **Enable** and enter a filtering start and stop date, if desired.
- 5. Highlight a sample name and click the right arrow to move the sample name to the selected list (on the right). Continue highlighting sample names and moving them to the selected list until you have selected all the samples for analysis.

6. Click OK.

The Analysis Parameters Editor dialog opens.

7. Select the appropriate analysis parameters or edit an existing parameter set and click **OK**. The batch processing begins and a window opens.

Figure 6-44 Sequence Analysis - Batch Processing

	atch																		
	4/06 16:34:41 Start Analysis 4/06 16:34:45 Sequence Test.	A02 0	C04341CK			I. D.		abarad											
	4/06 16:34:49 Sequence Test.																		
	4/06 16:34:53 Sequence Test.																		
	4/06 16:34:57 Sequence Test.																		
	4/06 16:35:01 Sequence Test.																		
	4/06 16:35:05 Sequence Test.																		
	4/06 16:35:09 Sequence Test.																		
	4/06 16:35:12 Sequence Test. 4/06 16:35:12 Analysis Comple		6042416	X Lompi	etea	1 : R	esult	stored											
#1 Z	4700 TO.33.12 Analysis Comple	atteu																	
1/24	1/06 16:35:11 Analyzed Data (Comput	ation Con	nleted	-	-	-			-	-	-	-			-	-	-	
	1/06 16:35:11 Base Seguence																		
	1/06 16:35:12 Base Sequence	: Conv	ert X Axis	to Base P	dum	04/24/06 16:35:11 Base Sequence: Compute Base Number Curve 04/24/06 16:35:12 Base Sequence: Convert X Axis to Base Numbers													
	1/06 16:35:12 Base Sequence		ute Initial	Base See	quen	nce													
4/24	1/06 16:35:12 Base Sequence	: Comp	ute Initial ute Final	Base See Base Seg	quen uen	nce ce													
1/24	1/06 16:35:12 Base Sequence 1/06 16:35:12 Base Sequence	: Comp : Comp	ute Initia ute Final ute Initia	Base See Base Seq Confiden	quen uen	nce ce													
1/24 1/24	1/06 16:35:12 Base Sequence	Comp Comp Refin	ute Initia ute Final ute Initia e Multiple	Base See Base Seq Confiden ts	quen luent ice V	nce ce /alue	es												
4/24 4/24 4/24 4/24	1/06 16:35:12 Base Sequence 1/06 16:35:12 Base Sequence 1/06 16:35:12 Base Sequence	: Comp : Comp : Refin : Comp	ute Initia ute Final ute Initia e Multiple ute Final	Base See Base Seq Confiden ts Confidence	quen luen ice V :ce V	nce ce /alue	es												
4/24 4/24 4/24 4/24	I/06 16:35:12 Base Sequence	: Comp : Comp : Refin : Comp	ute Initia ute Final ute Initia e Multiple ute Final	Base See Base Seq Confiden ts Confidenc	quen luen ice V :ce V	nce ce /alue	es												-
4/24 4/24 4/24 4/24	I/06 16:35:12 Base Sequence	: Comp : Comp : Refin : Comp : Perfo	ute Initia ute Final ute Initia e Multiple ute Final	Base See Base Seq Confiden ts Confidenc	quen luend lice V ce V	nce ce /alue	25 5	50	Tot					200	Tot				1
/24	V06 16:35:12 Base Sequence	: Comp : Comp : Refin : Comp	ute Initia ute Final ute Initia e Multiple ute Final rm Probal	Base See Base Seq Confiden ts Confidenc pility Analy	quen luen ice V :ce V	nce ce /alue	25 5	A 50 acc3		м	1	D	A	200 accy	Tot Err	м	1	D	A
/24	1/06 16:35:12 Base Sequence	: Comp : Comp : Refin : Comp : Perfo : Perfo Cap Pos	ute Initia ute Final ute Initia e Multiple ute Final rm Probal	Base Seq Base Seq Confiden ts Confidenco ility Analy Tot	quen luend lice V ce V	nce ce /alue	25 5			м	1	D	A	1		м	1	D	A
/24	VI06 16:35:12 Base Sequence Results Name Sequence Test A03_06042416KN	Comp Comp Comp Perfo Cap Pos	ute Initia ute Final ute Initia e Multiple ute Final rm Probal	Base Seq Base Seq Confiden ts Confidenco ility Analy Tot	quen luend lice V ce V	nce ce /alue	25 5			м	1	D	A	1		м	1	D	A
/24	Results Results Results Base Sequence M06 16:35:12 Base Sequence M06 16:35:12 Base Sequence M06 16:35:12 Base Sequence None Results Name Sequence Test A03_06042416KN Sequence Test A03_06042416KO Sequence Test A03_06042416KO	Comp Comp Comp Comp Perfo Pos A B	ute Initia ute Final ute Initia e Multiple ute Final rm Probal	Base Seq Base Seq Confiden ts Confidenco ility Analy Tot	quen luend lice V ce V	nce ce /alue	25 5			м	1	D	A	1		м	1	D	A
/24	Results Results Results Base Sequence V/06 16:35:12 Base Sequence Results Name Sequence Test.A03_06042416KO Sequence Test.030_06042416KO	Comp Comp Comp Comp Perfo Perfo A B C	ute Initia ute Final ute Initia e Multiple ute Final rm Probal	Base Seq Base Seq Confiden ts Confidenco ility Analy Tot	quen luend lice V ce V	nce ce /alue	25 5			M	1	D	A	1		M	1	D	A
/24	V/06 16:35:12 Base Sequence Results Name Sequence Test D03_06042416KO Sequence Test D03_06042416KO Sequence Test D03_06042416KO Sequence Test D03_06042416KO	Cap Pos A C D	ute Initia ute Final ute Initia e Multiple ute Final rm Probal	Base Seq Base Seq Confiden ts Confidenco ility Analy Tot	quen luend lice V ce V	nce ce /alue	25 5			M	1	D	A	1		M	1	D	A
/24	Results Results Results Base Sequence No 16 15:35:12 Base Sequence Name Base Sequence Base Sequence Sequence Test A03_06042416KN Sequence Test:003_06042416KA Sequence Test:003_06042416KA Sequence Test:003_06042416KA Sequence Test:033_06042416KA Sequence Test:033_06042416KA	Comp Comp Comp Comp Perfo Perfo A B C	ute Initia ute Final ute Initia e Multiple ute Final rm Probal	Base Seq Base Seq Confiden ts Confidenco ility Analy Tot	quen luend lice V ce V	nce ce /alue	25 5			M	1	D	A	1		M	1	D	A
1/24 1/24 1/24	V/06 16:35:12 Base Sequence Results Name Sequence Test A03_06042416KN Sequence Test D03_06042416KA Sequence Test D03_06042416KA Sequence Test D03_06042416KA Sequence Test D03_06042416KA Sequence Test D03_06042416KA	Comp Refin Perfo Perfo A B C D E F	ute Initia ute Final ute Initia e Multiple ute Final rm Probal	Base Seq Base Seq Confiden ts Confidenco ility Analy Tot	quen luend lice V ce V	nce ce /alue	25 5			M		D	A	1		M		D	A
1/24 1/24 1/24	Nofe 16:35:12 Base Sequence 106 16:30:100:124:164 Sequence Test 103:00:00:124:164 Sequence Test 103:00:00:241:164 Sequence Test 03:00:00:241:164	Comp Refin Perfo Perfo A B C D E F G	ute Initia ute Final ute Initia e Multiple ute Final rm Probal	Base Seq Base Seq Confiden ts Confidenco ility Analy Tot	quen luend lice V ce V	nce ce /alue	25 5			M			A	1		M			A
/24	V/06 16:35:12 Base Sequence Results Name Sequence Test A03_06042416KN Sequence Test D03_06042416KA Sequence Test D03_06042416KA Sequence Test D03_06042416KA Sequence Test D03_06042416KA Sequence Test D03_06042416KA	Comp Refin Perfo Perfo A B C D E F G	ute Initia ute Final ute Initia e Multiple ute Final rm Probal	Base Seq Base Seq Confiden ts Confidenco ility Analy Tot	quen luend lice V ce V	nce ce /alue	25 5			M			A	1		M 			A

Reanalyze a Batch

To reanalyze a batch:

- 1. After performing a batch analysis, select **Analysis > Reanalyze Batch**.
- 2. Select a new parameter set or edit the currently selected set from the Working Analysis Parameters dialog to obtain different results.
- 3. Click **OK** to start the reanalysis.

Note: To keep the results from the previous analysis, pin the results tab by clicking the **Pin** icon →. Otherwise, the system overwrites the results with any subsequent reanalyses.

View the Selected Batch Result

To view the selected batch result after performing a batch analysis:

- 1. Click the desired sample in the upper pane of the batch analysis window.
- 2. Select Analysis > View Selected Batch Sample Result.

Skip the Current Sample Analysis in a Batch

To skip the current sample during a batch analysis, select **Analysis > Skip Current Sample**.

Skip the Current Sample Set Analysis in a Batch

To skip the sample set during a batch analysis, select **Analysis** > **Skip Current Sample Set**. **Skip the Current Sample Plate Analysis in a Batch**

To skip the current sample plate during a batch analysis, select **Analysis** > **Skip Current Sample Plate**.

Restore the Original Sequence Data

With a results file open, select **Analysis** > **Restore Original Base Sequence** to return the result data to its original state, losing all edits.

Using Compare Mode

Select up to 15 results to compare against the open result, enabling the comparison of a maximum of 16 sequence results at one time, while in the Sequence Analysis module.

If you select more than 16 results from the Compare dialog, a message window opens to show the following warning: The maximum number of results allowed for comparison with the open result is 15. Please select again.

Compare Sequence Results

To compare sequence results:

- 1. Launch the Sequence Analysis module.
- 2. Select File > Open.
- 3. Select the Sequence Results tab and select one sequence result.
- 4. Click **OK** to open the result.
- 5. Click **Compare** on the Sample View toolbar:
- 6. Select up to 15 results.
- 7. Click OK.

The system processes all 15 results and the original result, and shows the results on the screen.

Note: The originally opened sequence result shows in the top pane.

To enable Compare Synch, select **Tools > Compare Synch**.

Zoom the Results

Click the **Zoom Mode** icon and zoom in on a region in the original sequence result and the other 15 results zoom into the same region.

Select **Tools** > **Unzoom All** and all 16 results zoom out so you can view the entire result.

Pan the Results

To pan results, click the **Pan Mode** icon

- Pan through the data in the original sequence and all 16 results pan together.
- Pan through the data in a result other than the original one and all 16 results pan together.
- Use the horizontal scroll bar to move the data along the X-axis. The data panes scroll synchronously.

Align the Results

To align results, click the Align Mode icon

- Select a point in the original result.
- Select points in the other results you want to align with the point in the original result.
- To access the Display Options dialog, double-click in the original result pane. If you choose the X-axis or the Y-axis range, the range changes in all of the panes.
- To disable **Compare Synch**, select **Tools > Compare Synch**.
- On the Sequence Analysis module Tools menu (refer to the section: **Tools** Menu), select **Zoom**, **Pan**, and **Align**, and the panes function independently of each other.
- To close the Compare tab, select **File > Close Tab**.

Sequence Result Report

The sequence result report includes additional information when certain Sample Elements are selected in the Report Format dialog. The following table describes what each sample element includes.

Option	Description
Header	Contains summary information for the sample, such as sample name, position, method under which it was run, sample plate name, analysis parameter set name, and capillary serial number. In addition, the sample note, property set, and consumables information associated with the sample will be printed.
	Note: This is the same information found on the General, Note, Property Set, and Consumables tabs in the Properties dialog.
Raw Data	Includes the unanalyzed data from the sample run.
Result Data	Shows data that has undergone final processing to produce result data. If the data has been analyzed using a sequence analysis parameter set, the data with each peak assigned a base is printed.
Result Output	Shows the final output in text form. If the data has been analyzed using a sequence analysis parameter set, the called sequence will be printed in text form. This section of the report corresponds to the base sequence text.
Current	Includes the trace associated with the current output of each capillary or the total current of the sample set run.
Voltage	Includes the trace associated with the voltage output of the system during the sample set run.
Analysis Log	Contains all of the steps used to perform the base-calling routine on the raw data.
Run Log	Contains all of the actions performed and instrument messages for the sample run.
Method Summary	Contains the details of the method used to perform the sample run. This is the information found on the Method tab of the Properties dialog.
Analysis Parameters	Shows the parameters used to produce result data and output for a given sample, including heterozygote detection and alignment. If the data has been analyzed using a sequence analysis parameter set, the sequence analysis parameter set will be printed. In addition, the alignment template and the alignment parameters information will be included in this section, when applicable.
	This information is available on the Analysis and Alignment tabs of the Properties dialog. It includes alignment information only if an alignment was performed.

 Table 6-14 Sequence Result Report Options

Option	Description
Quality Parameters	Includes the parameters that indicate that any given base is an A , C , G , or T along with a value to indicate the likelihood that the call is correct.
Trimming Log	Shows the parameters used in quality-based and sequence-based trimming. It includes the original sequence length, length after quality-based sequencing, length after sequence-based trimming, vectors trimmed, and base sequence in its original and trimmed forms. For sequence-based trims, an <i>X</i> replaces the trimmed bases that are located further than that specified in the Distance from End setting or if they are an internal match.
Alignment Results	Shows the text results of the alignment between a template sequence and a sequence result. The top row of text is the base number, the second line is the template sequence, the third line is the sample sequence, and the last line is the consensus.
Alignment Accuracy	Shows the statistical analysis of the accuracy of the sample sequence. This section groups the errors in the alignment into 50-base regions and shows the number and type of error for each region.

 Table 6-14 Sequence Result Report Options (continued)

- To define the report format and content, select **File > Report Format**.
- To print the report without previewing or formatting prior to printing, select **File > Print Report**.
- To view the report, select **File > Print Preview**.

The following example shows a sample report.

Figure 6-45 Sequence Results Report

Z	Project : Sequence Sample : testseq.A04_ Result : testseq.A04_		System : CEQ 8080	Operator : Instrument : CEQ 8080 (Ver. 7.0.26
Sequer	nce Results: testseq./	\04_03030518M	IG	
Created:	03/05/03 20:33:42	Project: System:	Default CEQ 8080 [Rev 0]	
Modified:	03/05/03 20:33:42	Project: System:	Default CEQ 8080 [Rev 0]	
Sample Nar Sample Plat Sample Plat Sample Pos Method:	te: te Barcode:	testseq.A04 testSeqLon 234567891 A04 LFR-a		
Instrument:		CEQ 8080	(Ver. 7.0.26)	
Analysis Pa	rameters:	DefaultSeq	uenceAnalysisParameters	
Note:				
Properties:				
Consumable	es:			
Capillary Ar Total Length Length of C:		608087 33.0 cm 30.0 cm 75.0 µm 9 2.0 days		
Gel Part Nu Gel Lot Nun Gel Algorith Hours on Ins	nber: m Type:	391438 CEQ Seque 0 49 hours	encing Gel	
Buffer Part I Buffer Lot N		608012 CEQ Seque	encing Separation Buffer	
	13 10:27:43			Page

Show Quality Parameters in a Report

To show the quality parameters in a report:

- 1. Select File > Report Format.
- 2. In the Sample Elements section, select Quality Parameters .
- 3. Click OK.

Figure 6-46 Report Format Dialog

Report Forma	at	×
Printer		
<u>N</u> ame:	Microsoft Office Document Image Writer	Properties
Status: Type: Where: Comment:	Ready Microsoft Office Document Image Writer Driver Microsoft Document Imaging Writer Port:	Colo <u>r</u> s
Page Layou	ič icape ments ar Analysis Log	ОК
E Result	Data Method Summary Output Analysis Parameters at I Quality Parameters	Cancel Help Options

- To view the quality parameters of an analyzed data set prior to printing, select File > Print Preview.
- To exit the **Print Preview** option, click **Close**.
- To print the quality parameters for that data set, select **File > Print Report**. The system prints the Quality Values as numerical values.

Figure 6-47 Quality	Parameters Report
---------------------	-------------------

Project : Default Sample : A.3.F01_01040516DS Result : A.3.F01_01102909QF Quality Parameters: A.3.F01_011029090							System : CEQ 2000XL 9QF				Ope	erator : Manual analysis Instrument : Unit07
Number	G	A 0			0	0.96	12	Migration 21.73	32	No	No	
2 3 4	T C C	0		0 0	250 0 0	0.86 0.82 0.82	12 10 10	21.84 21.84 21.89	47 68 81	No No No	No No No	
5 6 7	A	229 248 253	0 4 1	0 0 0	1 0 0	0.82 0.97 0.98	10 18 20	21.96 22.02 22.09	93 110 132	No No No	No No No	
8 9 10	G C C	2 0 1	253	252 0 0	0 0 0	0.99 1.00 1.00	20 38 31	22.14 22.19 22.26	154 173 192	No No No	No No No	
11 12 13	A G	252 3 254	0	0 251 0	0 0	0.98 1.00 1.00	18 36 36	22.34 22.39 22.48	207 229 250	No No No	No No No	
14 15	G A	0 254	1 0	253 0	0 0 0	1.00 1.00	36 36	22.54 22.63	272 295	No No	No No	
16 17 18	A	249 211 251	1 0 0	0 4 0	0 0 0	$1.00 \\ 1.00 \\ 1.00$	47 47 47	22.70 22.75 22.81	314 331 347	No No No	No No No	
19 20 21		0 252 248	1 0 0	251 0 2	0 0 0	1.00 1.00 0.99	47 38 22	22.85 22.93 23.00	366 384 403	No No No	No No No	
22 23 24	A	233 244 0	0	4 1 0	0 0 4	0.99 0.95 0.92	22 14 12	23.06	421 438 451	No No No	No No No	
25 26 27	Ť G	0 0 254	0	2 253 0	252 0 0	0.97 0.98 0.97	17 17 17 17	23.26 23.29 23.37	473 495 513	No No No	No No No	

The following table describes the categories on the quality parameters report.

Field	Description
Number	Number of the called base
Base	Single-letter designation of the called base (A , C , G , T , or other IUB code)
Α	Base identity score assigned by the base-caller (0 to 255)
С	Base identity score assigned by the base-caller (0 to 255)
G	Base identity score assigned by the base-caller (0 to 255)
Т	Base identity score assigned by the base-caller (0 to 255)
Call Score	Calculated probability of correctness indicated on a linear scale (0.00 to 1.00)
Quality Value	Probability of correctness presented on a logarithmic scale (1 to 100)

Table 6-15 Quality Parameters Report Categories	
---	--

Field	Description
Migration	Actual migration time (in minutes) in the raw data corresponding to the called bases
Data Point	Actual number of the data points in the analyzed data corresponding to the called bases
Insert	Indicates whether a base has been added manually to the base sequence
Edit	Indicates whether the base call has been changed manually

Table 6-15 Quality Parameters Report Categories (continued)

Magnify (Zoom) the Data

To magnify data:

- 1. Click the upper left point of the area to be magnified.
- 2. Drag the mouse cursor down to the lower right point of the area and then release the mouse button.
- 3. Repeat this procedure to increase magnification.

Note: Use the Unzoom icon 🔍 or Unzoom All icon 🔍 to zoom out.

Pan the Data

To pan back and forth in the data pane:

- 1. Select **Tools > Pan**.
- 2. Click anywhere in the raw or analyzed data panes and drag the mouse cursor left or right to move the display.
- 3. To deactivate the pan mode, select **Tools** > **Zoom** or click **Autoscale**.

Synchronize the Result Data with the Result Output

To synchronize the analyzed data with the base sequence:

- 1. Access the Sequence Analysis module.
- 2. Open the desired analyzed data.
- 3. Select Tools > Base Synch (Peak Synch) or click the Synch icon
- 4. Select a base or range of bases in the base sequence pane.

The corresponding peaks show between two hairline markers in sequence analysis.

Align Bases and Peaks

To align the bases in the base sequence pane with the peaks in the analyzed data pane:

- Select File > Open.
 The Open dialog opens.
- 2. Select the Results tab, highlight the desired sequence results, then click **OK**.
- 3. Click the **Align Mode** icon **to** visually align the peak with the base call.
- 4. Click the apex of the peak for which you want to view the alignment. (A hairline cursor shows through the apex of the peak to its corresponding base.)
- 5. Click Zoom, Pan, or Edit to exit Align mode.

Specify the Base Call Location

To specify the base call location:

- 1. Open a sequence results file.
- 2. Select **Tools** > **Bases on Top** to toggle the bases from the top to the bottom of the analyzed data pane or vice versa.

Use Audio Enable

To enable the audio feature:

- 1. With a sequence results file open, select **Edit > Audio Enable**.
- 2. Enter text into the base sequence pane.

Note: To enter text in the base sequence pane, Edit mode must be enabled.

The letter bases are announced as they are typed.

Use Audio Playback

To enable the audio playback feature:

- 1. With a sequence results file open, highlight a series of base sequence text.
- 2. Select Edit > Audio Playback.

Note: To use this feature, Edit mode must be enabled.

The highlighted base sequence text is announced.

View Color Calibrations

To view a color calibration for a sequence result:

- 1. Select **File > Open**. the Open dialog opens.
- 2. Select the Sequence Results tab, highlight the desired result name, then click OK.
- 3. Select **View > Parameters Used to Compute Sequence**. The Parameters Used dialog opens.
- 4. Click Color.
 - To view the color calibration prior to the run, select **Initial Values**.
 - To view the color calibration after the run, select Final Values.

Note: This is a read-only dialog. To make changes to the color calibrations, select **Analysis > Working Analysis Parameters > Edit > Color**.

• To add a successful color calibration to the list of available color calibrations, click **Save As** in the Color Calibration Editor dialog. Enter the desired name, then click **OK**.

Compute a New Color Calibration

To compute a new color calibration:

1. Select **Analysis > Working Analysis Parameters**. The Working Parameters dialog opens.

2. Click Edit.

The Sequence Analysis Parameters Editor dialog opens.

Note: If the 21 CFR Part 11 tools are in use, then the edit function is only available to users with Write access.

- 3. Click Color.
- 4. Select the **Compute new color matrix** check box.
- 5. Select the **Use Final Values as Initial Values** check box to use the final values for the initial values for the computation (recommended if the peak resolution is good).

Export a Sequence Result

To export from the Sequence Analysis module:

- 1. Launch the Sequence Analysis module and open a sequence result.
- 2. Select File > Export.

The Export dialog opens.

3. Select a target folder and file name.

Note: Do not use special characters (*I* : * ? \ " < > |) in the file name.

4. Select the desired format in the **Save as type** list.

Figure 6-48 Sequence Analysis Export Dialog

Export			? ×
Sample Elements Header Raw Data Result Data Quality Parameters Alignment Results Alignment Accuracy Options	Save in: 🗀	Export	⊨ 🗈 📸 ⊞•
Remove CEQ Tracking Suffix Resolve Filename Conflicts Apply Trimming	J File <u>n</u> ame: Save as <u>t</u> ype:	Sequence Test.A03_06042416KN SCF (Ver 3.00) (*.scf) SCF (Ver 3.00) (*.scf) SCF (Ver 2.10) (*.scf) Text (Tab Delimited) (*.txt) SEQ (Sequence Text only) (*.seq) FASTA (FASTA and QUAL) (*.fasta) PHRED (PHRED and SCF) (*.scf.phd.1) ESD (*.scf) CEQ (*.ccf)	Export Cancel

5. Click Export.

Sequence Analysis Module Export File Types

The following table describes the available export file types and options in the Sequence Analysis module.

Option	Description
SCF (.scf)	Use the standard chromatogram format (SCF) to store analyzed DNA sequence data for a single sample. The format describes both public and private data sections. The well-defined public data section contains the trace data points, called sequence, positions of each called base within the trace data, and call scores for each called base. It also includes a public comment section. The private section may store other information that most software packages ignore.
	The GenomeLab GeXP system uses the private data section to store sample data (including raw data along with current and voltage data). You can store sample data from either sequencing or fragment analysis samples. The notes and property sets for the sample are stored in the comment section. The GenomeLab System software supports SCF versions 2.1 and 3.0. (You would use this format, for instance, to export analyzed sequence data into Lasergene's SeqMan from DNASTAR). Third-party packages cannot currently view raw data, current, or voltage. However, custom applications can be written to view the data.
	Use the SCF format to transfer data from instrument to instrument, or to import data from third-party software into the Sequence Analysis module. Using SCF to exchange data between instruments removes information, such as sample plate name and capillary array serial number. To maintain exact copies of data including header information, use the CEQ format and import and export these data using the Data Manager module.
Text (Tab- Delimited) (.txt)	The tab-delimited format is valid for raw data, sequence results and fragment results. Select the Raw Data check box to include raw data, as well as current and voltage data. Select the Results Data and Results Output check boxes to include the sequence results. The format expresses the data points as text values that can be viewed in any text editor or in Excel.
SEQ (Sequence Text only) (.seq)	The SEQ format only applies to the sequence results portion (such as text bases) of analyzed sequence data. This format is used, for instance, to export data into Lasergene's SeqMan from DNASTAR. Selecting the SEQ format exports the result output (base sequence) automatically if the sample has been analyzed with a sequence analysis parameter set. If the sample has been analyzed with a fragment analysis parameter set, nothing is exported.

 Table 6-16 Sequence Analysis Module Export Options

Option	Description
FASTA (.fasta)	The FASTA format only applies to the sequence results portion (such as text bases) of analyzed data, and contains a comment line that is used to identify the data. Along with the text file, a quality values file is also generated (*.FASTA.QUAL) which lists the quality value for each base. (Quality values correspond to -10*log10[error rate]). The range for the quality values is 1 to 99. Edited and inserted bases have a value of 99. This format is used, for instance, to export data into PHRAP.
	For instance, in PHRED, a quality value of 10 means 1 error in 10. A quality value of 20 means an error rate of 1 error in 100. A quality value of 30 means an error rate of 1 error in 1,000. A quality value of 40 means an error rate of 1 error in 10,000, etc. Normally PHRED would export these quality values into PHRAP. In the case of exporting from the GenomeLab GeXP system, we bypass PHRED and export directly into PHRAP.
	Selecting the FASTA format exports the result output (base sequence) and the quality parameters automatically if the sample has been analyzed with a sequence analysis parameter set. If the sample has been analyzed with a fragment analysis parameter set, nothing is exported.
PHRED (.scf.phd.1)	The PHRED format produces two files: a PHRED file and an SCF file. A PHRED file is a text file composed of a SEQUENCE section containing a COMMENT and DNA section. The SEQUENCE section also contains the name of the analyzed result that was stored.
	The COMMENT section contains the file name of the associated SCF file (CHROMAT_FILE) that was produced along with the PHRED file. The values for ABI_THUMBPRINT and PHRED_VERSION are fixed text N/A . The values for CALL_METHOD and QUALITY_LEVELS are fixed as CEQ SEQUENCING and 99 , respectively for the GenomeLab GeXP system. The time stamp is the current time at the time of export.
	The DNA section is composed of the base-call (always lowercase), PHRED quality value, and the peak index (data point) of the analyzed data contained within the associated SCF file. Values are separated by spaces.
	Selecting the PHRED format exports the result data, result output (base sequence), and quality parameters automatically if the sample has been analyzed with a sequence analysis parameter set. If the sample has been analyzed with a fragment analysis parameter set, nothing is exported.

Table 6-16 Sequence Analysis Module Export Options (continued)

Option	Description		
ESD (.esd)	The electropherogram sample data (ESD) format exports raw data from the GenomeLab GeXP system to a third-party software package for analysis. This option exports only raw data and does not include any other information.		
CEQ (.cq*)	Selecting the CEQ format, the format native to our database, exports sample data, sequence or fragment results, sample plate results, sequence or fragment analysis parameters, sample plates, methods, locus tags (fragment data only), standards (fragment data only), and optical scan data. The sample elements are automatically selected in the Export dialog, which would export all of the associated information listed above. This format essentially takes a snapshot of the file and can retain all of the information belonging to that file.		
	This format is available only to import data into the Data Manager module. This format can be used to export data from the Sequence Analysis, Fragment Analysis, and Data Manager modules.		
	When exporting as CEQ data, the various data types are exported to files with the following extensions:		
	 Sequence analysis parameter = CQA 		
	 Sequence investigator = CQC 		
	 Fragment size standard = CQD 		
	 Fragment analysis parameter = CQF 		
	 Galvo scan cata = CQG 		
	 Fragment locus tags = CQL 		
	• Method = CQM		
	 SNP locus tag = CQN 		
	Sequence result = CQR		
	Sample data = CQS		
	• Sample table = CQT		
	 Fragment result = CQU 		
	Sample table result = CQX		

Table 6-16 Sequence Analysis Module Export Options (continued)

Option	Description
Remove CEQ Tracking Suffix	You can automatically remove the plate position coordinates (*.A01, *.B02, etc.) and the time/date stamp from the sample name which is normally generated by the GenomeLab System software on export. To do so, select the Remove CEQ Tracking Suffix check box.
Resolve Filename Conflicts	If the system encounters the same sample name at the destination, it usually overwrites the name (if you choose to remove the plate position coordinates and the time/date stamp which ensures a unique name). If you select the Resolve Filename Conflicts check box, the system increments the sample names on export when it encounters duplicate names. For example, if the system encounters the duplicate sample name of <i>Fred</i> on export, it renames the file exported to <i>Fred_1</i> .
Apply Trimming	Selecting this option removes the trims from the sequence before you export it and replaces internal trims with the letter <i>X</i> .

Table 6-16 Sequence Analysis Module Export Options (continued)

Customize a File Name Extension

To supply your own custom file name extension (or none at all) to exported files, modify the CEQ.ini file as follows:

Note: If there is no Export section in the CEQ.ini file, export any sample file to generate the Export section, then modify as needed.

Find the section (Export) and add/modify the appropriate entry.

- TXT_EXT for Text files, default is .txt
- SEQ_EXT for SEQ files, default is .seq
- FASTA_EXT for FASTA files, default is .fasta
- SCF_EXT for SCF files, default is .scf
- PHD_EXT for PHRED files, default is .scf.phd.1

To have no file extension added, set the entry to nothing (make sure there are no spaces after the equal sign).

• SCF EXT= Example Output FileName: SpaSample

To use a unique file extension, add or modify the entry with the desired file extension (do not add the leading dot (.), the system will add it).

Sequence Analysis Module

- SCF EXT=scf v3
- Output FileName: SpaSample.scf v3

To hide an entry and make the system use the default, either delete or place a semicolon (;) before it.

- SCF EXT=SCFV3
- Output FileName: SpaSample.scf

Export Data to a Third-Party Package

Note: Verify that the third-party analysis package accepts the data transferred using a command-line, and that it is compatible with the file types provided by the GenomeLab System software before attempting to perform this procedure.

To export sequence data to a third-party analysis package:

- Select File > Open. The Open dialog opens.
- 2. Select the appropriate tab, highlight the desired data, then click **OK**.
- 3. Select Analysis > Third Party Analysis Setup.
- 4. In the Third Party Analysis Setup dialog:
 - a. Specify the path and executable file of the third-party analysis package.
 - b. Select the appropriate **Export Data Format**.
 - c. Click **OK**.
- 5. Select **Analysis** > **Third-Party Analysis**. The Export dialog opens.
- 6. Enter the file name, then click **OK**. The system exports the data and launches the third-party package, showing the exported data.

This chapter provides an overview of the Sequence Investigator module, including its menu options, toolbars, and dialogs. It also shows you how to use this module to investigate and compare sequence analysis results.

Overview

Use the Sequence Investigator module to compare sequences to known reference sequences. For each comparison, you can use one or two new sequence results, in either orientation, to form a consensus for the comparison. The software automatically determines and matches the orientation of the sequences, forms the consensus, aligns the consensus with the reference sequence, and provides a detailed report of the differences.

The Sequence Investigator module provides a number of tools you can use to inspect and edit the data before generating the final report. The navigator toolbar allows you to jump to points of interest, from specific base sequences to a variety of discrepancies in the aligned data set. The discrepancy map provides an overview of the coverage of the reference sequence by the sequences and indicates the locations of the discrepancies. Together, these alignment, inspection, editing, and reporting features make Sequence Investigator module an ideal tool for identifying mutations or polymorphisms in DNA.

Main Window

The main window for the Sequence Investigator module has the following sections.

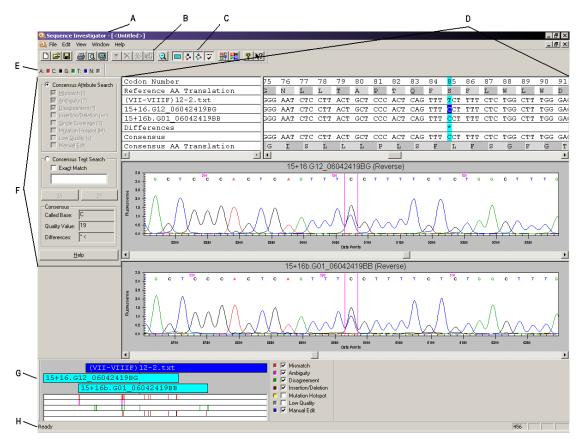


Figure 7-1 Main Window, Sequence Investigator Module

ltem	Description
А	Title Bar: Shows the module name, active document name, and standard
	Minimize, Maximize, and Close icons at the top right of the window.
В	Menu Bar: Refer to the section: Menu Bar.
С	Standard Toolbar: Refer to the section: Toolbars.
D	 Display Area: Use this area to graphically show the specific window or windows associated with the current task. This window has the following panes: Alignment Panes: Shows the text alignment results, including the base and codon numbering, reference and consensus amino acid translations, reference sequence, sample sequences, consensus sequence, and difference line. Trace Data Panes: Shows trace data and base sequence results for the selected sample sequences.

ltem	Description
E	Dye Colors Toolbar: Use these user-defined colors in the trace data view and the bases in the text view when the text coloring is activated.
F	Navigator Toolbar: Offers search and navigation tools within an alignment.
G	Discrepancy Map Toolbar: Shows color-coded locations of any type of attributes selected. You may view the coverage of the reference and sample sequences.
Н	Status Bar: Shows information concerning the current selection, including the position of the currently highlighted text.

Menu Bar

The following example shows the menu bar for the Sequence Investigator module.

Figure 7-2 Sequence Investigator Module Menu Bar

```
<u>File Edit View Window H</u>elp
```

The following topics describe each of these menus and their options.

File Menu

Click **File** to show the following menu.

Figure 7-3 File Menu, Sequence Investigator Module

File	
New	Ctrl+N
Open	Ctrl+O
Close	
Save	Ctrl+S
Save As	Ctrl+A
Export	
Preferences	Ctrl+F
Properties	
Report Format	
Print Preview	
Print	Ctrl+P
Print Desktop	
0 C:\CEQ System\Results\Investigate2.cqc	
Exit	

Option	Description
New	Prompts for a reference file from a Windows folder or disk, and then prompts for one or two sequence results from the current working database.
Open	Open an existing compared sequence result.
Close	Close the document.
Save	Save the current data to the same location with the same file name.
Save As	Save the current data with a newly assigned file name in a newly selected location.
Export	Export the current document as a text (*.txt) or a protein file (*.pro).
Preferences	Set the preferences for the Sequence Investigator module.
Properties	Show the properties of the current document.
Report Format	Set the format options used to print reports and adjust the printer settings.
Print Preview	Open a print preview of the active document of the active sample plate. A toolbar at the top of the window provides options for navigation, viewing, and printing the display. To restore the default screen, press Esc or click Close .
Print	Print the active document as specified in the Report Format dialog.
Print Desktop	Print the active desktop area as specified in the Preferences dialog.
Recent Objects	Use a shortcut to open previously opened documents.
Exit	Close the module.

Edit Menu

Click **Edit** to show the following menu.

Edit Insert Insert Delete Delete Replace > Trim Ctrl+T Search > Restore To Original Ctrl+R Quality Threshold... Ctrl+Q Flip Traces

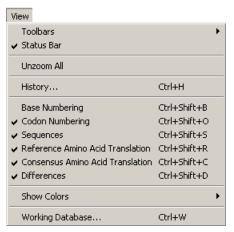
Figure 7-4 Edit Menu, Sequence Investigator Module

Option	Description
Insert	Insert a space before the currently selected position.
Delete	Delete the selected position.
Replace	Replace the highlighted position with a selected IUB code.
Trim	Remove contiguous selected bases that contain an end base in the sample sequence base-call text.
Search	Searche forward and backward for the attributes or text specified in the navigator toolbar.
Restore To Original	Restore the compared sequence result to its original state (removing all changes).
Quality Threshold	Change the quality threshold below which bases in the consensus are identified as having low quality.
Flip Traces	Move the sequence trace shown in the top trace pane and exchange its position with the bottom trace.

View Menu

Click **View** to show the following menu.

Note: A check mark next to an option indicates that the option is enabled.



Option	Description
Toolbars	Select which toolbars to show. Refer to the section: Toolbars.
Status Bar	Toggle to show or hide the status bar.
Unzoom All	Show each sequence in its entirety.
History	Open the history log.
Base Numbering	Toggle to show or hide base numbering.
Codon Numbering	Toggle to show or hide codon numbering.
Sequences	Toggle to show or hide the sequences.
Reference Amino Acid Translation	Toggle to show or hide the reference amino acid translation.
Consensus Amino Acid Translation	Toggle to show or hide the consensus amino acid translation.
Differences	Toggle to show or hide the sequence differences.
Show Colors	Toggle to show or hide the color used for text and background.
Working Database	Show the name of the database in use.

Window Menu

Click **Window** to show the following menu.

Figure 7-6 Window Menu

Window
Cascade
Tile Horizontally
Tile Vertically
Close All
Arrange Icons
↓ 1 <lintitled></lintitled>

Option	Description
Cascade	Cascade the open windows.
Tile Horizontally	Tile the windows in a horizontal orientation.
Tile Vertically	Tile the windows in a vertical orientation.
Close All	Close any currently active windows.
Arrange Icons	Automatically arrange the icons.

Help Menu

Click **Help** to show the following menu.

Figure 7-7 Help Menu

F	<u>l</u> elp	
	Help <u>T</u> opics	
Using Help About GenomeLab System		sing Help
		bout GenomeLab System
	S	CIEX Home Page

Option	Description
Help Topics	Select this option to show the Help topics. By default, the Contents show in the navigation pane on the left and the introductory topic shows in the topic pane on the right. Use the Contents to select and print specific Help topics. In the navigation pane, use the Search and Index tabs to search for information by topic, index entry, and/or keyword.
Using Help	Select this option to open the Contents window on how to use the Windows Help system.

Option	Description
About GenomeLab System	Select this option to access software and system information.
SCIEX Home Page	Select this option to access the SCIEX Home Page on the Internet.

Toolbars

The toolbars for the Sequence Investigator module provide quick and easy access to commonly used menu items. Each toolbar contains icons that correspond to commonly used menu items. Click the icon to execute the function. By default, all the toolbars appear when you first open the module.

Standard Toolbar

The Sequence Investigator module's Standard toolbar contains icons that correspond to common menu options.

Figure 7-8 Standard Toolbar, Sequence Investigator Module



lcon	Description
D	New: Prompts for a reference file from a Windows folder or disk, then prompts for one or two sequence results from the current working database.
2	Open: Open an existing compared sequence result.
	Save: Save the current data to the same location with the same file name.
5	Print: Print the active document as specified in the Report Format dialog.
Q	Print Preview: Open a print preview of the active document of the active sample plate. A toolbar at the top of the window provides options for navigation, viewing, and printing the display. To restore the default screen, press Esc or click Close .
9	Print Desktop: Print the active desktop area as specified in the Preferences dialog.

lcon	Description			
₹	Insert: Insert a space before the currently selected position.			
×	Delete: Delete the selected position.			
*	Trim: Remove contiguous selected bases that contain an end base in the sample sequence base-call text.			
5	Restore to Original: Restore the compared sequence result to its original state (removing all changes).			
Q	Unzoom all: Show both sequence traces in their entirety.			
	Toggle Sequences: Toggle to show or hide the sequences in the text view.			
\$	Toggle Reference AA Translation: Toggle to show or hide the reference amino acid translation line in the text view.			
Ş	Toggle Consensus AA Translation: Toggle to show or hide the consensus amino acid translation line in the text view.			
.	Toggle Differences: Toggle to show or hide the differences line in the text view.			
립무	Dye Color Coded Text: Toggle to show the base text in the specified dye colors or in black and white.			
	Dye Color Coded Text Block: Toggle to show the base text background in the specified dye colors.			
8	Help Topics: Open the Help System. By default, the Contents show in the navigation pane on the left and the introductory topic shows in the topic pane on the right. Use the Contents to select and print specific Help topics. In the navigation pane, use the Search and Index tabs to search for information by topic, index entry, and/or keyword.			
N ?	Context-Sensitive Help: Click this icon and then on a menu item to open the Help topic related to the selection.			

Dye Colors Toolbar

The Sequence Investigator module's Dye Colors toolbar identifies the colors assigned to dyes shown in the trace data view and for the bases in the text view.

To change any of these color assignments, double-click the desired icon and use the Colors dialog to choose another color.

Figure 7-9 Dye Colors Toolbar, Sequence Investigator Module



lcon	Description			
A (red)	d is the default color assigned to the adenine nucleotide in the data view.			
C (black)	lack is the default color assigned to the cytosine nucleotide in the data ew.			
G (green)	Green is the default color assigned to the guanine nucleotide in the data view.			
T (blue)	Blue is the default color assigned to the thymine nucleotide in the data view.			
N (gray)	Gray is the default color assigned to ambiguous nucleotides other than A, C, G, or T.			

Using the Sequence Investigator Module

This section shows you how to use the Sequence Investigator module to investigate and compare sequence analysis results.

To open the Sequence Investigator module, on the Main Menu, click the **INVESTIGATOR** icon

Create a Reference File

The reference file is a text file you can create using Notepad or any other text editor. It may be composed of one or two sections: the sequence and, optionally, specific nucleotide or amino acid mutations. To create the file, enter the text of the sequence starting in the first line of the text editor. You may break the sequence text into several lines.

Note: To create a reference file from the Sequence Analysis module, export the data in *.seq format or choose *.txt with only **Result Output** checked.

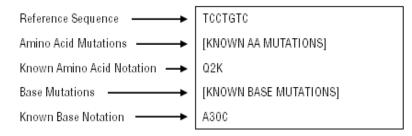
To enter known protein or nucleotide mutations:

- 1. On the line below the reference sequence, type an open bracket, followed by KNOWN AA MUTATIONS and/or KNOWN BASE MUTATIONS, then a closed bracket.
- 2. On the next line, type the current reference amino acid or base, the position number using the amino acid or base number, then the expected amino acid or base change.

The following example shows a sample reference.

🕞 reference1.txt - Notepad 📃 🗖	×
<u>Fi</u> le <u>E</u> dit Format <u>V</u> iew <u>H</u> elp	
TCATTGACAGTCCAGCTGTCTTTTTCTGGCAGCACTATAGGCTGTACTGTCCATTTATCAGGATGGAGTTCATAACC CATCCAAAGGAATGGAGGTTCTTTTCTGATGTTTTTGTCTGGTGTGGTAAGTCCCCACCTCAACAGATGTTGTCTCA GCTCCTCTATTTTGTTCTATGCTGCCCTATTTCTAAGTCAGATCCTACATACA	4
[KNOWN AA MUTATIONS] K157EQX A159G A160R L161V L162A A163EGV F164V F165IL P166S X167IM A168G P169L P171AS L172P D173L E174G P175LQ	
[KNOWN BASE MUTATIONS] G488N C489N T492N T493N A501N G510N C511N	

Figure 7-10 Sample Reference



Note: The system creates a default Reference folder during software installation. You may store your reference files in that location or in any other folder. When you create a new comparison, navigate to the appropriate folder to select your reference.

Select Reference and Sequence Results

- 1. Open the Sequence Investigator module.
- Select File > New.
 An Open dialog shows the contents of a reference folder.
- 3. Select the sample reference and click **Open**. The Open Sequence Results dialog opens.

Figure 7-11 Open Dialog

Open							? X
Look jn: [Heterozygote and Alignment tutorial	•	÷	£	Ċ	:::: -	
	12-2 RC.txt						
(VII-VIIIF)							
poor genom							
File <u>n</u> ame:	(VII-VIIIF)12-2.txt			-		<u>O</u> per	n
							· .
Files of <u>type</u> :	Reference files (*.txt)			•		Canc	

4. Select one or two results and click **OK**.

Note: To select two results, left-click the first result, press and hold the **Ctrl** key, then left-click the second result.

equence Results			
	EQ OQ TESTIN	G_GENESIS OK	
- Filter			
Start Date: 01-17-	2006 🚊 🗖	Enable Cancel	
End Date: 01-17-	2006	Refresh Help	
,			_
Name	Date-Time	Sample	▲
15+16.G12_0604	04/26/06 1	15+16.G12_0010040334	
15+16b.G01_060		15+16b.G01_00110221F	
Seq Test.A03_06	03/02/06 1	Seq Test.A03_06030116	
Seq Test.A03_06	03/02/06 1	Seq Test.A03_06030116	
Seq Test.A04_06	03/02/06 1	Seq Test.A04_06030118	
Seq Test.A04_06	03/02/06 1	Seq Test.A04_06030118	
Seq Test.A05_06	03/02/06 1	Seq Test.A05_06030120	
Seq Test.A05_06	03/02/06 1	Seq Test.A05_06030120	
Seq Test.B03_06	03/02/06 1	Seq Test.B03_06030116	
Seq Test.B03_06	03/02/06 1	Seq Test.B03_06030116	
Seq Test.B04_06	03/02/06 1	Seq Test.B04_06030118	
Seq Test.B04_06	03/02/06 1	Seq Test.B04_06030118	
Seq Test.B05_06	03/02/06 1	Seq Test.B05_06030120	
Seq Test.B05_06	03/02/06 1	Seq Test.B05_06030120	
Seq Test.C03_06	03/02/06 1	Seq Test.C03_06030116	
Seq Test.C03_06	03/02/06 1	Seq Test.C03_06030116	
Seq Test.C03_06	03/02/06 1	Seq Test.C03_06030116	•
•		► F	

Figure 7-12 Open Sequence Results Dialog

 If two results are in different projects, select (none) as the Project Name to show all available results files for a given database. A new window opens.

Note: By default the standard, navigator, dye color, and discrepancy map toolbars are docked. If they are not shown, select **View** > **Toolbars** and enable the desired toolbar.

Numbering

Base and codon numbering start at the beginning of the reference sequence. The system uses these numbers as a navigational reference point.

The column position numbering begins at the first base in the comparison. This may be a reference base, a sample sequence base, or a consensus base, depending on the alignment of the sequences being compared. The selected column number appears in the lower right-hand corner of the status bar. The History Log records all edits made in the Sequence Investigator, and their column number.

View Reference Amino Acid Translations

When the system generates the comparison, it translates the nucleotide sequence of the reference file into an amino acid sequence and shows it above the reference base sequence line. From the beginning of the reference, the bases are separated into groups of three nucleotides (codons). Each group is translated into an amino acid which is shown above the codon. Each codon is shaded to show its boundaries. (If an inserted space exists, signified by a dash "–", the space is included in the codon.)

The amino acid designation is left-justified above the codon. If two amino acids are possible, the letters designating the possible amino acids appear. If the system detects more than two possible amino acids at any location, it shows an asterisk instead.

To show or hide the reference amino acid sequence, select **View > Reference Amino Acid Translation**. The screen shows or hides the reference amino acid translation, depending on whether the item is checked or unchecked.

View Sequence Traces

When you first perform a comparison, select one or two sequences to form the consensus. After performing the comparison, the system shows the traces for these sequences. When you click any position in alignment view, the corresponding peaks in the traces move to the center of the trace view, framed by two vertical lines.

When you first open or create a comparison, the system scales the traces to improve readability.

- To zoom each sequence pane individually and repeatedly, left-click and hold the mouse button while dragging the pointer to form a box around the desired area, then release the mouse button.
- To unzoom both traces, click the **Unzoom All** icon **Q**. The traces in both panes revert to a completely unzoomed state, showing the entire range of the sequence results.
- To unzoom one of the two open data traces, right-click in the desired trace pane and select **Unzoom Pane**. The selected trace shows the entire range of the result.

You cannot edit sequence traces in the Sequence Investigator. However, you may edit sequence traces using the Sequence Analysis module. If you use an edited result for your comparison, your edited bases appear in lowercase.

View Consensus Amino Acid Translations

When the system generates the comparison, it translates the consensus nucleotide sequence into an amino acid sequence and shows it below the consensus sequence. The amino acid translation starts at the first base relative to a reference codon. The consensus is also segmented into codons, which are translated into their amino acids.

If edits occur in the consensus, a retranslation of the consensus amino acid translation occurs automatically and the system automatically updates the discrepancy map and differences line.

Each codon is shaded to show its boundaries. If a heterozygote occurs, the system notes up to two possibilities. If more possibilities exist, an asterisk shows instead.

The consensus sequence is editable within the Sequence Investigator module. Edited bases appear in lowercase letters.

Use Quality Values

- If you are using a single sequence, the quality value assigned to each base in the consensus match each base in the sequence.
- When using two sequences and the bases agree, one of two calculations occur:
 - If the orientation of the two sequences is opposing, the system adds two quality values.
 - If the orientation is the same, the system uses the higher quality value.
- If the bases disagree, the system performs a calculation to determine the probability of error for that position, providing a quality value which is the difference between the higher and lower value.

To set the value at which bases in the consensus are considered low quality, select **Edit** > **Quality Threshold**. Low quality bases are identified in the differences line, the navigator toolbar, and the discrepancy map.

View Differences

The GenomeLab GeXP system uses codes to denote the type of discrepancy encountered when performing the comparison. These codes show in the differences section of the alignment pane. To turn the differences line on or off, select **View > Differences**. The highest level code shows in the differences line in the location where the discrepancy occurred.

Note: Any consensus position may have multiple difference codes associated with it. The codes have a hierarchy; the system shows the code with the highest precedence. To view all difference codes associated with any position, select **View > Toolbars > Navigator**. The Differences field in the Navigator toolbar shows the difference codes in order of precedence.

Navigate and Edit

The navigator toolbar allows you to search for locations of interest in the consensus based on attributes, as well as search for specific bases and view their associated quality values and difference codes.

If the navigator toolbar is hidden from view, select **View > Toolbars > Navigator**. The system shows the navigator toolbar. When docked, the toolbar shows to the left of the screen display.

If you need more space for your screen display, you may move the navigator toolbar so it floats over the screen display.

- To reposition a docked toolbar, select any background area of the dialog, press and hold the left mouse button while dragging the toolbar to a convenient position on the screen, then release the mouse button.
- To dock a floating toolbar, left-click the toolbar's title bar, press and hold the left mouse button while dragging the toolbar to the lower-left corner of the screen, then release the mouse button.

Note: For details on how to use the search and editing capabilities provided on the navigator toolbar, click **Help**.

Figure 7-13 Navigator Toolbar - Floating

Navigator		
Image: Consensus Attribute Search Image: Mismatch (!) Image: Ambiguity (?) Image: Disagreement (*) Image: Disagreement (*)		
Consensus Text Search		
<u> </u>		
Consensus		
Called Base:		
Quality Value:		
Differences:		
Help		

When editing the consensus, follow these guidelines:

- Complete all trim operations before performing other types of editing. Using any other editing operation disables the trim feature.
- You may insert and delete spaces in the reference sequence, but not change bases.
- Select a single consensus base if you want to perform base editing.

Note: The system adds all editing operations to the history log along with the date, time, and position of the operation.

Complement the Sequence

When the system performs a comparison, it determines which orientation, forward or reverse, to provide a better fit with the reference. If the system determines that the sequences generate a better comparison in the opposite orientation, it automatically reverse complements the sequences.

To manually reverse complement a sample:

1. Right-click the desired trace data pane.

2. Select Reverse Complement.

The trace and associated bases are reverse complemented.

Flip the Traces

You may want to switch the order of sequence shown.

To move the sequence trace shown in the top trace pane and exchange its position with the bottom trace:

- 1. Select Edit > Flip Traces or right-click in one of the trace data panes.
- 2. Select Flip Traces.

This exchanges the position of the sequences in both the alignment pane and the trace panes.

Trim the Bases

When you first open or create a compared sequence result, the sequences used in the comparison appear in the alignment pane. You can then trim the ends of the sequences.

If sequences are not shown, select **View** > **Sequences** to show the sequences in the alignment pane.

To trim the sequence:

- 1. Select a group of sequence bases at either end of the sequence.
- 2. Click the **Trim** icon to remove unwanted bases from the alignment. This deletes the bases from the text and trace data views.

Note: You may only trim at either end of the sequence. You may not trim within the sequence.

Note: The system adds trim operations to the history log, along with bases trimmed with date and time of trim operation. To access the history log, select **View > History**.

After each trim, the system automatically aligns and shows the alignment text and traces again.

Search the Consensus for Specific Attributes

To search the consensus for specific attributes:

- 1. Select Consensus Attribute Search.
- 2. Select the attributes that you want to define as the search criteria.
- 3. Click >> or << to search the consensus in the specified direction.

Note: You can also press Ctrl and the arrow keys to perform the search.

When the search finds consensus bases that conform to the search criteria, it highlights them.

Search the Consensus for Specific Bases

To search for a specific base or contiguous multiple bases:

- 1. Select the **Consensus Text Search** check box.
- 2. Enter the bases in the field.
- 3. Specify the search criteria:
 - To search for the exact match for text, select the Exact Match check box. Use the forward and back arrow buttons or press Ctrl and the arrow keys (← and →) to search backwards and forwards through the base sequence for more text searches.
 - To search for a base without requiring an exact match, clear the **Exact Match** check box. The search result includes all exact matches as well as all IUB codes with an overlapping base.

Note: For example, a search for the ambiguity code **K** not only results in the bases **G** or **T**, but also result in any ambiguity code that contains **G** or **T**. The search stops on all ambiguity codes other than **M**. (The bases represented by **M** are **A** and **C**.) Also, searching for an **A**, **C**, **G**, or **T** matches any ambiguity codes containing the base. For example, searching for **A** results in any ambiguity code containing **A** (specifically, **M**, **R**, **W**, **U**, **H**, **D**, and **N**).

Edit the Consensus

To edit the base selected by the search:

- 1. Type the desired base or select **Edit** > **Replace**.
- Select the desired base or IUB ambiguity code.
 To automatically jump to the next base that matches your search criteria once you have completed the edit, select **Forward** or **Backward** on the Edit tab of the Preferences dialog.

Show the Discrepancy Map

The discrepancy map toolbar allows you to look for areas of interest in relationship to the reference. You may view the coverage of the consensus and sample sequences. You may also view the color-coded locations in the experiment of any type of attributes of interest that you specify.

Figure 7-14 Discrepancy Map Toolbar

(VII-VIIIF) 12-2.txt	■ 🔽 Mismatch ■ 🔽 Ambiguity
Copy of 1+3.F03_00101217FG	 Ambiguity Disagreement
1+3.H03_00101221	Insertion/Deletion
	 Mutation Hotspot Low Quality
	Manual Edit
Ready	

- The top bar in the discrepancy map represents the reference sequence.
- The second and third bars (if two sample sequences were selected) show the coverage of the sample sequences in relation to the reference.
- The bottom area represents the consensus and shows the color-coded regions of interest associated with the specified attributes.

A red marker behind the reference, sample, and consensus bars corresponds to the selected area in the trace data view and the selected base in the trace text view. You can use this toolbar to navigate to desired areas by clicking on any point of interest. The alignment and trace data panes refresh and show the area.

For a global view of the desired attributes, select the corresponding check boxes on the right side of the map. For each selected attribute, the system adds a new bar to the consensus area showing the locations where selected attributes exist.

Change the Color Coding of the Attribute Display

To change the color code of the attribute display:

- 1. Click the color well to the left of the attribute. The color palette for the selected well opens.
- 2. Select a new color.
- 3. Click **OK**. The discrepancy map uses the newly selected color.

Print a Report

You may print a report for the active compared sequence result. To set up a report:

- 1. Select File > Report Format.
- 2. Select the default printer from the **Printer Name** list.
- 3. Set your preferred page layout by selecting **Portrait** or **Landscape**.
- 4. Select the option elements you want shown in your report.

Element	Description
Header	Prints information such as the name of the compared sequence and the name of the sample sequences as well as the database and project where the sample resides.
Alignment	Prints the reference amino acid translation, reference, sequences, difference codes, consensus, and consensus amino acid translation.
History Log	Prints a history of changes you performed in the alignment.
Statistics	Prints the statistics of the report, such as the number of discrepancies before and after editing.
Mutations	Prints all mutations, including known and novel bases and amino acids.

Table 7-1 Report Display Elements

Export the Sequence

You may export the active compared sequence in either of three formats, as described in the following table.

Table 7-2 Export Formats

Format	Description
text (*.txt)	Exports the base sequence of the consensus.
tab delimited text (*.txt)	Exports data as a single line and all carriage returns (\downarrow) are replaced with tabs (\rightarrow).

Format	Description			
protein (*.pro)	Exports the amino acid sequence. To set the protein format, select File > Preferences and choose one of the following format in the Preferences dialog.			
	 Condensed: Provides all protein translations in the same text line and designates heterozygotes between brackets. 			
	Expanded: Includes all possible translations on different lines.			
	The following example compares the differences between condensed and expanded protein formats.			
	KVS[IM]LPLV[LP]K or KVSILPLVLK KVSMLPLVPK KVSMLPLVLK KVSILPLVLK KVSILPLVPK			

 Table 7-2 Export Formats (continued)

To export the active compared sequence:

- 1. Select File > Export.
- 2. Choose the desired format.
- Click OK to export the data. The system shows an hourglass while exporting the data.

Note: If a file with the same name exists in the selected directory, the system prompts you to rename the new file or confirm that you want to overwrite the existing file.

Save the Data

To save a new document:

- Select File > Save. A Save As dialog opens, prompting you to enter a file name and folder location.
- 2. Select the folder location and enter a file name.
- 3. Click Save.

The system saves the data to the file for later retrieval.

Note: If the document has previously been saved, select **File > Save** to save the document immediately.

- 4. To change the name or location of an existing file:
 - a. Select File > Save As.
 A Save As dialog opens, prompting you to enter a file name and folder location.
 - b. Select the folder location and enter a file name.
 - c. Click Save.

The analysis of fragment or allele data is performed with the GenomeLab System software, by using Studies. A Study represents a collection of quality sample results used for locus tag generation, genotyping, quantitation, or AFLP profile generation. Ultimately, a Study contains a common group of sample results that are analyzed and exported together.

This chapter provides an overview of the Fragment Analysis module, including its menu options, toolbars, and dialogs. It shows how to use this module to work with Studies, to run a fragment analysis, to set up parameters, to use result properties, and to show options. It also describes how to work with, report, and export fragment analysis functions.

Overview

The Fragment Analysis module processes fragment data from the GenomeLab GeXP system platform and provides size and allele information for detected peaks. The system organizes parameters that identify alleles as locus tags that you can customize. You can then view, export, and print the analysis results.

Analysis parameter sets provide parameters specific to an experiment, allowing size calibration and locus tag assignment as necessary, when processing data.

Data analysis can be a manual function or set to run automatically. Edit and reanalysis functions provide the capability to verify the accuracy of the data.

Graphically view, analyze, edit, compare, and print data of the following list types:

- Raw data
- Fragment data
- Fragment lists
- Information about locus tags and assigned alleles

Current data and voltage data are also available.

Raw data, current data, and voltage data cannot be edited.

Main Window

The main window for the Fragment Analysis module has the following sections.

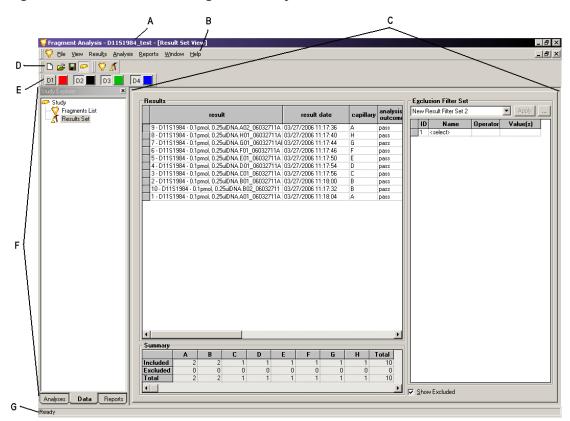


Figure 8-1 Main Window, Fragment Analysis Module

Label	Description
A	Title Bar: Shows the module name (Fragment Analysis) and the name of the currently open Study.
В	Menu Bar: Refer to the section: Menu Bar.
С	Display Area: Graphically shows the opened data.
D	Toolbar: Refer to the section: Toolbars.
E	Dye Colors - Double-clicking on a dye color opens the Color dialog. Change the dye color shown for that particular dye. Clicking on the dye name (D1 , D2 , D3 , D4) removes that dye trace from all traces.
F	Study Explorer: Shows and provides access to all of the parameters available in the selected Study. These are divided into the tabbed sections: Analyses, Data, and Reports, which you can select at the bottom of the Study Explorer frame.

Label	Description
G	Status Bar: Shows information concerning the current selection.

Menu Bar

The following example shows the menu bar for the Fragment Analysis module.

Figure 8-2 Fragment Analysis Module Menu Bar

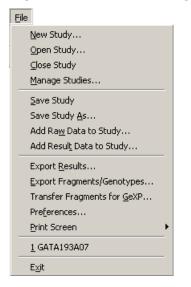
```
<u>File View Results Analysis Reports Window Help</u>
```

The following topics describe each of these menus and their options.

File Menu

Click File to show the following menu.

Figure 8-3 File Menu, Fragment Analysis Module



Option	Description	
New Study	Start a new Study from either raw data or analyzed results.	
Open Study	Open an existing Study.	
Close Study	Close the active Study.	
Manage Studies	View and select all Studies saved to the database.	

Option	Description		
Save Study	Save the new or edited Study.		
Save Study As	Save the current Study with a new name.		
Add Raw Data to Study	Select additional data (raw data) to add to the Study.		
Add Result Data to Study	Select additional data (previously analyzed result data) to add to the Study.		
Export Results	Export data in one of the following formats:		
	Text (tab delimited) (*.txt)		
	• CEQ (*.cq)		
	• CRV (*.crv)		
Export	Export the fragment / genotype data in one of the following formats:		
Fragments / Genotypes	CSV (comma delimited) (*.csv)		
	Linkage (Pedin) (*.pre)		
	Discovery Manager (DMPopulation) (*.txt)		
	Genotype Summary Report (tab delimited) (*.gsr)		
Transfer Fragments for GeXP	Export fragment data in a *.csv file compatible with the CSV Transformer Tool.		
Preferences	 Set the following preferences: Select whether to show the Study window when the Fragment Analysis module is launched. 		
	Specify whether to graph all electropherograms by size or time.		
	 Set the number of electropherograms to be cached. 		
	 Select the initial view when selecting the Single Result View of a sample. 		
	 Specify whether to show a warning message before resetting all manually included or excluded bins during an AFLP analysis. 		
	 Specify whether to show a warning message before reanalysis if old results in the study will be replaced by new results. 		

Option	Description	
Print Screen	Open a sub-menu with options to print an image of the computer desktop or application window.	
Recent Studies	View a list of the most recently opened Studies.	
Exit	Close the module.	

View Menu

Click **View** to show the following menu.

Note: A check mark next to an option indicates that the option is enabled.

Figure 8-4 View Menu, Fragment Analysis Module

⊻ie	w	
	Eragment List	F2
	Resul <u>t</u> s Set	F4
	L <u>o</u> cus List Editor	F7
	<u>G</u> enotype Summary	F6
~	<u>S</u> tatus Bar	
~	Study <u>E</u> xplorer	
	Toolbars	Þ

Option	Description	
Fragment List	Show the fragment list, which contains information for all analyzed fragments included in the Study, such as size, height, area, locus, and allele ID.	
Results Set	Open the Results Set View window, which contains information for all of the analyzed samples included in the Study.	
Locus List Editor	Open a dialog that creates the LocusList.txt file used for the Genotype Summary View (GSV).	
Genotype Summary	Show a Study's genotype information (loci and alleles).	
Status Bar	Toggle to show or hide the status bar.	
Study Explorer	Show the Study Explorer, which provides access to all of the parameters available in the selected Study.	
Toolbars	Select which toolbars to show. Refer to the section: Toolbars.	

Results Menu

Click **Results** to show the following menu.

Note: A check mark next to an option indicates that the option is enabled.

Figure 8-5 Results Menu, Fragment Analysis Module

Results
Show Excluded Ctrl+F
Show Capillary Summary

Option	Description	
Show Excluded	Show fragment results that were excluded from the fragment results grid.	
Show Capillary Summary	Show a table that identifies the number of samples that are included and excluded in the result set within each capillary.	

Fragments Menu

Click **Fragments** to show the following menu.

Figure 8-6 Fragments Menu, Fragment Analysis Module



Option	escription	
Show Excluded	Show fragments that were excluded from the fragment list.	
Manually Add Peaks	Manually select peaks to include in the fragment list.	
Save Fragment Grid As CSV	Save the fragment grid as a comma-delimited file (*.csv)	

Analysis Menu

Click **Analysis** to show the following menu.

Figure 8-7 Analysis Menu, Fragment Analysis Module

Analysis	
Anal	ysis <u>P</u> arameters
New	AFLP Analysis
New	Binning Analysis
New	Pea <u>k</u> Ratio Analysis
Run	LOH Analysis

Option	Description	
AnalysisView the current working analysis parameters, edit the working analysisParametersParameters (maintained in memory for the duration of the application session), and select a previously saved parameter set to be used in analysis.		
	Note: If the 21 CFR Part 11 tools are in use, then the edit function is only available to users with Write access.	
New AFLP Analysis	Start a new AFLP analysis. Refer to the section: Perform AFLP Analysis.	
New Binning Analysis	Start a new binning analysis. Refer to the section: Perform Bin Analysis.	
New Peak Ratio Analysis	Start a new peak ratio analysis. Refer to the section: Calculate Peak Ratios.	
Run LOH Analysis	Run a loss of heterozygosity (LOH) analysis. Refer to the section: Perform LOH Analysis.	

Reports Menu

Click **Reports** to show the following menu.

Figure 8-8 Reports Menu, Fragment Analysis Module

<u>R</u> eports	
<u>N</u> ew Report	
<u>R</u> efresh	F5

Option	Description	
New Report	Select a template and context type for the report.	
Refresh	Refresh the display of the selected report.	

Window Menu

Click **Window** to show the following menu.

Figure 8-9 Window Menu

Window
Cascade
Tile Horizontally
Tile Vertically
Close All
Arrange Icons
↓ 1 <lintitled></lintitled>

Option	Description
Cascade	Cascade the open windows.
Tile Horizontally	Tile the windows in a horizontal orientation.
Tile Vertically	Tile the windows in a vertical orientation.
Close All	Close any currently active windows.
Arrange Icons	Automatically arrange the icons.

Help Menu

Click **Help** to show the following menu.

Figure 8-10 Help Menu

F	<u>l</u> elp	
	н	elp <u>T</u> opics
	Ū	sing Help
	A	bout GenomeLab System
	S	CIEX Home Page

Option	Description
Help Topics	Select this option to show the Help topics. By default, the Contents show in the navigation pane on the left and the introductory topic shows in the topic pane on the right. Use the Contents to select and print specific Help topics. In the navigation pane, use the Search and Index tabs to search for information by topic, index entry, and/or keyword.
Using Help	Select this option to open the Contents window on how to use the Windows Help system.

Option	Description
About GenomeLab System	Select this option to access software and system information.
SCIEX Home Page	Select this option to access the SCIEX Home Page on the Internet.

Toolbars

The Fragment Analysis module provides four toolbars that activate some of the most commonly used commands: Study, Dye Colors, View, and EgramBand.

The following tables describe the function of each of the toolbar icons.

Study Toolbar

The following example shows the Fragment Analysis module's Study toolbar.

Figure 8-11 Study Toolbar, Fragment Analysis Module



lcon	Description
D	New: Create a new Study from raw data or result data.
2	Open: Open an existing or a recent Study.
	Save: Save the new or edited Study.
₽	Study Explorer: Open the Study Explorer.

Dye Colors Toolbar

The following example shows the Fragment Analysis module's Dye Colors toolbar.

Figure 8-12 Dye Colors Toolbar, Fragment Analysis Module



Icon	Description
D1 (red)	Red is the default color assigned to Dye #1 in the fragment data pane and D1 is the default name.
D2 (black)	Black is the default color assigned to Dye #2 in the fragment data pane and D2 is the default name.
D3 (green)	Green is the default color assigned to Dye #3 in the fragment data pane and D3 is the default name.
D4 (blue)	Blue is the default color assigned to Dye #4 in the fragment data pane and D4 is the default name.

View Toolbar

The following example shows the Fragment Analysis module's View toolbar.

Figure 8-13 View Toolbar, Fragment Analysis Module



lcon	Description
V	Fragment List: Open the Fragment List.
\$	Result Set: Open the Result Set View window.

EgramBand Toolbar

The following example shows the Fragment Analysis module's EgramBand toolbar.

Figure 8-14 EgramBand Toolbar, Fragment Analysis Module

	Y Height	52215.49	÷	×Width	96.90042	- · ·	1	$\overline{\mathbf{Q}}$		
--	----------	----------	---	--------	----------	---------	---	-------------------------	--	--

lcon	Description	
Y Height 52215.49	EgramBand Y Axis: Scale the Y axis.	
XWidth 96.90042	EgramBand X Axis: Scale the X axis.	

lcon	Description
	EgramBand Zoom tool: Zoom in on a selected area of the Overlay Graph electropherogram display. This option is the default selection in Overlay Graph mode.
	EgramBand Subset Selection tool: Select a subset of data to be shown in Stacked Graph mode. This option is only available in Overlay Graph mode.

Note: Portions of this toolbar are only available when viewing sample results in Single Result, Stacked Graph, and Overlay Graph View modes. Showing the selected sample results in one of these modes will automatically show the appropriate EgramBand components for the selected display mode.

Using the Fragment Analysis Module

To open the Fragment Analysis module, on the Main Menu, click the **FRAGMENTS** icon **V**.

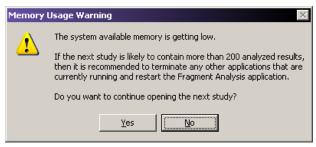
😽 Fragment Analysis - New Stud	dy *									_ 8 ×
Eile View Resul <u>t</u> s Analysis R	eports	Window Help								
🗋 D 🚅 🖬 🔗 🛛 🏹 🚿										
	D4	1								
D1 D2 D3 D3	U4									
	_									_
Study	- <u>-</u>	Result Set View							_ 🗆	×
							F 1 b O b			1
		Results		1			n Filter Set			
		result	result date	capillary	analysis outcome	ID New Hest	ult Filter Set 2	▼ Operator	Apply	
		DATS 93-D4.A01_06042512ZM	04/25/2006 12:59:56	A	pass	1 <s< td=""><td></td><td>Uperator</td><td>value(sj</td><td></td></s<>		Uperator	value(sj	
		DATS 94-D4.B01_06042512ZK	04/25/2006 12:59:50	B	pass		618607	1		
		DATS 96-D4.C01_06042512ZI DATS 97-D4.D01_06042512ZF	04/25/2006 12:59:44	C D	pass					
		DATS 97-04.001_060425122F	04/25/2006 12:59:36	E	pass pass					
		DATS 100-D4.F01_060425122C	04/25/2006 12:59:20	F	pass					
		DATS 101-D4.G01 0604251226	04/25/2006 12:59:12	G	pass					
		DATS 102-D4.H01_06042512Z4	04/25/2006 12:59:06	Н	pass					
					A. A					
		Summary				1				
		A B C D) E F G	н	Total					
		Included 1 1 1			8					
		Excluded 0 0 0 Total 1 1 1	0 0 0	0 0						
			1 1 1	1 1	8					
					Þ	Show E	xcluded			
Analyses Data Reports										
Ready										

Figure 8-15 Fragment Analysis: New Study Window

Performance Safeguards Controller Resources

The system checks the available controller resources before it starts processing results. If the system determines that the resources needed to complete the analysis are not available, a warning message shows. If this occurs, close all applications, restart the Fragment Analysis module, and attempt the analysis again.

Figure 8-16 Memory Usage Warning



Database Size Warning

If a database that is larger than 1,700 megabytes is selected and you try to perform additional data analysis steps, the following warning message shows.

Figure 8-17 Database Size Warning



Working with Studies

A Study is a set of multiple sample results together with the results of analyses performed on the group of sample results. You can use the Study Wizard to create a new Study easily. The Study Wizard guides you through the process of creating a new Study starting from either raw data or previously analyzed fragment results.

The Study window opens automatically when you start the Fragment Analysis module. You can open a new or existing Study immediately from this window or at any time while working within the Fragment Analysis module.

To access the Study window, select **File > New Study** or **File > Open Study**.

Create a new Study from	
👽 💿 Baw Data	
C Analyzed Results	
C Open an existing Study	
Existing Recent	
Study Name	Date-Time
D11S1984_test GATA193A07_test1	3/27/2006 11:32:33 AM 3/27/2006 11:40:28 AM
GATA193A07_test2	3/27/2006 12:44:52 PM
Don't show this dialog box again	

Figure 8-18 Study Window - Creating or Opening a Study

You can create a new Study from either **Raw Data** or previously **Analyzed Results**. Make your selection using one of the option buttons and click **OK** to continue.

The Study Wizard guides you through the steps of creating a Study. After the system analyzes the samples, you may include additional results in the new Study.

To open an existing Study, select it from the list of names available on the Existing or Recent tab. The **Date-Time** column indicates when the Study was created or last modified. When you have made your selection, click **OK** to open the existing Study.

Select the Components of the New Study

The first step in creating a new Study involves manually filtering data from the database by selecting the samples you would like to include in your Study (this is first-level filtering).

After the system analyzes these samples, you may further filter the sample results (second-level filtering) in the Result Set View.

Finally, you may filter the generated fragment and allele data (third-level filtering) in the Fragment List. This leaves a collection of identified fragments and alleles that exactly match the requirements of your Study.

Select Raw Data for the New Study

When you select the **Raw Data** check box and click **OK**, the Study Wizard opens, showing the Select Raw Data window.

Select Raw Data			
Raw Data	List View	Plate View	
Analysis Parameters	P		
Analyze Data	Project: AFLP 06 TP2.20		
Result Data	·		and the second se
	✓ Filter by date		Raw Data
	Start: 4 /25/2001 💌 End: 7 /25/2001 💌		
	- Baw Data		
	Available:	Selected: (8)	
	Name Date	▲ Name	Date
	DATS 103-D4.A02_01060520G 6/5/2001 10:06:30 PM	DATS 102-D4.H01_01	060518QY 6/5/2001 8:24:03 PM
	DATS 105-D4.802_01060520G 6/5/2001 10:06:31 PM	DATS 101-D4.G01_01	
	DATS 107-D4.C02_01060520G 6/5/2001 10:06:32 PM	DATS 100-D4.F01_010	
	DATS 110-D4.D02_01060520G 6/5/2001 10:06:34 PM	> DATS 99-D4.E01_010	
	DATS 111-D4.E02_01060520G 6/5/2001 10:06:35 PM	DATS 97-D4.D01_010	
	DATS 113-D4.F02_01060520G 6/5/2001 10:06:37 PM	DATS 96-D4.C01_010	
	DATS 114-D4.G02_01060520G 6/5/2001 10:06:38 PM DATS 115-D4.H02_01060520G 6/5/2001 10:06:39 PM	DATS 94-D4.B01_010	
	DATS 113-04.H02_0106052260 6/5/2001 10:06:33 FM DATS 93-D2.A03 010605226D 6/5/2001 11:49:09 PM	>> DATS 33-D4.A01_010	0016QT 6/0/2001 6:23:53 FM
	DATS 94-D2.803_010605226D_6/5/2001 11:49:10 PM		
	DATS 96-D2.C03 010605226D 6/5/2001 11:49:11 PM	<<	
	DATS 97-D2.D03 010605226D 6/5/2001 11:49:13 PM		
	DATS 99-D2.E03 010605226D 6/5/2001 11:49:14 PM		
	DATS 100-D2.F03 010605226 6/5/2001 11:49:16 PM		
	DATS 101-D2.G03 010605226 6/5/2001 11:49:17 PM		
	DATS 102-D2.H03_010605226_6/5/2001 11:49:18 PM	-	
	DATE 103 D3 404 010005334 CIC 0001 1 31 51 4W		
	Help	<< <u>P</u> revious	Next>> Einish Cancel

Figure 8-19 Select Raw Data Window

Use this window to select the raw data to be analyzed from a database of samples that have been run in a particular projects. The system includes the selected raw data in your Study and analyzes it as described in the following steps.

Note: To view an individual sample electropherogram and other collected parameters prior to adding the sample to your Study, right-click a highlighted sample and select the **Show Single Sample View** check box.

The List View and Plate View tabs provide you with two ways to select raw data. Each selection method provides the same sample information presented in different ways.

List View Tab

The List View tab allows you to select from a list of samples contained in a specific database and/or collected during a particular time frame.

- 1. Select the **List View** tab in the Select Raw Data window.
- 2. Select the project folder that contains the sample data that you wish to include in the Study from the **Project** list.

Note: If the desired project is not listed, it may be located in a different database. To enter a different database, you must close all applications and set the working database. Refer to the section: Set the Working Database or the Help System for more information.

- 3. Filter data: If the data of interest was run during a particular time period, you can filter the data by selecting the **Filter by date** check box and choosing a range of dates to be searched. When enabled, the system shows only those samples that were run during the specified time period.
- 4. Select samples from the Raw Data / Available area:
 - To select the samples to be included in your Study, click to highlight the sample, then click the right-arrow button to move the selection to the Raw Data / Selected area.
 - To select all of the listed samples to be included in your Study, click the double-rightarrow button to move them to the Raw Data / Selected area.
 - To remove samples from your Study, click to highlight the samples in the Raw Data / Selected area and click either of the left-arrow buttons to remove them from your selected samples.

Note: You may select data from multiple projects to be included in your Study as long as they are located in the same database. Multiple results from multiple databases may not be combined into the same Study. Continue making selections from the list of available projects and data until all of the desired samples have been added to the Raw Data / Selected area.

5. Click **Next** to proceed to the Analysis Parameters window.

Plate View Tab

The Plate View tab allows you to make your selections from a visual representation of the sample plate.

1. Select the **Plate View** tab in the Select Raw Data window.

Steps Raw Data Analysis Parameters	List View Pla	ate View
Analyse Data Result Data	Project: AFLP 06 TP2:20	Baw Data Legend: Image: Constraint of the second
	I I <thi< th=""> <thi< th=""> <thi< th=""> <thi< th=""></thi<></thi<></thi<></thi<>	Selected Wells: Name Date
	Help	<< Brevious Next >> Einish Cancel

Figure 8-20 Selecting Raw Data from the Plate View Tab

2. Select the project folder that contains the sample data that you want to include in the Study from the **Project** list.

Note: If the desired project is not listed, it may be located in a different database. To enter a different database, you must close all applications and set the working database. Refer to the section: Set the Working Database or the Help System for more information.

- 3. Select the plate from the **Plates** list. A visual representation of the plate shows in the Current Plate area of the window.
 - To select the samples for your Study, click the well location of the sample of interest. The sample ID moves to the Current Plate / Selected Wells area of the window, indicating that the sample will be included in the Study.

Note: Refer to the Raw Data Legend area of the window for information regarding sample well selection.

- To remove samples from your Study, click the well location of the sample you would like to remove. The sample ID moves to the Current Plate / Selected Wells area of the window, indicating that the sample has been removed from the Study.
- To select or remove entire rows or columns of wells, click the corresponding row letters or column numbers.

• To select the entire plate to be included in your Study, click the red box in the upper-left corner of the plate.

Note: You may select Study data from multiple projects and/or multiple plates. Continue making selections from the list of available projects and plates to add all of the desired samples to the Raw Data / Selected area.

4. Click Next to proceed to the Analysis Parameters window.

Select an Analysis Parameter Set

The second step in creating a new Study from raw data involves defining the parameters to use when analyzing selected samples. Analysis parameters are used to process the fragment data to estimated fragment sizes and to identify alleles.

Figure 8-21 Analysis Parameters Window - Selecting a Fragment Analysis Parameter Set

쯋 Analysis Parameters				×
Steps Raw Data Analysis Parameters Analyze Data Result Data			Select Analysis Parameter Set FA600 Edit	
			Parameter Set Summary Project: Default Date Modified: 3/27/2006 1:55:54 PM Dye Mobility Calibration: NoCorrection Size Standard: SizeStandard-600 Model: Quartic Locus Tags Date Image: Name Date	
	Help]	<< Previous Next >> Finish	Cancel

Use the Analysis Parameters window to create, select, or edit the analysis parameter set for analyzing the data selected for the new Study. Your choice of a particular set of analysis parameters depends on the particular fragments you are attempting to identify.

Note: If the 21 CFR Part 11 tools are in use, then this function is only available to users with Write access.

The GenomeLab System software has a set of default analysis parameters, used to estimate unknown fragment lengths. The calibration curve is based on the migration times of the size standard fragments. The default set of analysis parameters cannot be edited.

The default parameters are as follows:

- **DEFAULT FRAGMENT ANALYSIS PARAMETERS:** Used for applications such as STR and AFLP.
- **DEFAULT GeXP ANALYSIS PARAMETERS:** Used for the Gene Expression application.
- **DEFAULT SNP ANALYSIS PARAMETERS:** Used for the SNP application.

Select a Saved Analysis Parameter Set

1. In the Select Analysis Parameter Set area of the window, select the parameter set from the list.

The Parameter Set Summary area of the window shows summary information about the selected analysis parameter set. These include:

- Date and time the analysis parameter set was created or last modified.
- Selected size calibration standard, calibration curve, and optional dye mobility correction.
- Currently assigned locus tags that have been selected for this Study.
- 2. Click **Next** to proceed to the Analyze Data window.

Note: If the available parameter sets do not apply to your Study, you can create a new set of analysis parameters or edit an existing set to meet the requirements of your Study. Refer to the section: Define Fragment Analysis Parameters, or the Help System for more information.

Analyze the Data

With the new data selected and the analysis method defined, the third step in creating the Study is to analyze the data.

The Analyze Data window shows all samples selected for analysis, the date each was collected, the status (unanalyzed) of each sample, and the parameter set being applied to the Study.

1. To begin the analysis, click **Analyze**. As the system proceeds with the analysis, the progress bar becomes active and the lower dialog shows the analysis log for each sample as it is analyzed.

Note: To stop the analysis while it is in progress, click **Stop**. The system stops the analysis when it has finished with the sample in progress.

alysis Parameters alyze Data	Sample	Date	Status	Date Analyzed
t Data	DATS 102-D4.H01_01060518QY	6/5/2001 8:24:03 PM	pass	4/25/2006 12:59:07 PM
Data	DATS 101-D4.G01_01060518QY	6/5/2001 8:24:01 PM	pass	4/25/2006 12:59:14 PM
	DATS 100-D4.F01_01060518QY	6/5/2001 8:24:00 PM	pass	4/25/2006 12:59:21 PM
	DATS 99-D4.E01_01060518QY	6/5/2001 8:23:59 PM	pass	4/25/2006 12:59:30 PM
	DATS 97-D4.D01_01060518QY	6/5/2001 8:23:57 PM	Unanalyzed	
	DATS 96-D4.C01_01060518QY	6/5/2001 8:23:56 PM	Unanalyzed	
	DATS 94-D4.B01_01060518QY	6/5/2001 8:23:54 PM	Unanalyzed	
	DATS 93-D4.A01 010605180Y	6/5/2001 8:23:53 PM	Unanalyzed	
	PreProcessing: channel 3 raw data RMS r PreProcessing: Estimating baseline for cha PreProcessing: channel 4 average baselin PreProcessing: channel 4 raw data RMS r	annel À le level (RFU): 2077.442146 noise (RFU): 46.558699		
	PreProcessing: Estimating baseline for cha PreProcessing: channel 4 average baselin	annel 4 ie level (RFU): 2077.442146 noise (RFU): 46.558699 in e spectra ta. dyes: 2		•

Figure 8-22 Analyze Data Window - Analysis Currently in Progress

When the analysis is complete, the status column indicates if the analysis succeeded or failed for each sample included in the Study.

- 2. To review the analysis log, highlight a sample in the upper samples list and scroll through the analysis information in the lower analysis log area of the window.
- 3. Click **Next** to proceed to the Select Result Data window.

Select Additional Results to Add to the Study

Add result data to your new Study in this fourth step. Additional result data may include samples that were previously analyzed under a different set of analysis parameters than those analyzed in the preceding steps of the Study currently being developed.

The Select Result Data window is identical to the Select Raw Data window shown in the first step of the Study Wizard.

- 1. For an explanation of the features available in the Select Result Data window, refer to the section: Select Raw Data for the New Study.
- 2. Select any additional results that you would like to add to the new Study and click **Finish** to complete the Study.
- 3. To save your new Study, select **File > Save** or **File > Save As**.

Define Fragment Analysis Parameters

Note: If the 21 CFR Part 11 tools are in use, then this function is only available to users with Write access.

The following sections describe how to edit a parameter set or create an entirely new set of analysis parameters to meet the specific requirements for your samples.

You can create or edit parameter sets using the Fragment Analysis Parameters window. This window contains a number of tabs in which you define the general parameter set information, set locus tag parameters, select an analysis method, assign quantitation values, and define several other factors that affect how your data is analyzed.

You can access the Fragment Analysis Parameters window either while developing a Study from within the Study Wizard. Refer to the section: Select Raw Data for the New Study. You can also access it at any time while working within the Fragment Analysis module by selecting **Analysis** > **Analysis Parameters**.

Using either method, the Analysis Parameters window opens, showing the currently saved parameter sets available for selection.

- To create a new parameter set, click New.
- To edit an existing parameter set, click Edit.

The following illustration shows the Edit Fragment Analysis Parameters window.

Figure 8-23 Edit Fragment Analysis	s Parameters Window	- Creating or Editing a Param	neter
Set			

😽 Edit Fragment Analysis Parameters	
Parameter Set Name: DefaultGeXPAnalysisParameters Project: Default General Analysis Method Quantitation Parameter Set Date created: 12/20/2005 11:04:02 AM	SIR Locus Tags SNP Locus Tags Advanced
Date modified: 12/20/2005 11:04:02 AM Peak Criteria	Allele Identification
Slope threshold: 10 ★ Relative peak height threshold: 1 ★ %	SNP Analysis Size Estimation and Allele ID Confidence Confidence level: 95 🛨 %
Help Save As	Save Cancel

General Tab

The General tab is the first tab that shows when you open the Fragment Analysis Parameters window. This tab contains general parameter set information and peak identification properties.

Parameter Set Information

Parameter Set information depends on whether you are creating a new parameter set or editing an existing parameter set:

- If you are creating a new parameter set, enter a descriptive name in the **Parameter Set Name** field and select the project where you want to save the parameter set.
- If you are editing an existing parameter set, you may choose to save the edited parameters under a different name. To do this, click Save As and enter the new name in the Parameter Set Name field. You may also choose to save the edited parameters under the original name. The default set of fragment analysis parameters cannot be edited.

The General tab's Parameter Set area shows the date and time the parameter set was created and modified.

Peak Criteria

You can edit the following Peak Criteria options:

Option	Description
Slope threshold	Specify the minimum rate of the signal increase on the leading edge of peaks in order for a peak (standard or unknown) to be detected.
	• Enter a value between 1 and 1,000. The default value is 10.
	• The slope is proportional to the height of the peak and depends on the width of the peak. You can set this parameter to detect peaks of interest while eliminating smaller or broader peaks that may not be of interest.
	• The slope threshold is set relative to the baseline noise, as the baseline noise varies for each dye trace. Although you can define only one slope threshold value, the system adjusts for each dye, depending on the noise detected for that dye trace.
Relative peak height threshold	Set a minimum height criterion that unknown peaks must meet before being shown in the fragment list or annotated in an electropherogram.
(%)	• Enter a value between 0 and 100%. The default value is 1%.
	 The unknown peaks above this set value (%) relative to the second highest peak in each dye trace will show in the fragment list and the sizes will show in the electropherogram.
	This value does not apply to standards.
	• To size the peaks only, clear the Allele Identification check box.

Allele Identification

The Allele Identification options let you add either of the following parameters:

Option	Description
Identify STR Alleles	Select this option to include allele identification as specified in the allele lists of the selected Short Tandem Repeat (STR) locus tags.
SNP Analysis	Select this option to include Single Nucleotide Polymorphism (SNP) analysis in the parameter set.

Fragment Analysis Module

Note: You may include only one of these options in the same analysis.

Size Estimation and Allele ID Confidence

Confidence level: Set this level as required for your analysis. The confidence level (default value 95%) is equal to one minus the significance level of the built-in statistical tests used for allele identification and for computing apparent fragment size confidence intervals.

Analysis Method Tab

Set the size standard and model. The model selected should be the best fit for the size standard selected, as it is used to generate the size calibration model, which estimates sizes for the unknown fragments.

roject:	Default					
·						
<u>G</u> eneral	Analysis <u>M</u> ethod	titation	S <u>T</u> R Locus	Tags SN <u>P</u> L	ocus Tags 🎽	Ad <u>v</u> anced
Cine stands	rd: SizeStandard-600	•	Selected	Peaks:		
Size stariua	iu. Joizeotanualu-600		Use	Number	Size	-
Dye:	D1			1	60	
				2	70	
Size Standa	rd Fit Coefficient: 0.57		ম	3	80 90	
			 ▼	4 5	100	
Model:	Quartic	_	V	6	120	
	1.	<u> </u>	V	7	140	
Red	quires a minimum of 5 selected	<u>peaks.</u>		8	160	
				9	180	
				10	190	
– Migratio	n Variable			11	200	
_			ম	12 13	220 240	
	ation time			14	240	
O Mobi	ility		▼	15	280	-
				40		
			Select	All Deselec	t All	

Figure 8-24 Fragment Analysis Parameters Window - Analysis Method Tab

Size standards are sets of DNA fragments of known sizes, run with a sample and used to create the size calibration model. The system uses this model to relate the known fragment size to either migration time or mobility for estimating the size of the unknown fragments.

Note: Refer to the Help System for information on creating size standards.

Set Size Standards

To ensure the system finds the standards and makes correct associations between the fragment peaks and the fragment list, size standards must meet the following two requirements:

- Fragments in the larger half of the size range should be spaced uniformly. For example, the peaks in the range of 200 to 400 nucleotides of the **SizeStandard-400** are 20 nucleotides apart.
- Fragments in the smaller half of the size range should include a set of at least three fragments that are spaced approximately half the distance of those that are in the larger fragment size range. For example, in the **SizeStandard-400**, the peaks in the larger half of the size range are spaced 20 nucleotides apart, while some in the smaller half of the size range are spaced 10 nucleotides apart.

Note: Custom Size Standards can be created and defined in the Data Manager module. Refer to the section: Create New Size Standards.

Set the Analysis Method

To set the analysis method:

- Select the standard to run with the sample from the Size Standard list. The size standards available are SizeStandard-400, SizeStandard-600, and SizeStandard-80. Your selection determines which size standards become available for selection in the Selected Peaks list to the right.
- 2. Select the peaks you want to use in the **Selected Peaks** list:
 - To use all fragments in the selected standard, click Select All.
 - To select individual fragments in the selected standard, select the check box next to each of the fragment sizes you want to run with the sample.
 - If you do not want to use any of the standard sizes, click Deselect All.

Note: If you do not use a size standard when analyzing the data, the unknown fragments will not be sized.

3. Note the parameters provided in the following fields for the selected size standard:

Option	Description
Dye	Shows the dye label on the size standard fragments.

Option	Description
Size Standard Fit Coefficient	Shows the standard deviation of the differences between the actual fragment sizes and the apparent sizes of the standard fragments determined from a large number of repeated runs. This parameter is sometimes called the <i>lack-of-fit</i> .

4. Select the empirical model you want to use for generating the curve that best fits the identified standard fragments from the **Model** list.

Note: The relationship between fragment size and mobility (or migration time) in the GenomeLab GeXP system with replaceable gels is nonlinear and is not described with sufficient accuracy with any theoretical model to yield reliable fragment size estimates. However, polynomials of varying degrees serve as practical empirical models that will accurately account for this nonlinearity.

Based on the selected standard, you can use one of these following models:

- **Linear Fit** is a straight line model, which is useful only for a set of size standards spanning a very small range.
- **Quadratic Fit** adequately accounts for the nonlinear dependence of mobility on size under some conditions, but usually over relatively short size ranges that are well separated from the size limits of the separation system.
- **Cubic Curve** generally represents the curvature in a time or mobility versus size plot well enough that errors in estimated sizes are dominated by other factors, such as effects of primary structure on mobility. This is usually the best choice for routine use with the **SizeStandard-400** set.
- **Quartic Curve** is a fourth-degree polynomial that provides more accurate size estimates than a cubic curve when used for calibrations that span a long size range or extend into size areas where the size selectivity of the separation system is very nonlinear. This is usually the best choice for routine use with the **SizeStandard-600** set.
- Local Southern method estimates the size of a sample fragment from its mobility and that of the four standard fragments closest in size. If you select this method, the X Variable options are grayed-out and mobility is automatically selected.
- 5. Set the **Migration Variable** fields to either migration time or mobility as the independent variable for the Model. Size is the dependent variable.
 - **Migration time**: Select this option to use the average migration times and sizes of the standard fragments to construct a size calibration.
 - **Mobility**: Select this option to use fragment velocity (cm/s) divided by the separation potential (V/cm) with the sizes of the standard fragments to construct a size calibration.

Quantitation Tab

The Quantitation tab allows you to set the parameters for calculating the quantity of all peaks by comparing their heights or areas to a single peak of the same color whose quantity is known.

Figure 8-25 Edit Fragment Analysis Parameters Dialog - Quantitation Tab

💛 Edit Fragment Anal	ysis Parameters				
Parameter Set Name:	FA600				
Project:	Default				
General	Analysis <u>M</u> ethod	Quantitation	S <u>I</u> R Locus Tags	SN <u>P</u> Locus Tags Adv	anced
<u>s</u> enteral	· and fore <u>m</u> eaned				
ID Stand	dard		Calculate Using	9	- II
C Siz	:e		C Height		
⊙ Tim	ne		Area		
Quantita	ation Values				- II
	Time +/-	Window	Amount	Units	
D1 0	0	min 🛛	.00 E O	g 🗸	
D2 0	0	min C	.00 E O	• g 🔻	
D3 0	0	min 🖸	.00 E O	• g 💌	
D4 0	0	min 🛛	.00 E O	• g •	
Help		Save As		Save	Cancel

Once you have entered the information for the known peak into this window, the system looks for the reference peak in each trace using the specified size or time. Based on this information, the system can then calculate the quantity of each of the remaining peaks in the sample.

ID Standard

In the ID Standard area of the window, select one of the following options as the method the system uses to identify your reference peak:

Option	Description
Size	Select this option to identify your reference peak based on the fragment size specified in the Size field listed for each of the four dyes.

Option	Description
Time	Select this option to identify your reference peak based on the migration time specified in the Time field listed for each of the four dyes.

Calculate Using

In the Calculate Using area of the window, select the method the system uses to calculate the quantity of your reference peak:

Option	Description
Height	Select this option to calculate the quantity of your reference peak based on its peak height.
Area	Select this option to calculate the quantity of your reference peak based on its peak area.

Quantitation Values

Set the parameters for each dye. Enter values for each dye selection:

Option	Description
Size/Time	 These fields depend on the selected ID Standard: If you selected Size as the ID Standard, the first field next to the dye requires you to enter the size of the reference peak (nucleotides) for that dye. If you have selected Time as the ID Standard, the first field next to the dye requires you to enter the migration time of the reference peak (minutes) for that dye.
+/- Window	Enter a variable quantity to consider when identifying the reference peak. For example, if you listed the size of your reference peak as 90 nucleotides, you may enter a value of 1 in the +/- Window field. This value should compensate for any variability that might arise in identification of the reference peak.
	Note: The values entered in these fields are based on the ID Standard used, either +/- nucleotides (for Size) or +/- minutes (for Time).
Amount	Enter the known quantity of your reference peak.
E	Enter this value as the exponential notation.

Option	Description
Units	Select the units for the quantity entered into the Amount field. Values may be any of the following:
	Moles (mol)
	• Grams (g)
	Molarity (M)
	• (g/L)

STR Locus Tags Tab

The STR Locus Tags tab allows you to select the Short Tandem Repeat (STR) locus tags used for allele identification with the current parameter.

Note: Although the STR Locus Tags tab is similar in appearance and functionality to the SNP Locus Tags tab, the two types of Locus Tags are used for different purposes. STR Locus Tags use size to define allele fragments having a single specified dye label in a specified size range. SNP Locus Tags use multiple dye labels for fragments of a single specified size to define specific SNP variants.

The system uses locus tags to define a particular locus of interest. Each available locus tag is configured with a specific set of parameters that define the manner in which the system identifies detected fragments as allele fragments. These parameters include the fragment size range, repeat unit length, the dye used, and an allele list associated with the locus tag.

When you select a locus tag and perform an analysis, the system identifies the alleles present within the locus region using the estimated size of the fragments and the allele list.

Note: The system estimates sizes only if you specified a size standard (refer to the section: Analysis Method Tab) and calls alleles only if an allele list exists for the selected locus tag (refer to the section: Locus Tag Tab).

You can choose STR locus tags from a list of available STR locus tags previously configured for analysis. This tab also gives you access to the STR Locus Tag Editor, where you can edit existing or create new STR locus tags to meet the specific requirements of your samples.

Available:		STR Locus Tags SNP Locus Tags Advan
Name TPDX TH01 Penta E Penta D New Locus Tag Locus 1 GATA193A07_test2 GATA193A07 D9S2157 D8S1179 D7S820 D3S2387_2 D3S2387 D3S1358	Date ▲ 10/10/2005 6.38:10 P 10/10/2005 6.38:10 P 10/10/2005 6.38:10 P 10/10/2005 6.38:10 P 10/10/2005 6.38:10 P 8/9/2001 5:23:06 PM 4/10/2001 5:57:18 P 3/27/2006 12:44:51 P 4/19/2001 3:22:06 P 8/11/2001 11:54:19 A 10/10/2005 6:38:10 P 10/10/2005 6:38:10 P 8/11/2001 8:34:08 A 8/11/2001 7:31:46 A 10/10/2005 6:38:10 P 10/10/2005 6:38:10 P	Name Date GATA193A07_2 8/11/2001 10:07:40 A > < <

Figure 8-26 Edit Fragment Analysis Parameters Window - Selecting STR Locus Tags

Select STR Locus Tags

Select one or more locus tags you want included in your parameter set:

- To select an individual locus, select it in the **Available** list, then click the **right-arrow** (>) to move the selection to the **Selected** list.
- To select all available locus tags, click the double-right-arrow (>>) to move the selections to the Selected list.
- To remove locus tags from your parameter set, select the locus tag in the **Selected** list and click either of the left-arrow buttons to remove them from your parameter set.
- To edit a locus tag, highlight the selection and click **Edit Locus** to open the STR Locus Tag Editor window.
- To create a new locus tag, click **New Locus** to open the STR Locus Tag Editor window.

Note: For information about creating or editing locus tags, refer to the section: **STR Locus Tag** Editor.

SNP Locus Tags Tab

The SNP Locus Tags tab allows you to select the locus tags used for SNP analysis with the current parameter set.

Use SNP locus tags to detect the presence of specific Single Nucleotide Polymorphisms (SNPs) in the group of sample data selected for the Study. SNP locus tags are much the same in many respects as STR locus tags. Each SNP locus tag is configured with a specific set of parameters that tell the system what fragment size to look for and to assign an allele name based on the color (dye label) of the identified fragment.

The results produced from a SNP analysis looks the same as those produced by an STR analysis. Fragments identified as alleles of a locus are labeled as such in the fragment list. No more than one fragment labeled with a given dye can belong to the same locus (up to four peaks can be labeled as alleles of the same locus, but each must be labeled with a different dye).

You can choose SNP locus tags from a list of available SNP locus tags previously configured for analysis. This tab also gives you access to the SNP Locus Tag Editor, where you can edit existing or create new SNP locus tags to meet the specific requirements of your sample.

<u>G</u> eneral	Analysis <u>M</u> eth	od <u>Q</u> uantit	ation	∫ s <u>i</u>	R Locus Tags	SNP Locus Tags	Ad <u>v</u> anc
Available:					Selected:		
	Name	App Frag Size			Nam	e App Frag	g Size
795512-L	44	41.2					
914994-L	29New SNP Lo	32.1	A				
937275-L	43	66.2	Α	>			
944227-L		53.3					
944819-L		72.1	A	<			
	eterozygous A/	35.38	A				
	omozygous C	26.38		>>			
	omozygous T	48.87					
SNP1		18.47	A	<<			
SNP2		25	Α	~ ~ ~			
SNP3		30.39	A				
SNP4		16.7	A				
SNP5		23	A				
SNP6		25			L .		
•			•		•		•

Figure 8-27 Edit Fragment Analysis Parameters Window - Selecting SNP Locus Tags

Select SNP Locus Tags

Select one or more locus tags you want included in your parameter set:

- To select an individual locus, select it in the **Available** list, then click the**right-arrow (>)** to move the selection to the **Selected** list.
- To select all available locus tags, click the double-right-arrow (>>) to move all of them to the Selected list.
- To remove locus tags from your parameter set, select each locus tag in the **Selected** list and click either of the left-arrow buttons to remove them from your parameter set.
- To edit a locus tag, select it and click **Edit Locus** to open the SNP Locus Tag Editor window.
- To create a new locus tag, click **New Locus** to open the SNP Locus Tag Editor window.

Note: For information about creating or editing locus tags, refer to the section: SNP Locus Tag Editor.

Run a SNP Analysis

The following list identifies the three tasks you must complete before running a SNP analysis.

- 1. Select the **SNP Locus Tags** as part of the analysis in the SNP Locus Tags tab of the Analysis Parameters window. Refer to the section: **SNP Locus Tags Tab**.
- 2. Select **SNP Analysis** in the General tab of the Analysis Parameters window. Refer to the section: General Tab.
- 3. Select an appropriate **SNP Size Standard** (Size Standard-80) in the Analysis Method tab of the Analysis Parameters window. Refer to the section: Analysis Method Tab.
 - A size standard with exactly two fragments must be selected for SNP analysis to succeed.
 - The size standards must bracket all expected SNP fragments.
 - Both of these fragments must be selected.
 - The only valid model for a two-fragment standard is the Linear model.

These three requirements enable you to successfully run the SNP analysis, even if you do not select the mobility calibration, but the values obtained might vary slightly.

If you want to correct for the mobility differences between the reference fragments and each of the four possible dye-labeled SNP fragments, use the Advanced tab of the Analysis Parameters window to select the **SNP Dye Mobility Calibration**. Refer to the section: Advanced Tab.

Note: The SNP Ver. 1 Dye Mobility Calibration is for use with the GenomeLab SNP Primer Extension Kit and the SNP Ver. 2 calibration is for use with the GenomeLab SNPStart Primer Extension Kit.

Advanced Tab

Set additional fragment analysis parameters. From this window, you can assign dye mobility correction values to compensate for the varying effects of different dyes on fragment mobility, select a standard mobility reference, and indicate whether to use the dye spectra from sample data or from saved system spectra.

	Analysis Parame						_ [□]
Parameter Set Na	ame: GATA1934	.07					
Project:	Default						
<u>G</u> eneral	Analysis <u>M</u> et	hod Quar	ntitation S	<u>T</u> R Locus Tags	SN <u>P</u> Locus Tag	S Ad <u>v</u> ance	ed
	- Mobility						
	Dye Mobility Ca	libration:	NoCorrection			· .	
		D1	D2	D3	D4	T	
	Coef. 1	0.000000	0.000000	0.000000	0.000000		
	Coef. 2	0.000000	0.000000	0.000000	0.000000		
	Coef. 3	0.000000	0.000000	0.000000	0.000000		
	Coef. 4	0.000000	0.000000	0.000000	0.000000		
	Standard mobility	v reference:	[None]		<u> </u>	3	
ſ	-Dye Spectra						
	O Use calculation	ated dye spectra					
	O Use system	n dye spectra	₽ D1	₽ D2 🖪	🗖 D3 🔽 D4		
Help		S	ave As		9	Gave Ca	ncel

Mobility

Set up the mobility parameters for the current analysis.

Option	Description
Dye Mobility Calibration	Specifies the calibration from the options available in the list. Based on your selection, this option adjusts migration times or mobility to compensate for the varying effects of different dyes on fragment mobility. To choose a value, select any of the following options from the list:
PA ver.1	Select this option when analyzing samples made from primers labeled with the phosphoramidite dyes (D2-PA, D3-PA, or D4-PA).
AE ver.2	Select this option to improve calibration and size estimates for primers labeled with the active ester dyes (D1, D2, D3, or D4).
AE ver.1	Select this option for the Fragment Analysis test sample.

Option	Description
SNP ver. 1	Select this option when performing a SNP analysis using the GenomeLab SNP Primer Extension. The SNP analysis can proceed if you do not select this mobility correction, but the values obtained may vary slightly.
SNP ver. 2	Select this option when performing a SNP analysis using the GenomeLab SNPStart Primer Extension Kit.
No Correction:	Keep this default value when you do not want the system to make any compensation for dye effects on fragment mobility.

Note: When performing a Fragment Analysis Test Sample, you must use the **AE ver.1** dye mobility calibration for correct sizing of the fragments.

Dye Spectra

Dye Spectra values are calculations of the relative emission intensities of each dye in each of the four detection channels. The system calculates these values during each analysis and uses them to transform the four electropherogram channels to dye signal traces for each of the dye labels present in the sample.

When unable to calculate a dye spectrum from the sample, the analysis process terminates unless system dye spectra are available.

The **Dye Spectra** options allow you to select an existing set of dye spectra to use instead of estimating the dye spectra from each sample.

Note: This option can shorten the analysis time by up to 50%.

The system stores only one set of system spectra, used automatically when the system cannot estimate one or more of the needed spectra from the sample data.

Select one of these **Dye Spectra** options:

Option	Description
Use calculated dye spectra	This default setting specifies using the set of dye spectra obtained from the sample data.
Use system dye spectra	When you select this option, you must also select each Dye check box for the dye traces you want generated. This setting applies the system dye spectra to the data, instead of estimating the dye spectra from the current sample being run.

Note: The system dye spectra is established when running the Fragment Analysis Test Sample during installation when viewing the Dye Matrices window for a sample result in which spectra for all four dye labels were estimated.

After you run a Fragment Analysis Test Sample, the system shows a Spectral Matrix of this sample in the single result view under the Dye Matrices tab, which provides a **Save as System Dye Spectra** option.

STR Locus Tag Editor

The STR Locus Tag Editor allows you to create a new Short Tandem Repeat (STR) Locus Tag or edit an existing one to be used for STR analysis. You can access this editor from the STR Locus Tag tab of the Analysis Parameters window. Refer to the section: STR Locus Tags Tab.

roject: GATA193A07 Date last modified: 4/19/2001 3:22:06 PM Locus Tag Allele ID Criteria Locus Information Locus Name: GATA193A07 Dye: D3 Fragment length range between: 340 340 and 393 nt Hepeat unit length: 4 4 nt Hoidy: 2 Primer Label Forward: 5' to 3' Numeric Start at: 1 Generate Allele List	.ocus Tag Editor			
roject: GATA193A07 Date last modified: 4/19/2001 3.22:06 PM Locus Tag Allele ID Criteria Locus Information Locus Name: GATA193A07 Dye: D3 ▼ Fragment length range between: 340 and 393 nt Repeat unit length: 4 ÷ nt # of repeats in shortest Allele: 1 * Allele Naming Convention Nominal size Allehabetic Start at A # Generate Allele List ID Nominal Size (nt) Apparent Size (nt) Std. Dev. (nt) Num. Points Comment I 340 340.8 0.00 0 I 2 344 344.17 0.00 0 I 2 344 344.2 340.8 0.00 0 I 2 344 344.2 0 I 3 348 348.26 0.00 0 I 2 344 344.2 0 I 3 348 348.26 0.00 0 I 2 344 348.2 348.2 0 I 2 344 344.17 0 I 2 344 344.17 0 I 2 344 344.17 0 I 2 34	ocus Tag: GATA193A07	Save As		
Locus Information Locus Name: GATA193A07 Dye: D3 Primer set name: GATA193A07 Dye: D3 Fragment length range between: 340 and 393 nt Repeat unit length: 4 4 nt # of repeats in shortest Allele: 1 Allele Naming Convention Shortest Allele size: Nominal size Shortest Allele size: Numeric Start at: Allele List Image: Start at: ID Nominal Size (nt) Apparent Size (nt) Std. Dev. (nt) Num. Points II 340 340.08 0.00 0 2 344 344.17 0.00 0 3 348 348.26 0.00 0 3 348 348.26 0.00 0 5 356.49 0.00 0 0	roject: GATA193A07 Date last modified: 4/19/200			
Locus Name: GATA193A07 Dye: D3 Fragment length range between: 340 and 393 nt Repeat unit length: 4 # of repeats in shortest Allele: 1 Allele Naming Convention • Nominal size Notest Allele size: CAlphabetic Start at A • Numeric Start at 1 Benerate Allele List Numeric Start at 1 Benerate Allele List Benerate Allele List Benerate Allele List Benerate A	Locus Tag Allele ID Criteria			
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Repeat unit length: 4 nt # of repeats in shortest Allele: 1 Allele Naming Convention 1	Dye: D3 S GenBank Accession:			
Repeat unit length: 4 # of repeats in shortest Allele: 1 * Nominal size Shortest Allele size: * Alphabetic Start at: * Alphabetic Start at: * Numeric Start at: * Ide List * Ide List * Ide Alge and a start at: * Ide Alge and a start at: <	Fragment length range between: 340 and 393 nt	. Insta		
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5 356 356.49 0.00 0 6.2 358 358.20 0.00 0				
6.2 358 358.20 0.00 0				
	16 360 0.00 U			
		F		
Help OK Cance		OK Cancel		

Figure 8-29 Locus Tag Editor

The Locus Tag Editor contains two tabs for creating or editing locus tags: the Locus Tag tab and the Allele ID Criteria tab. The top of the window shows the locus name and project.

You can save new or edited locus tags to a selected project for use in additional analyses.

Locus Tag Tab

Use the Locus Tag tab to define the general locus and supplementary reporting information. These parameters include the fragment size range, repeat unit length, and dye to be used. This is the minimum amount of information necessary to generate an allele list.

Locus Information

The Locus Information area of the window contains parameters used to generate a list of expected alleles in your analysis. Locus Information includes the following fields:

Option	Description
Locus Name	Enter a locus identification in this field. This is an optional field.
Dye	Select the dye to be used for the locus from the list.
Fragment length range between	Enter the fragment length range (the minimum and maximum fragment size associated with the locus) in these two fields. This defines the range in which the system searches for the particular fragment.
Repeat unit length	Enter the length of the repeat unit that represents the allele.
# of repeats in shortest Allele	Select the number of repeats in the shortest allele from the list. Variant alleles that contain a particular repeat are designated by a decimal, followed by the number of bases in the partial repeat. For example, a variant allele with the designation 5.3 contains 5 complete repeat units and a partial repeat unit of 3 nucleotides.

Naming Alleles

Select the method in which the system labels the identified alleles in the fragment list. For example, you can label the alleles the same as their expected fragment size in nucleotides (163, 164, 167,... etc.), in alphabetic order (A, B, C,...etc.), or in simple numeric order (1, 2, 3,...etc.).

Select the method in which to name the alleles in the fragment list:

Option	Description
Nominal size	Select this option to label the alleles by their nominal fragment size.
Alphabetic	Select this option to label the alleles in lettered order, then select the letter in the Start at field.
Numeric	Select this option to label the alleles in numeric order, then select the number in the Start at field.

Note: With the exception of **Ploidy**, the following fields are not required for any part of the analysis. They are used for supplementary reporting purposes only.

Ancillary Information

The Ancillary Information area of the window allows you to select any supplementary reporting information to include with the locus tag parameters. This area includes the following fields:

Option	Description
Primer set name	Enter the name of the primer set.
GenBank Accession	Enter the GenBank Accession Number.
Repeat unit sequence	Enter the sequence of the repeat unit associated with the locus tag.
Ploidy	Enter the number of alleles associated with the locus tag.

Primer Label (Optional)

The Primer Label area of the window allows you to indicate (for supplementary reporting purposes) on which strand the primer is labeled with the dye. You may choose one of these options:

Option	Description	
Forward	Select this option if the primer is dye labeled on the forward strand.	
Reverse	Select this option if the primer is dye labeled on the reverse strand.	
None	Select this option if you do not want to indicate which strand the primer is dye labeled.	

Primer Sequence (Optional)

The Primer Sequence area of the window allows you to indicate (for supplementary reporting purposes) the specific sequence of the primer used. This can be up to 128 characters.

Option	Description
Forward	If you labeled the primer on the forward strand, enter the sequence of the primer in the 5' to 3' direction.
Reverse	If you labeled the primer on the reverse strand, enter the sequence of the primer in the 5' to 3' direction.

Generate an Allele List

After setting the locus values, click Generate Allele List to begin creating the allele list.

The system generates an allele list from the information provided. The allele list starts at the low end of the fragment length range. It creates an allele at each perfect repeat length until it reaches the end of the fragment length range.

Column	Description
ID	Uses the allele naming convention described in the section above.
Nominal Size	Shows the generated fragment lengths as the initial integer values. You can modify these values to represent the allele sizes that show when the data is analyzed. The nominal sizes are also shown in the apparent size column, but these will usually be edited or regenerated.
Apparent Size	Shows the fragment lengths, which are either entered using the locus tag editor, generated by interpolation or regression from an incomplete list, or generated from the automatic binning analysis feature. Refer to the section: Perform Bin Analysis.

The Allele List defines each allele under the following columns:

When apparent fragment lengths are user-entered or generated by interpolation or linear regression, you must enter the nominal sizes. When the apparent fragment lengths are generated by binning analysis, the nominal sizes are automatically generated.

Refer to the following pages for more information regarding generation of the allele list using interpolation or linear regression.

- Std. Dev. and Num. Points: Describe the number of points included in each bin and the sample standard deviation. Both of these columns are generated using the automatic binning feature (refer to the section: Perform Bin Analysis) and are empty for allele lists not based on a bin analysis.
- **Comment**: Shows any user comments associated with the allele.

Interpolation and Linear Regression

You can generate an allele list from a list of nominal sizes and a partial list of apparent sizes using Interpolation and linear regression.

- Interpolation calculates the apparent fragment sizes between two selected points.
- Linear regression calculates the *line of best fit* for the selected alleles. The system uses this line to determine the apparent sizes for all points not selected. This option requires you to select more than two apparent fragment sizes.

To generate an allele list using one of these methods:

- 1. Individually edit the apparent fragment sizes within the allele list from which you want to generate interpolation or a linear regression line.
- 2. Select the alleles you want to use for generating the allele apparent sizes:
 - To select the rows within the allele list, click the row header of the allele list.
 - To select multiple consecutive alleles, press and hold the **Shift** key while dragging through the headers.
 - To select nonconsecutive rows, press and hold the **Ctrl** key while selecting each of the rows with the left mouse button.
- Right-click your selection and select Interpolate or Regression. The Interpolation or Linear Regression function replaces existing or missing apparent fragment sizes or supplies the generated values.

Allele ID Criteria Tab

The Allele ID Criteria tab contains the parameters used to accurately identify your alleles and to set these "true" alleles apart from various other anomalies identified in the fragment list.

A sample fragment represents a particular allele if its apparent size is similar to the size of a particular allele fragment in the allele list. However, because both the sequence and size of a fragment affects its mobility, the nominal allele size will not likely be the same as its apparent size. Therefore, the system identifies the alleles based on their "closeness" to an apparent size, using a user-defined confidence level.

Confidence Interval

You can either enter the confidence interval manually or generate it automatically using the bin analysis. When automatically generated, the value that shows depends on the overall standard deviation computed in the bin analysis and the user-specified confidence level (default = 95%). When you manually enter the confidence interval, the system uses the entered value and the confidence level to estimate an overall standard deviation.

Increasing the confidence interval increases the width of the bins. You should set the confidence interval to a value less than 0.5 nt.

If you enter a value of zero in the **Confidence Interval** field, the system uses only the standard deviation of the estimated sample fragment size in the statistical test used to identify a fragment as corresponding to an allele.

When you enter the confidence interval, the allele identification will not correspond to an error probability estimated from actual data, but is still a useful relative indicator of the "goodness" of the match.

- If would like to overwrite the confidence interval value generated by automatic binning, select the **Overwrite system confidence interval** check box.
- If you are overwriting the system generated value, specify a confidence interval (number of nucleotides (+/-)) in the field provided.

Figure 8-30 Locus Tag Editor Window - Allele ID Criteria Tab

Locus Tag Allele ID Criteria Stutter Definition ✓ Search for Stutter Stutter detection window width ↓ repeats Maximum relative stutter peak height ↓ Detect stutter shorter than alelle ↓ Detect stutter longer than alelle ↓ Allele List	Save <u>A</u> s wified: 4/19/2001 3:22:06 PM urious Peak Detection Detect spurious peaks Maximum height for spurious peaks <u>30 </u> <u>=</u> ≈ ≈
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System generated allele confidence interval 0.00 +/- nt ☑ Overwrite system confidence interval 0.5 +/- nt ID Nominal Size (nt) Apparent Size (nt) Std. Dev. (nt) Nt 1 340 340.08 0.00 2 344 344.17 0.00 3 348 348.26 0.00 4 352 352.33 0.00	
System generated allele confidence interval 0.00 +/- nt ☑ Overwrite system confidence interval 0.5 +/- nt ID Nominal Size (nt) Apparent Size (nt) Std. Dev. (nt) Nu 1 340 340.08 0.00 2 344 344.17 0.00 3 348 348.26 0.00 4 352 352.33 0.00	
ID Nominal Size (nt) Apparent Size (nt) Std. Dev. (nt) Nu 1 340 340.08 0.00 2 344 344.17 0.00 3 348 348.26 0.00 4 352 352.33 0.00	Detect +/- A Use +A peak to call Allele
2 344 344.17 0.00 3 348 348.26 0.00 4 352 352.33 0.00	m. Points Comment
3 348 348.26 0.00 4 352 352.33 0.00	0
4 352 352.33 0.00	0
	0
IS 1266 : 26679; UUU	0
	Π
6.2 358 358.20 0.00 6 360 360.55 0.00	
0 300.33 0.00	0
	0

Stutter Definition

Stutter consists of small peaks, generally DNA amplification artifacts that form as a result of halted polymerase activity. When this happens, allele fragments form that differ in length from the true allele by an integral number of repeats (such as n-1, n-2, etc.). The absolute size of a fragment is a function of the variety of repeats (for example, dinucleotides, trinucleotides).

The Stutter Definition area of the window allows you to set the parameters for which the system can identify these peaks as stutter and not as true alleles.

Option	Description
Search for Stutter	Select this option if you want the system to search for stutter. When selected, you must also choose at least one of the stutter options below it.
Stutter detection window width	Select the number of repeats to specify the width of the window around each allele in which the system detects the stutter.
Maximum relative stutter peak height	Enter the height percent of the stutter relative to the true allele below which detected peaks are considered stutters. Peaks above this threshold are considered unknown alleles.
Detect stutter shorter than allele	Select this option to detect stutters shorter than (occurring prior to) the allele.
Detect stutter longer than allele	Select this option to detect stutters longer than (occurring after to) the allele.

Spurious Peak Detection

Spurious peaks are generally small trace artifacts detected as a result of sample contaminants. These peaks tend to have a different shape than fragment peaks but often fall within the size range of a locus tag and are detected. However, they generally do not meet the criteria to be identified as known alleles and are therefore labeled as "spurious" peaks.

The Spurious Peak Detection area allows you to direct the system to look for spurious peaks in your analysis. You can specify a height threshold under which the system identifies a peak as a contaminant and not include it in your allele list.

To enable this option:

- 1. Select **Detect spurious peaks** if you want the system to remove any peaks that are below the maximum height threshold for spurious peaks.
- 2. In the **Maximum height for spurious peak** field, enter the percent of the spurious peak relative to the true allele below which detected peaks are considered spurious peaks.

+A Detection

+A Detection is an allele calling criteria used to compensate for a common DNA polymerase phenomenon of adding a nontemplate-directed deoxyadenosine to the ends of PCR products. The result is a mixture of PCR products, some of which are template "true length" (called -A products) while others are one nucleotide longer (called +A products).

The ratio of these two types of products can vary greatly between different reactions and primers. The result of having different fragment sizes from a single allele complicates the interpretation of fragment analysis data. Instead of one peak for one allele, there may be two that represent the same allele spaced one nucleotide apart.

The +A Detection area of the window allows you to specify how the system will differentiate between the true allele and the +A allele product.

Note: You can select more than one of these check boxes at a time. Read through the following descriptions or refer to the tables below to see how these selections affect +A Detection and the allele peak assignments.

Option	Description	
Apparent size includes +A	Selecting this check box indicates that the sizes entered into the Apparent Size column of the allele list were for the +A fragments. The parameter affects where the system looks for peaks when identifying alleles.	
Detect +/- A	 Selecting or clearing this check box determines: When the Detect +/- A check box is selected, the system identifies in the fragment list either the +A or –A peaks. The system then identifies one of them as the true allele based on the selection in the Use +A peak to call Allele check box. It designates the other peak as either +A or –A accordingly. 	
	 When the Detect +/- A check box is not selected, the system identifies one of the two alleles as the true allele based on the selection in the Use +A peak to call Allele check box. It considers other peaks on the fragment list as unknown alleles. 	
Use +A peak to call Allele	 Select this check box if the +A form is expected to be the higher peak and leave it unchecked if the –A form is expected to be the higher peak. If the Use +A peak to call Allele box is selected, the system identifies the +A peak as the true allele in the fragment list when the Detect +/- A box is also selected. It designates the other peak as either +A or –A accordingly. 	
	• If the Use +A peak to call Allele box is cleared, the system identifies the +A peak as the true allele in the fragment list when the Detect +/- A box is also cleared. The other peak will be included in the fragment list as an unknown allele.	
	• If the Detect spurious peaks check box is selected and the peak falls below the maximum height designation for spurious peaks, the other peak will be included in the fragment list as a spurious peak.	

Note: The only time that the **Use +A peak to call Allele** check box is selected while the **Detect +/-A** check box is cleared is when there is complete conversion of the PCR product to +A. When neither the **Use +A peak to call Allele** nor the **Detect +/-A** is selected, no +/-A labeling can occur.

In general, the reproducibility of size estimates is better for higher peaks than for lower peaks. Therefore, it is good practice to direct the system to use the higher of the peaks associated with the allele to identify the allele.

Additionally, it is generally a good idea to check the **Detect +/- A** option so the system correctly categorizes peaks. If the option is incorrectly configured, the alleles are still identified, but the +A and –A peaks are frequently categorized as unknown alleles and the system reports Too many alleles in the Comment column of the fragment data table.

+A Detection	Use +A peak to call Allele	Apparent size includes +A
	Cleared	Selected
Use +A peak to call Allele Selected	No size adjustment.	One nucleotide is subtracted from the apparent size of the allele in the allele list.
Apparent size includes +A Cleared	One nucleotide is added to the apparent size of the allele in the allele list.	No size adjustment.

Table 8-1 Apparent Size Adjustments

Table 8-2 Truth Table for Setting +A Allele Identification

+A Detection	Detect +/- A	Detect +/- A		
	Selected	Cleared		
Use +A peak to call Allele Selected	All fragments assumed to be +A. The +A fragment is identified as the allele in the allele table. Fragments without +A (if any) are identified as –A peaks or spurious peaks.	All fragments assumed to be +A. The +A fragment is identified as the allele in the allele table. Fragments without +A (if any) are identified as unknown alleles or spurious peaks.		

+A Detection	Detect +/- A	Detect +/- A		
	Selected	Cleared		
Use +A peak to call Allele Cleared	All fragments assumed not to be +A. Any fragments without +A are identified as the allele in the allele table. Fragments with +A are identified as +A peaks or spurious peaks.	All fragments assumed not to be +A. Any fragments without +A are identified as the allele in the allele table. Fragments with +A are identified as unknown alleles or spurious peaks.		

Table 8-2 Truth Table for Setting +A Allele Identification (continued)

SNP Locus Tag Editor

Use the SNP Locus Tag Editor to create a new SNP locus tag or edit an existing one to be used for SNP analysis. You can access this editor from the SNP Locus Tag tab of the Analysis Parameters window. Refer to the section: SNP Locus Tags Tab.

Figure	8-31	SNP	Locus	Tag	Editor	Window	- (Creating	a New	Locus	Tag
5		-						J 20			

💖 SNP Locus Tag Eo	ditor	
Locus Tag:	SNP1	Save As
Project:	ddSNP Allele ID	
Locus Name:	SNP1	
Apparent Fragment Size:	18.47	
Ploidy:	2	
Allele ID's for		
D1:	A	
D2:	С	
D3:	G	
D4:	Т	
Accession #:		
Primer Sequence:		
Help	OK	Cancel

The Locus Tag Editor provides the following fields:

	•		
Field	Description		
Locus Tag	Enter or edit the name of the locus tag in this field.		
Project	Select the project to which the new or edited locus should be saved.		
Locus Name	Enter the identification of the locus in the field. This is the locus tag name that appears in the fragment list for all fragments that belong to this locus.		
Apparent Fragment Size	Enter a real number greater than 0 and less than or equal to 150. This represents the estimated size in nucleotides that the user expects for fragments belonging to this locus.		
Ploidy	This field is inactive for this system release.		
Allele ID's for	This area provides fields for Allele IDs D1 , D2 , D3 , and D4 . Each field accepts 8 character alphanumeric values used to label the Allele IDs in the fragment list. The default values are A, T, G & C. These fields cannot be blank.		
Accession #	This field is not used for analysis and is for supplementary reporting purposes only. This field is optional.		
Primer Sequence	This field is not used for analysis and is for supplementary reporting purposes only. This field is optional.		

Table 8-3 Locus Tag Editor Fields

Apparent sizes of SNP probe fragments as estimated with SNP reference fragments often differ more from actual sizes than the STR allele fragments do. This is due to the effects of primary structure on fragment mobilities.

Therefore, it is important that the apparent size entered into the SNP locus tag definition is a known value. This is best determined in preliminary runs as the average apparent size of all of the possible variants that might occur in a Study.

Because the effects of different dye labels are relatively greater on shorter fragments, it is essential that in include the **Dye Mobility Calibration** in the parameter set for a SNP analysis.

Filter the Result Set

The first steps in creating a Study involve applying a set of fragment analysis parameters to analyze the raw sample data. These parameters are specifically configured to process the fragment data for a particular sample or set of samples. The outcome of this analysis is a result set of data consisting of characterized fragments and identified alleles.

You can then use specific filters to scrutinize and selectively isolate results based on the parameters of the applied filters. In other words, the types of exclusions that you apply to the data filter out all unwanted results from your Study. This process is known as *result set filtering*.

Access the Result Set View Window

You can view the Result Set View window while working with a Study using one of these methods:

- Select View > View Result Set.
- Click the Results Set icon

😽 Fragment Analysis - New Study *						
File View Results Analysis Reports	; <u>W</u> indow <u>H</u> elp					
D1 D2 D3 D4						
Study Explorer			_	_		
	-					
Study	🖗 Result Set View					
- Binnings						1
Peak Ratios	Results				Exclusion Filter Sel	
	result	result date	capillary	analysis outcome	New Result Filter Set 1	
	DATS 93-D4.A01_06042512ZM	04/25/2006 12:59:56	A	pass	ID Name	Operator Value(s)
	DATS 94-D4.B01_06042512ZK	04/25/2006 12:59:50	В	pass	1 <select></select>	
	DATS 96-D4.C01_06042512ZI	04/25/2006 12:59:44	С	pass		
	DATS 97-D4.D01_060425122F	04/25/2006 12:59:36	D	pass		
	DATS 99-D4.E01_06042512ZC DATS 100-D4.F01_06042512Z9	04/25/2006 12:59:26	F	pass		
	DATS 100-04.F01_0604251225	04/25/2006 12:59:20 04/25/2006 12:59:12	G	pass pass		
	DATS 102-D4.H01_0604251224	04/25/2006 12:59:06	H	pass		
		0112012000 12:00:00				
	•			Þ		
		DEF		Tatal		
	A B C Included 1 1 1		i H 1 1	Total 8		
	Excluded 0 0		0 0			
	Total 1 1 1	1 1 1	1 1	8	•	
				Þ	Show Excluded	
Analyses Data Reports						
Ready						

Figure 8-32 Result Set View Window - Second-Level Filtering

The Result Set View window provides three key elements:

Option	Description
Fragment Results table	Shows all analyzed samples, both included and excluded, that have been selected for the Study. The columns that show are user-defined and can be modified to show a number of different system variables and information.

Option	Description
Exclusion Filter Set area	Where the user-defined exclusion filters are selected and applied to the data. Each column contains a menu of parameters to be applied as exclusion filters. The system comes equipped with one predefined sets of exclusion filters. You can create, modify, and save additional filter sets.
Summary area	Shows a table that identifies the number of analyses that have been affected by the application of exclusion filters. As the result set is manipulated, you can view the number of samples that have been excluded from your Study based on your filter set parameters.

Note: The filter sets applied to the Result Set data (second-level filtering) are different from the filter sets applied to the Fragment List data (3rd Level Filtering). In the Result Set, you apply filters to an entire sample that might contain numerous fragments. In the Fragment List, you apply filters to individual fragments that have been identified within the various samples. For more information, refer to the section: Fragment Lists.

Customize the Result Set View Window

You can define the fragment result components available in the Result Set View window. You can also modify them using right-click commands. These commands allow you to select specific columns to show in the fragment results, customize the arrangement of fragment results information, and modify several other parameters of the fragment results.

For details about using the Column Selector window, the Column Sort window, and other display parameters, refer to the section: Customize the Results List.

Apply Exclusion Filters to the Result Set Data

A *filter set* is a collection of any number of filters that are either pre-defined by the system or user-defined. The purpose of the filter set is to exclude any results that do not meet the criteria of the Study and to include results that do.

There are many filters available. You must apply each one using an operator and a value. You can apply any number of filters to a result set and save the filter sets for future use.

You can assign filter sets to filter out samples with excessive baseline noise, excessively broad peaks, no size standard, out of calibration range, etc.

This *second-level filtering* leaves only the results you are sure of and saves you the time of removing unwanted fragment data later.

For example, suppose that your result set contains multiple samples collected under two different sets of fragment analysis parameters. One of these parameter sets used the

SizeStandard-400 to determine the fragment sizes while the second parameter set used the **SizeStandard-600** to make the determination.

To exclude any data that were analyzed with the **SizeStandard-600**:

- 1. Open the list in the Name column of the Filter Set area and select SS (Size Standard).
- 2. Open the list in the **Operator** column of the Filter Set area and select =.
- 3. Open the list in the Values column of the Filter Set area and type in SizeStandard-600.
- 4. Click **Apply** at the top of the Filter Set area.

All samples in the fragment results table that used the **SizeStandard-600** are highlighted in teal (visible by selecting this parameter for display). This indicates that they have been excluded from the Study. The Excluded column in the Summary area has increased by the number of samples using the **SizeStandard-600**.

Find Exclusion Filters

You can find all of the exclusion filters in the list in the Name column of the Filter Set area.

After selecting the name of the filter to use, you must select an operator (such as +, <, >=) and a value to be applied to the filter.

Note: Refer to the Help System for detailed descriptions of the available exclusion filters and their use.

View the Summary

The Summary area shows a table that identifies the number of samples that have been affected by the application of exclusion filters. As you manipulate the result set, you can view the number of samples excluded from your Study based on your filter set parameters.

The Summary area shows the following:

- The number of results that are included in result set within each sample capillary (**A-H**). As samples are filtered out of the result set by the exclusion filters, they are moved from this row to the "excluded" row.
- The number of results that have been excluded from the result set by the exclusion filters.
- The total number of all samples, both included and excluded, that are part of the result set.

View Electropherogram Data

You can view individual sample traces at any time, either individually or in selected groups along with the parameters used or collected during the separation and analysis steps. Sample traces can also be stacked or overlaid on top of one another for comparison purposes. All of these

features are available through the right-click menus whenever a single or collection of samples is highlighted.

Single Sample View Window

The Single Sample View window shows all of the available information for an individual data sample, including the collected trace views, fragment data, separation and analysis parameters, calibration curve, run log, and more. The top of the window shows the name of the individual data sample selected. All of the information pertaining to the data sample is distributed under several different tabs within this window.

Note: Refer to the Help System for detailed descriptions of each of the available tabs on the Single Sample View window.

To access the Single Sample View window:

- 1. Right-click the highlighted sample (or multiple samples) from any of the data or fragment lists.
- 2. Select Show Single Result.

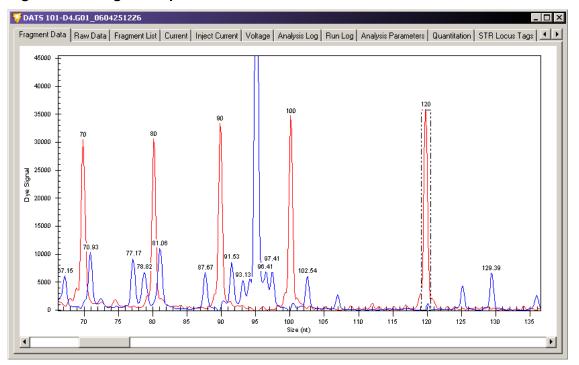


Figure 8-33 Single Sample View Window

Tab	Description
Fragment Data	Shows a plot of the dye signal traces.
Raw Data	Shows a plot of the raw, four-channel electropherogram.
Fragment List	Shows a list of all fragments that meet the minimum relative height criterion set in the parameter set. This view shows all available fragment properties.
Current	Shows a graph of the separation current versus time for the duration of the separation event.
Inject Current	Shows a graph of the injection current versus time for the duration of the injection event.
Voltage	Shows a graph of the separation voltage versus time for the duration of the separation event.
Analysis Log	Shows the list of messages generated by the primary analysis program during the analysis of the selected result. The analysis log contains information that can help determine the cause of an analysis failure.
Run Log	Shows the list of messages generated by the GenomeLab GeXP system during the pre-separation and separation events of the eight samples that include the individual selected result. Use the run log to troubleshoot the data in the case of a run failure.
Analysis Parameters	Shows a summary of the fragment analysis parameters applied to the raw data in the selected result.
Quantitation	Shows a summary of the peak information used to estimate fragment amounts within the selected result.
STR Locus Tags	Shows a list of STR locus tags that have been applied to the raw data in the selected result.
SNP Locus Tags	Shows a list of SNP locus tags that have been applied to the raw data in the selected result.
Size Calibration	Shows a description of the size standards and calibration curve used to calculate the sizes of the unknown fragments in the selected result.
Dye Matrices	Shows the dye spectra and dye mobility calibration parameters used in generating the selected result.
General/Notes	Shows a list of system and user-generated notes specific to the selected result (for example, sample name, dates, database location, and hardware information).

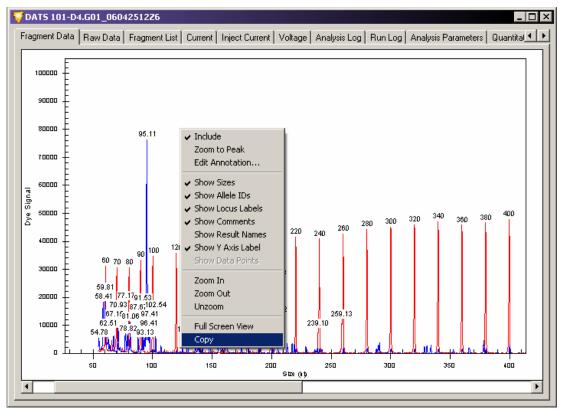
Tab	Description
Property Set	Shows a list of user-defined property fields and the values associated with the selected result. These parameters may be edited.
Method/ Consumables	Shows a summary of the separation methods, consumable parts and lot numbers that were used with the selected result.

Trace Copies

To copy a trace as an image to the clipboard:

1. Right-click the trace and select **Copy**.

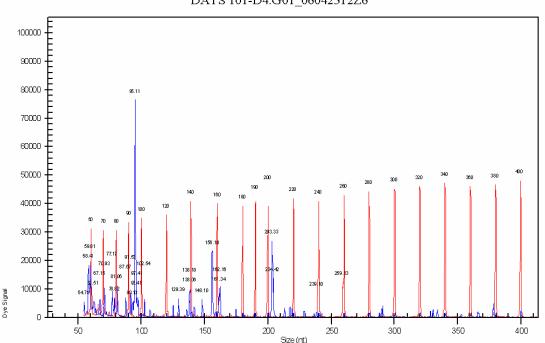
Figure 8-34 Copying a Trace to the Clipboard



The system copies subtitles with the image.

2. To paste the image in other software, use the **Paste** command.

Figure 8-35 Trace with Labels



DATS 101-D4.G01_06042512Z6

Show a Stacked Graph

The Stacked Graph function aligns individual selected traces by size or time. Zooming and scrolling on the X-axis is synchronous between all plots selected while the Y-axis of each plot selected is independently scaled, based on the available data.

To access this function:

- 1. Right-click the selected samples in the fragment list.
- 2. Select Show Stacked Graph.

Show an Overlay Graph

The Overlay Graph function superimposes the electropherograms of selected results on a single X/Y plot.

To access this function:

1. Right-click the selected results from any of the results or fragment lists.

2. Select Show Overlay Graph.

This function has two different modes. The default mode, **Zoom**, allows you to draw a rectangular box around a selected area to zoom into. The second mode, **Subset Selection**,

allows you to select a group of peaks and add them to a Stacked Graph view. To switch between the two modes, select the appropriate icon from the EgramBand toolbar.

Include and Exclude Results

You can exclude results automatically using filter sets or manually by selecting the **Exclude Results** command. You can re-include any excluded results at any time using the **Include Results** / **Peaks** command available in the right-click menu.

- To re-include results that were excluded in the Study, right-click the selected samples from any of the data or fragment lists and select **Include Results**. When showing excluded results, the screen uses a color code:
 - Teal shading identifies results excluded automatically using filter sets.
 - Maroon shading identifies results excluded manually.
 - Light gray shading identifies previously excluded results that have been re-included.
- 2. To hide both filtered and manually excluded results from view, clear the **Show Excluded** command in the right-click menu.

Note: Refer to the Help System for detailed descriptions of each of these and other display parameters.

Reanalyze the Results

The Fragment Analysis module contains a Reanalyze Results feature that allows you to select one or more samples from the Result Set View window to be reanalyzed. This allows you to apply a different set of fragment analysis parameters to a previously analyzed data set to pick up missed peaks or to avoid picking up unwanted peaks. The data resulting from the reanalysis automatically replaces the older Result Set data, allowing you to quickly continue with your Study.

To reanalyze the results:

- 1. Select one or more results in the Result Set View window.
- 2. Right-click the selected results and select **Reanalyze Results**.

The following options are available:

Option	Description
From Sample Data	Replaces the previous analysis result with a complete new analysis from raw data.
Using New Parameters	Allows you to modify the analysis parameters previously used.

Option	Description
Using Additional/ Edited Locus Tags	Does not change the basis analysis, but allows you to modify allele identification.

When selected, a message window opens prompting you to verify that you want to replace the selected results with reanalyzed results.

3. Click **Yes** to proceed with the reanalysis. The appropriate Fragment Analysis Parameters dialog opens, allowing you to select a new or an edited set of parameters to use for reanalyzing the data.

💛 Analysis Parameters	;	×
Steps Analysis Parameters Analyze Data		Select Analysis Parameter Set
		Parameter Set Summary Project: Default Date Modified: 8/11/2001 10:07:44 AM Dye Mobility Calibration: NoCorrection Size Standard: SizeStandard-400 Model: Cubic Locus Tags GATA193A07_2 B/11/2001 10:07:40 AM B/11/2001 10:07:40 AM
	Help	<< Erevious Next >> Errish Cancel

Figure 8-36 Selecting a Parameter Set to Be Used for Reanalysis

Note: For details on selecting, creating, editing, and saving fragment analysis parameter sets, refer to the section: Define Fragment Analysis Parameters.

- 4. Select an Analysis Parameter Set.
- 5. Click **Next** to proceed to the Reanalyze Data window.

alyze Data	Sample	Date	Status	Date Analyzed
	DATS 93-D4.A01_01060518QY	6/5/2001 8:23:53 PM	pass	4/25/2006 1:55:17 PM
	DATS 94-D4.801_01060518QY	6/5/2001 8:23:54 PM	pass	4/25/2006 1:55:24 PM
	DATS 96-D4.C01_01060518QY	6/5/2001 8:23:56 PM	pass	4/25/2006 1:55:30 PM
	DATS 97-D4.D01_01060518QY	6/5/2001 8:23:57 PM	pass	4/25/2006 1:55:37 PM
	DATS 99-D4.E01 010605180Y	6/5/2001 8:23:59 PM	Unanalyzed	
	DATS 100-D4.F01_01060518QY	6/5/2001 8:24:00 PM	Unanalyzed	
	DATS 101-D4.G01 01060518QY	6/5/2001 8:24:01 PM	Unanalyzed	
	DATS 102-D4.H01 01060518QY	6/5/2001 8:24:03 PM	Unanalyzed	
	Analyze Current Trace: End STATUS: Data dimensions, 10790 points + STATUS: Constant velocity migration offse STATUS: Constant velocity migration offse	t: 1.00 min.		A
	STATUS: Data dimensions, 10790 points x STATUS: Data rate: 2.0 Hz	t: 1.00 min. I rank map window: 22 points to 7204		▲ ▼

Figure 8-37 Reanalyze Data Window

Note: For details regarding the data analysis process, refer to the section: Analyze the Data.

6. Click **Analyze**. During analysis, the system activates a progress bar and shows an analysis log for each sample analyzed. When finished, the status column indicates if the reanalysis passed or failed for each sample selected for reanalysis.

Note: To stop an analysis currently in progress, click **Stop**. The system stops the analysis after completing the sample in progress.

- 7. Review the analysis log:
 - a. Select a sample in the upper samples list.
 - b. Scroll through the analysis information in the lower analysis log area of the window.
- 8. Click Finish to return to the Result Set View window. When finished reanalyzing the data, the system removes the initially selected results from the Study and replaces it with the reanalyzed results. The screen refreshes the Result Set View window, including the Summary area, with the new data and reapplies the selected filters to the entire set of data.

Note: Refer to the Help System for detailed descriptions of the reanalysis process.

Fragment Lists

The Fragment List window allows you to view and modify the results of the fragment data identified in the Study. You may perform a third-level of filtering on this fragment list by applying additional exclusion filters.

Access the Fragment List Window

To access the Fragment List window, use one of these methods:

- Select View > View Fragment List.
- Click the Fragment List icon

The Fragment List window opens to show a table of all analyzed fragments included in the Study.

💛 Fragment List _ 🗆 × Fragments: Exclusion Filter Set . New Fragment Filter Set 1 💌 Apply std frag est frag pk area pk height RN dye size std loc size (nt) size (nt) (rfu) (rfuxmm) ID Name Operator Value 1 2 3 size std DATS 93-D4.A01 58.46 16989 16727 dve D1 59.80 DATS 93-D4,A01 No 11000 10888 spurious peak 6187 = Yes DATS 93-D4.A01 70.93 No 4739 4 <select) DATS 93-D4.A01 78.85 3183 4107 No DATS 93-D4 A01 No 81.13 5057 6919 87.74 DATS 93-D4.A01 No 2006 3342 DATS 93-D4.A01 6519 91.61 4182 No DATS 93-D4.A01 95.18 31758 49648 No DATS 93-D4,A01 No 96.50 4032 6285 DATS 93-D4.A01 97.47 2818 4343 No DATS 93-D4.A01 No 102.63 2295 4024 DATS 93-D4.A01 No 129.46 2670 4848 DATS 93-D4 A01 No 138.16 3651 6483 DATS 93-D4.A01 No 148.20 3221 5784 DATS 93-D4.A01 156.23 10845 16745 No DATS 93-D4 A01 No 161.46 4555 8287 Ö DATS 93-D4.A01 162.26 4912 8939 No DATS 93-D4.A01 174.57 2139 3860 No 16676 DATS 93-D4.A01 203.43 12094 No DATS 93-D4 A01 No n 204 60 6093 8432 DATS 93-D4.A01 228.74 5064 No 2732 0 DATS 94-D4.801 58.18 544 No 753 DATS 94-D4.B01 No 59.62 321 627 Π DATS 94-D4 B01 540 No 60.63 DATS 94-D4.B01 No 70.29 471 842 DATS 94-D4.801 No ñ 80.69 605 891 DATS 94-D4.B01 No Π 90.24 489 928 DATS 94-D4.B01 95.12 No 1306 2176 DATS 94-D4.B01 No 100.46 591 1242 Show Excluded

Figure 8-38 Fragment List Window

You can modify the columns to show different system variables and information.

You can use the Filter Set area to define and select exclusion filters you want applied to the fragment data. Create, modify, and save filter sets. Each **Filter Set** column provides a right-click menu of parameters you can use to apply as exclusion filters.

Note: The Filter Sets applied to the Result Set data (second-level filtering) are different from the Filter Sets applied to the Fragment List data (third-level filtering). You can apply filters to the Result Set to filter an entire sample that may contain numerous fragments. You can also apply filters to the Fragment List to filter individual fragments identified within the various samples.

Apply Exclusion Filters to the Fragment List

Use the exclusion filter set is to exclude any fragments that do not meet the criteria of the Study and to include fragments that do.

Note: Refer to the Help System for information and examples regarding the application of various filters to the fragment list.

The system provides many filters, which you can apply using an operator and a value. You can apply any number of filters to a result set and save it for future use.

You can assign filter sets to filter out fragments in which no confidence interval was estimated, those with asymmetric peaks, stutter peaks, etc.

This third-level filtering keeps only the sample data with fragments that you are certain of, which saves you the time of "weeding out" inaccurate fragment data later in the analysis.

Find Exclusion Filters

You can find all of the exclusion filters in the list in the Name column of the Filter Set area.

After selecting the name of the filter to use, you must select an operator (such as +, <, >=) and a value to be applied to the filter.

Note: Refer to the Help System for detailed descriptions of the available exclusion filters and their use.

Customize the Fragment List

You can define and modify the Fragment List components using the right-click menus. These commands allow you to select specific columns to show in the fragment list, customize the arrangement of fragment list information, and modify several other parameters of the fragment list.

For details regarding the Column Selector window, the Column Sort window, and other display parameters, refer to the *Customizing the Fragment List* section of the Help System.

Manually Select Peaks for the Fragment List

Manually select peaks you want to add to or clear from a fragment list or building a new one from scratch.

To access this function:

- 1. Right-click within the fragment list and select **Manually Select Peak**. When selected, the system asks if you want to clear the existing fragment list.
- 2. Choose one of the following:
 - Click No if you want to keep the list but add or remove peaks from it.
 - Click Yes if you want to discard the system-generated list and start over.

Export a Fragment List to a CSV File

You can export the fragment list to other third-party spreadsheet programs.

To export the fragment list:

- 1. Right-click the selected samples from any of the data or fragment lists.
- 2. Select **Export to CSV File**. The Export window opens.
- 3. Select a name and location to save the file.

Note: For more information regarding data export, refer to the section: Export Fragment Lists or Genotypes from a Study.

Show a Stacked Graph

The Stacked Graph function aligns individual selected traces by size or time. Zooming and scrolling on the X-axis is synchronous between all plots selected while the Y-axis of each plot selected is independently scaled, based on the available data.

To access this function:

- 1. Right-click the selected samples in the fragment list.
- 2. Select Show Stacked Graph.

Perform Bin Analysis

Binning is a one-dimensional clustering, by size, of fragments compiled from multiple samples. You can use bin analysis to generate an allele list that represents the population you are studying. For example, you might want to use this to establish a database of information on alleles and possible anomalies associated with them (such as imperfect repeats).

Binning of alleles from multiple samples allow you to create a new allele list or update an existing allele list for a locus tag based on the collection of fragment results.

During bin analysis, the system groups fragments by size to form bins characterized by an average apparent size and standard deviation of the fragments in the bin.

Set Up a Bin Analysis

Bin analysis requires an exiting result set, either from a previously saved Study or a new Study created exclusively for this purpose.

To set up a bin analysis:

- 1. Open the result set you want to analyze.
- 2. Select Analyze > New Binning Analysis.

Note: As an alternative, you can access **Bin Analysis** from the **Analyses** tab of the Study Explorer.

The New Binning Analysis window opens.

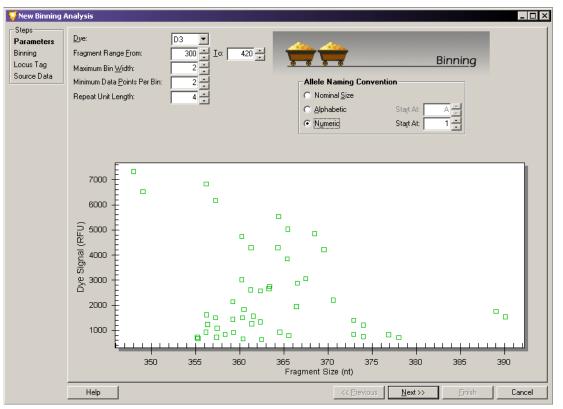


Figure 8-39 New Binning Analysis Window - Bin Parameters

Note: Bin analysis requires a minimum of 10 samples.

Define Parameters

The first step in creating a new bin analysis is to set the bin analysis parameters. These parameters identify the fragment list data you want to include in the bin analysis. By setting these parameters you define the appearance of the view generated. After they are set, the system uses the information from the selected result set to generate a scatter plot. The plot consists of all of the analyzed fragment data shown as a function of the peak height (y-axis) and fragment length (x-axis).

To set the bin analysis parameters:

1. Select the dye used during the raw data analysis from the **Dye** list.

Note: You can examine only one dye at a time during each bin analysis.

- 2. Enter the fragment range (the minimum and maximum fragment size) in the **Fragment Range From** and **To** fields.
- 3. Enter the **Maximum Bin Width** (maximum difference between the smallest and largest fragments within a bin) in nucleotides.
- 4. Enter the **Minimum Data Points Per Bin** (the limiting number of points necessary to form a bin in the scatter plot).
- 5. Enter the length of the repeat unit of the allele in the **Repeat Unit Length** field.
- 6. Select the preferred allele naming convention. Refer to the section: Naming Alleles.
- 7. Click Next.

The system applies the new parameters to the scatter plot and assigns the bins as specified.

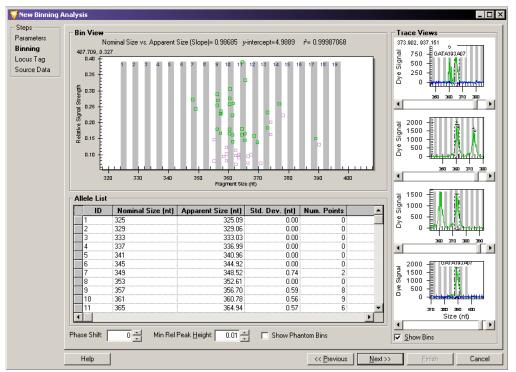


Figure 8-40 New Binning Analysis Window - Bin Analysis View

View Bin Analysis

After applying the bin analysis parameters to the selected data, the system shows the results of the bin analysis. This bin analysis view is composed of three areas representing the selected results.

Bin View

The Bin View consists of a scatter plot diagram showing the **Fragment Size** (X-axis) versus the **Relative Signal Strength** (Y-axis). The points plotted represent the results selected when you created the Study. The bins are defined and visible with the majority of the result points within their borders.

Allele List

The system develops the initial Allele List from information provided in the first window of the Bin parameters plus the data from the fragment list. After completing the analysis, you can correct the allele list using the following options:

Option	Description
Phase Shift	Enter an integer value (nucleotide) in either direction to move all identified bins. This overrides the system-determined position of perfect repeat alleles.
Minimum Relative Peak Height	Enter a value to exclude any data points generated from small noise peaks. The relative peak height is calculated in the color and size range specified in the initial binning parameters window.
Show Phantom Bins	Select Show Phantom Bins to highlight the area of all system-generated phantom bins.

The system generates phantom bins at all intervening positions for whole number nominal sizes not already assigned to a bin. Phantom bins encompass expected fragment sizes (based on other observed sizes) that may or may not be present in the data set.

To convert phantom bins to real bins (alleles), right-click the desired bin location and select **Create Allele**. The system adds a line to the Allele List representing the new bin, which you should check for consistency.

Trace Views

At the bottom of the Trace Views area, select **Show Bins** to show the bins with each trace shown in the stacked trace views.

Investigate Points

Position your mouse-pointer over any point in the Bin View plot and left-click to select it.

The Trace View shows a trace for the selected point. You can use this trace as a visual comparison to other points. The visual comparison of the two traces help you confirm assigning a data point to an already defined bin or to a new bin of its own.

The system assigns a **Name** and **Bin ID** using the naming and ID conventions selected in the bin analysis parameters window. The ID assigned by the system reflects the bin number with a decimal point and the number of bases over that of the bins.

For example: ID = 5.2 indicates a fragment with 5 repeats and 2 extra bases. If you prefer a different allele ID, you can click the system-suggested ID and make changes. The final classification is up to you.

Continue selecting points and viewing their traces until you are satisfied with all the points in the histogram.

Fragment Analysis Module

The system stores the information for a new allele or the modified information of an existing one, which you can apply in later records. The system automatically recalculates the statistics based on the edited results.

Update the Locus Tag and Allele List

After completing the bin analysis, the system generates an allele list based on the information gathered during the analysis. It updates the list, but at this point has saved it to the database. The system prompts you for the locus tag to save to the generated allele list.

Figure 8-41 New Binning Analysis Window - Updating Locus Tag

🤴 New Binning A	Analysis						
Steps Parameters Binning Locus Tag Source Data	s Locus Tag: Project: Default Date last modified: Locus Tag Allele ID Criteria Locus Name: Dye: D3 Primer set name: GenBank Accession: Fragment length: 4 nt H of repeats in shortest Allele: 1 Primer Label Primer Sequence						
					 ⊂ Reverse ⊙ None 	Forward: Reverse:	5' to 3'
	Allele List						
	ID	Nominal Size (nt)	Apparent Size (nt)	Std. Dev. (nt)	Num. Points	Commen	t 🔺
	1	325	325.09	0.00	0		
	2	329	329.06	0.00	0		
	3	333	333.03	0.00	0		
	4	337	336.99	0.00	0		
	5	341 345	340.96 344.92	0.00 0.00	0		
	7	349	344.52	0.00	2		
				0.14	<u>.</u>		
	Help				<< <u>P</u> revious	Next >> Eini	sh Cancel

- 1. You can use this window to select an existing **Locus Tag** from the list or add any **Ancillary Information** to the selected locus tag at this time.
- 2. Save the new data to the selected locus tag to update the allele list with locus information, including **Dye**, **Size**, and **Repeat Unit Length**.

Note: For more information about locus tag parameters, refer to the section: Locus Tag Tab or SNP Locus Tag Editor.

The system saves the following information:

- Observed Size (bin mean)
- Bin Minimum & Maximum
- Standard Deviation
- # of Data Points
- Minimum Peak Height
- X and Y coordinates of all the data points in the plot (stored as additional information)

The system automatically updates the allele list with the new information obtained during the bin analysis.

Review the Source Data

After applying the bin analysis parameters to the data, selecting the reference and trace points, and updating the locus tag and allele list, you can review the source data for all included samples before completing the bin analysis.

The Fragment Results table and Summary area list all of the data samples included in the bin analysis. You may view and compare individual samples manually to include or exclude individual samples or groups of samples from the analysis and reanalyze selected samples.

Figure 8-42 New Binning Analysis Window - Reviewing the Source Data

Click Finish to add the bin analysis to your Study.

Calculate Peak Ratios

After creating a Study, you can begin peak ratio calculations. Peak ratio calculations analyze a Study result to quantify the amount of DNA present in a fragment. The system calculates the DNA quantity as a relative comparison to a user-defined reference peak.

Start a Peak Ratio Calculation

When viewing an exiting Study, select **Analysis** > **New Peak Ratio** to calculate peak ratios.

The Peak Ratios window opens to show all of the result data and the selected parameters used to quantify the amount of DNA present in your sample.

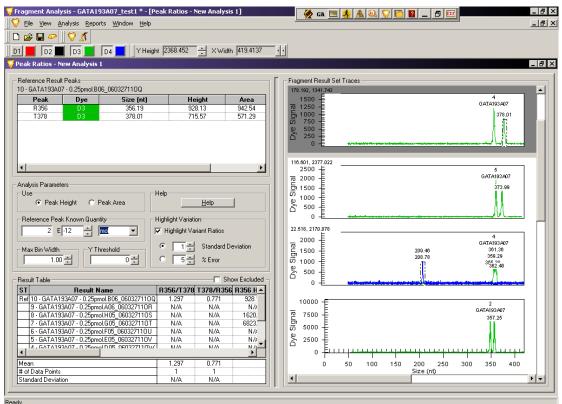


Figure 8-43 Peak Ratios Window

Reference Result Peaks

This area shows the selected reference and test peak information. You select the reference result, which contains the best reference and test peaks for the system to use for a relative analytical comparison of trace peaks.

You may select any number of test peaks, but only one reference peak for each peak ratio analysis.

Analysis Parameters

Option	Description
Use Peak Height	Select this option to perform a quantitation based on peak height in relative fluorescence units (RFU).
Use Peak Area	Select this option to perform a quantitation based on peak area (mm × RFU).

Option	Description
	(Optional) If desired, enter the known quantity of your reference peak in the field. You must enter this value in scientific notation. Select the units for the quantity from the list. The options are moles , grams , molarity , and g/L . The system uses this information to calculate the quantity of DNA in the other results.

Highlight Variation

Option	Description
Highlight Variant Ratios	Select this option to enable the system to highlight any result ratios that deviate from average values. The criteria for variant ratios are specified by either the Standard Deviation about the mean or the % Error .
Standard Deviation	Select this option to base the system criteria for identifying any variant ratios on the number of standard deviations from the mean. Enter the standard deviation number as the value a ratio must meet before the system considers the ratio a variant.
% Error	Select this option to enable the system to base its criteria for identifying any variant ratios on the percentage of error. Enter the percent value of error the system allows a ratio to reach before it considers the ratio to be variant.

Peak Ratio Result Table

The system performs the analysis based on the selected reference and at least one test peak.

• **Show Excluded**: Select this option if you want the result table to show peaks excluded from the analysis.

The Peak Ratio Result table shows details in the following columns:

Column	Description
Status	Shows an ${\ensuremath{\mathbb R}}$ if the result has been selected as a reference.
Result Name	Shows the result name.
Ratio R/T	Shows the ratio of the reference peak to the test peak.
Ratio T/R	Shows the ratio of the test peak to the reference peak.
Timestamp	Shows the time the quantitation was performed.
Sample Name	Shows the sample name.

Column	Description
Reference Peak Height	Shows the height of the selected reference peak.
Test Peak Height	Shows the height of the selected test peak.
Test Peak Quantity	Shows the calculated quantity of the test peak based on user-supplied reference values.
Comments	Shows any user comments associated with the result.

Mean, Data Points, and Standard Deviation

The lower-left area of the Peak Ratio window shows system-generated values for each of the calculated columns in the Peak Ratio Result table. Based on the reference and test peaks selected, the system calculates the **Mean**, **# of Data Points**, and **Standard Deviation**.

Select a Reference Trace

A reference trace is a user-selected trace assigned as a reference by the system for comparative analysis of peak height and area. You can select the reference trace from either the Peak Ratio Result table or from the Fragment Result Set Traces areas of the window.

- To select the reference trace from the Peak Ratio Result table, right-click your selection and select **Set Result As Reference**. The system highlights the result trace with a dark background and moves the trace to the top of the Result Set Traces area of the window to distinguish it as the reference result trace. It marks the fragment result with an R in the left-most column to distinguish it as the reference trace.
- To deselect the reference result, right-click the reference result and select Set Result As Reference again to clear the check box. The system removes the R from the deselected trace and returns it to its original highlight status and position in the Fragment Result Set Traces area of the window.
- To select the reference peak from the Result Set Traces area, right-click your selection and select **Reference**. The system highlights the result trace with a dark background and moves the trace to the top of the Result Set Traces area of the window to distinguish it as the reference result trace. It marks the corresponding result with an R in the left-most column to distinguish it as the reference peak.
- To deselect the reference result, right-click the reference result trace and select **Reference** again to toggle the command to clear the check box. The system removes the R from the deselected trace returns it to its original highlight status and position in the Fragment Result Set Traces area of the window.

Select a Reference Peak

A reference peak is a trace peak you select from the reference result trace. The system compares the reference peak to the test peak, calculates a relative length, height, and area, then adds the results to the Peak Ratio Results table.

- To select the reference peak, right-click an individual peak from the selected **Result Set** Trace located at the top of the Fragment Result Set Traces area of the window and select
 Reference Peak. The system then calculates the length, area, and height of the test peaks
 by comparison and enters this information into the Quantitation Result table.
- To deselect the reference peak from the Reference Result Peaks table, right-click the reference peak and select **Set Peak As Reference** again to clear the check box.
- To deselect the reference peak from the Fragment Result Set Traces area, right-click the reference peak and select **Peak** > **Reference** to clear the check box.

Select a Test Peak

A test peak is a trace peak that you select from the reference result trace. The system compares the test peak to the reference peak, calculates a relative height and area, then adds the results to the Peak Ratio Results table.

- To select the test peak, right-click an individual peak from the selected **Result Set Trace**, located at the top of the Result Set Traces area of the window, and select **Test Peak**. The system calculates the area and height by comparing it with the reference peak, then enters this information into the Peak Ratio Result table. It uses each selected test peak to calculate ratios with the corresponding reference peak of the same trace.
- Select test peaks in the same manner until all desired peaks are identified.
- To deselect a test peak from the Reference Result Peaks table, right-click the test peak and select **Set Peak As Test** to clear the check box.
- To deselect a test peak from the Fragment Result Set Traces area, right-click the test peak and select **Peak > Test** to clear the check box.

Perform AFLP Analysis

Amplified Fragment Length Polymorphism (AFLP) analysis is a highly sensitive DNA fingerprinting technique that relies on the selective amplification of restriction fragments from a total digest of genomic DNA.

The GenomeLab GeXP system includes AFLP fingerprint analysis, which allows you to rapidly score AFLP fingerprints with output in a text format, which is compatible with most statistical and pattern matching system. The AFLP analysis tool is a cluster analysis module that scores the presence or absence of a fragment of a given size per sample.

Use the AFLP Feature

The AFLP feature takes all results from the Study and creates bins from fragments with sizes that fall within a user-defined bin width.

For each of the possible fragments in the Study, the system assigns a 1 if it finds a fragment in that sample and assigns it a 0 if the fragment is absent. It then creates a table of 1s and 0s. You can export the text to most other statistical analysis and spreadsheet software.

Open a New AFLP Analysis

To start a new AFLP analysis, select **Analysis** > **New AFLP Analysis**.

The New AFLP window opens.

Note: You can also access this from the Analyses tab of the Study Explorer.

Figure 8-44 New AFLP Window

😽 New AFLP	
<u></u>	AFLP
Analysis Parameters	Dyes
Maximum Bin <u>W</u> idth 1.00 <u>→</u> nt	📕 🗆 D1
Y Threshold 0 🕂 RFU	■ ▼ D <u>2</u>
Exclude Fully Populated Bins	
Exclude Samples w/o Qualifying Peaks	□ D <u>4</u>
Help	K Cancel

Set Up ALFP Analysis Parameters

The analysis parameters area allows you to set filtering parameters for including or excluding fragment results in your AFLP analysis.

Option	Description
Maximum Bin Width	Sets the limiting value given to the width of the individual bins. The default value is 1.0 nt. Enter the value in nucleotides. Within each dye label, the system clusters the fragments with the sizes that fall within the set bin width and creates a bin with the size rounded to the next integer.

Option	Description
Y Threshold	Serves as a exclusion filter, defining the lowest acceptable value (peak height) for the Y-axis. The default value is zero relative fluorescence units (RFU). Set the Y-threshold in RFU. The system excludes fragments with heights less than this threshold value from the clustering process.
Exclude Fully Populated Bins	Select this option to exclude bins represented in all samples (such as non-polymorphic fragments).
Exclude Samples without Qualifying Peaks	Select this option to exclude samples that have no qualifying peaks.

ALFP Dyes

The **Dyes** fields determine the dye colors the system uses during the cluster analysis.

If the results list contains samples with fragments that have more than one dye (either multiple dye-labeled fragments pooled in a sample or multiple samples with single dye-labeled fragments in each sample), you can select the appropriate dyes for export.

Start a New AFLP Analysis

After setting the analysis parameters, click **OK** to generate the new AFLP analysis.

Figure 8-45 AFLP Analysis Window

Bin	Dye	Samples	Fragments	XMin	XMax	XMean	XVar	YMean	1	2	3	4	5	6	7	8	
55	D4	3	3	54.14	55.03	54.65	0.14	3824	0	0	0	0	1	0	1	1	
61	D4	2	2	60.63	61.04	60.83	0.04	1104	0	1	0	0	0	0	0	1	
62	D4	3	3	61.99	62.52	62.34	0.06	4200	0	0	0	0	1	0	1	1	
67	D4	3	3	66.71	67.15	67.00	0.04	7705	0	0	0	0	1	0	1	1	
77	D4	5	5	76.92	77.17	77.11	0.01	8534	0	0	0	1	1	1	1	1	
79	D4	4	4	78.62	78.85	78.78	0.01	4957	1	0	0	0	1	0	1	1	
38	D4	5	5	87.61		87.66	0.00	4291	1	0	1	0	1	0	1	1	
30	D4	1	1	90.24	90.24	90.24	0.00	928	0	1	0	0	0	0	0	0	
32	D4	7	7	91.45	91.65	91.53	0.01	7611	1	0	1	1	1	1	1	1	
94	D4	4	5	93.09	94.07	93.51	0.20	5101	0	0	0	0	2	1	1	1	
94	D4	1	1	94.23	94.23	94.23	0.00	1186	0	0	0	0	0	0	0	1	
	D4	7	7	96.39	96.66	96.47	0.01	8172	1	0	1	1	1	1	1	1	
97		6	6	97.37	97.62	97.45	0.01	6033	1	0	1	0	1	1	1	1	
	D4	1	1	100.46		100.46	0.00	1242	0	1	0	0	0	0	0	0	
	D4	4	4	102.49		102.63	0.02	4552	1	0	0	0	1	0	1	1	
	D4	1	1	120.12	120.12		0.00	1306	0	1	0	0	0	0	0	0	
	D4	5	5	125.14		125.33	0.12	12678	0	0	1	1	1	1	0	1	
30	D4	7	7	129.39	130.19	129.53	0.07	5463	1	1	1	0	1	1	1	1	
36	D4	1	1	135.95	135.95	135.95	0.00	300	0	1	0	0	0	0	0	0	
	D4	1	1	139.45	139.45	139.45	0.00	1470	0	1	0	0	0	0	0	0	
	D4	2	2		142.56	142.17	0.15	3303	0	0	0	0	1	0	0	1	
	D4	1	1	146.68	146.68	146.68	0.00	2008	0	0	0	0	0	0	0	1	
	D4	7	7			148.20	0.04	7961	1	0	1	1	1	1	1	1	
	D4	1	1	150.38			0.00	17729	0	0	0	0	1	0	0	0	
56	D4	7	7		156.57		0.02	21863	1	0	1	1	1	1	1	1	
	D4	1	1	160.42			0.00	1546	0	1	0	0	0	0	0	0	
	D4	7	7	161.31			0.03	10588	1	0	1	1	1	1	1	1	
	D4	7	7	162.16	162.63	162.26	0.02	10067	1	0	1	1	1	1	1	1	
75	D4	1	1	174.57	174.57	174.57	0.00	3860	1	0	0	0	0	0	0	0	
	D4	1	1	180.41	180.41	180.41	0.00	1610	0	1	0	0	0	0	0	0	
	D4	1	1	190.41	190.41	190.41	0.00	1660	0	1	0	0	0	0	0	0	
:00	D4	1	1	200.39	200.39	200.39	0.00	1559	0	1	0	0	0	0	0	0	
03	D4	7	7	203.06	203.43	203.33	0.01	22969	1	0	1	1	1	1	1	1	
	D/I	7	_7			204 39			1	n		1	1	1	1	1	_
		Bin: <u>F</u> ragn Bin: Binar				ully <u>P</u> opul		ıns alifying Peal	Max	iimum Bin 🛽	<u>M</u> idth	1.00 🕂	Sample A	xis Colum	ns 🔻	He Tim	elp

View the Cluster Analysis

After completing the clustering operation, the system shows a table showing the results of the AFLP analysis. You can format this table as either rows or columns of data for easy export to a third-party software program for further analysis.

This table presen	ts the data in rows	or columns as follows:
-------------------	---------------------	------------------------

Row/Column	Description
Bin	Shows the bin number. It assigns bins with A and B suffixes if two adjacent bins fall in the same integer after rounding the Bin X Mean.
Dye	Shows the dye included.
Samples	Shows the sample count within the bin.
Fragments	Shows the fragment count within the bin.
X-Min	Shows the minimum fragment size within the bin.
X-Max	Shows the maximum fragment size within the bin.
X-Mean	Shows the mean fragment size within the bin.
X-Var	Shows the fragment size variation within the bin.
Y-Mean	Shows the mean peak height within the bin.

Row/Column	Description
Numbered headings	Show the number of sample components (peaks/fragments) within each bin for each sample.

Set Cluster Analysis Parameters

The selections available at the bottom of the AFLP Cluster Analysis window provide additional settings that affect the values and output format of the fields that show.

Select one of the following Sample Bin options:

Option	Description
Fragment Count	Shows the number of components within each sample bin as either zero components, one component, or multiple components.
Binary Presence	Indicates the binary presence or absence within each sample bin.
Fragments Max Y	Shows the absolute peak height of the fragments within each sample bin.

Select one or more of the following options to modify the details that show:

Option	Description
Exclude Fully Populated Bins	Excludes bins that are represented in all samples (such as non-polymorphic fragments).
Exclude Samples without Qualifying Peaks	Excludes samples that have no qualifying peaks
Show Excluded Elements	Highlights all fragment data that was excluded from the analysis.
Sample Axis	Arranges the table presentation by either Rows or Columns . As an alternative, you can switch between these views using the right-click menu. To do this, select the row or column heading, then select Transpose .

The following fields let you make additional adjustments to both the width and threshold:

Option	Description
Maximum Bin Width	Sets the limiting value given to the width of the individual bins. The default value is 1.0 nt. Enter the value in nucleotides. Within each dye label, the system clusters the fragments with the sizes that fall within the set bin width and creates a bin with the size rounded to the next integer.
Y Threshold	Serves as a exclusion filter, defining the lowest acceptable value (peak height) for the Y-axis. The default value is zero relative fluorescence units (RFU). Set the Y-threshold in RFU. The system excludes fragments with heights less than this threshold value from the clustering process.

Note: You can reduce the chance of finding multiple components in a bin by decreasing the bin width.

Customize the AFLP Table

The components of the cluster analysis are user-defined. You can modify them using the rightclick menus. These commands allow you to select specific columns to show in the fragment list, customize the arrangement of fragment list information, and modify several other parameters of the fragment list.

Note: For details about how to use the Column Selector window, Column Sort window, and other display parameters, refer to the section: Customize the Results List.

Export the AFLP Analysis Results

To export the AFLP analysis results:

- Select File > Export ALFP. The Export AFLP window opens.
- 2. Select a file name and destination folder for the data.
- 3. Click Save.

Some third-party software may require that you export the sample information in a specific format. When choosing to show the sample names in rows, you can show the alleles in separate columns or in one column. You can include the Bin Labels in the file by checking the appropriate boxes in the Export File Format for file export.

The **Dyes** parameters determine the dye color the system uses during the cluster analysis. If the results list contains samples with fragments that have more than one dye (either multiple dye-labeled fragments pooled in a sample or multiple samples with single dye-labeled fragments in each sample), you can select the appropriate dyes to be exported.

Perform LOH Analysis

The Fragment Analysis module comes equipped with a macro that is intended to detect loss of heterozygosity (LOH) between alleles from Normal (reference) samples and those from other samples.

In LOH analysis, pairs of samples to be compared must have the same "root" names followed by characters that allow the distinction between reference and test samples or loci. All data to be analyzed reside in a temporary file created by the System Fragment Analysis application.

The macro scans the fragment results contained in the Study to determine the loci to include in the LOH analysis. For each result in the Study, a list of alleles is then generated at each locus. The peak heights of alleles identified in similarly named samples are then compared and their status determined (LOH, AI, MI, Homozygous, NoNorm Alleles).

To start LOH analysis:

1. Select **Analysis** > **Run LOH Analysis** to launch Microsoft Excel. The LOH.xml file opens, showing the START sheet.

📧 Mi	croso	oft Exc	el - LOI	H.xls [R	ead-Only]							
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-												
2												
3		_				Peak Height						
4		Rat	ios for	Detect	ing LOH (1-10	0%):	20	.	LOH Thr	eshold		
5												
6		bd i u	•	.1:65		Peak Height						
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16							Normal in	i same well				
17		_			Start							
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19 20												
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14 4	K K	\ Inct	ructions	STAR	т							► ►
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Kead}	/										NOM	

Figure 8-46 Run LOH Analysis

Note: You must select **Enable Macros** to run the LOH analysis. Otherwise, a prompt reminds you to enable this feature.

2. From the LOH.xls START sheet, set the LOH Threshold and the AI Threshold if desired.

- 3. Specify the unique character combination used to identify Normal samples or loci (i.e., Norm).
- 4. Specify whether Normal and Tumor samples are in the same well:
 - If Normal and Tumor samples are run in the same well, select the corresponding check box.
 - If Normal and Tumor samples are in different wells, clear this check box.
- 5. Click Start.

Note: Do not use the keyboard or the mouse during LOH analysis.

6. If desired, save the results when the processing is complete.

Note: To save the data correctly, you must save the LOH analysis results as a Microsoft Excel Workbook (*.xls) file type. You must also change the file name from the default name shown in the Save As dialog. Otherwise, the system saves result as a temporary file, which it will overwrite in the next LOH analysis.

Note: If more than two alleles are found for a locus, the last allele listed for the locus will be followed by an asterisk.

Customize the Results List

The components of the results list are user-defined. You can modify them using the rightclick menus. These commands allow you to select specific columns to show in the results list, customize the arrangement of information, add or remove columns, and modify several other parameters of the results shown. Customized lists are available in the Result Set View, Fragment List, AFLP Analysis, and Peak Ratio tables.

Use the Column Selector Window

The Column Selector window contains a list of all possible columns that you can show in the list. You can select these columns individually or in groups, arrange them in any order, and lock them so they always remain in view when scrolling through the fragment list.

Access the Column Selector Window

To access the Column Selector window, right-click the column header area of the list to show, then select **Select Columns**.

The Column Selector window opens.

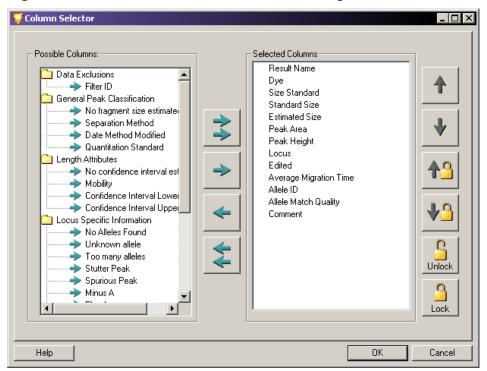


Figure 8-47 Column Selector Window - Selecting Columns to Show in the Table

Note: The application area in which you select the Column Selector window (such as Result Set View, Fragment List, or AFLP Cluster Analysis) determines which columns are available to show.

Note: Refer to the Help System for detailed descriptions of the available columns and their use.

Select the Columns to Show

- Highlight the column selection in the **Possible Columns** area of the window and click the **right-arrow** to move the selection to the **Selected Columns** area of the window.
- To select all columns to show in the list, click the **double-right-arrow**.
- To remove columns from the **Selected Columns** area, highlight the column selection and click the **left-arrow** to move the selection back to the **Possible Columns** area.
- To remove all columns from the list, right-click the header and select the **Hide Column**.

Arrange the Column Display

You can arrange the left-to-right order of columns in the list by ordering the columns top-tobottom in the **Selected Columns** area of the Column Selector window:

- Highlight the column selection in the **Selected Columns** area and click either the **up-arrow** or **down-arrow** buttons to move the column to the selected location.
- Order the columns top-to-bottom in the **Selected Columns** area relative to the left-to-right order you want the columns to show in the Fragment List table.

Lock the Column Position

You can lock selected columns into position so they remain in view as you scroll across the remaining columns in the list. This helps when the results list contains multiple columns of data which require left-to-right scrolling and you want to keep a particular column (such as the result name or status) always in view.

• To lock a column, highlight the column in the **Selected Columns** area of the Column Selector window and click **Lock**. A lock icon shows next to the selected column to indicate that the column position is locked.

Note: This also locks all columns positioned above the lock location in the **Selected Columns** area, and thus to the left of the locked position in the list. If this includes columns that you do not want locked, use the positioning arrows to move the columns out of the lock range.

- If the view is filled with locked columns, you may not be able to scroll through the grid. If this happens, reduce the number or widths of the locked columns.
- To move the lock position to a different column location, click **up-arrow/lock** or **down-arrow/ lock** in the Column Selector window.
- To remove the column lock, click **Unlock** in the Column Selector window.
- To lock and unlock columns directly from the fragment result table, right-click the column header and select **Lock Column** or **Unlock Column**.

Sort the Results List

You can sort the components of the results list in ascending or descending order for any of the column parameters that show. For example, you can sort the results list by ascending bin number, descending bin fragment count, and so on. You can sort columns in the Result Set View window and the Fragment List, AFLP Analysis, and Peak Ratios tables.

Sort an Individual Column

- 1. Right-click the header of the column you want to sort and select **Sort Column**. The system sorts the column in either ascending or descending order, depending on the order in which it was previously arranged.
- 2. To sort the same column in the reverse order, right-click the header and select **Sort Column** again.

Use the Column Sort Window

1. To access the Column Sort window, right-click the column header area of the fragment list and select **Sort Columns**.

Figure 8-48 Column Sort Window - Sorting the Results List Data Columns

쯋 Column Sort	
Sort by Result Name	 Ascending Descending
Then by Sample Name	 Ascending Descending
Then by Slope Threshold	 Ascending Descending
Help	OK Cancel

- 2. To sort a column:
 - a. Select the column parameter you want to be sort from the list.
 - b. Select the order in which you want the parameter sorted (Ascending or Descending).
 - c. Repeat the first two steps for each additional columns that you want to sort.
 - d. Click **OK** to sort the list.

Note: See the Help System for more information on these and other display modifications.

Report the Results

The GenomeLab System software includes a powerful suite of utilities for creating reports. It also allows you to export data and results to other third-party software packages.

💛 Fragment Analysis - New Study *	- 🗆 🗵						
Eile <u>View A</u> nalysis <u>R</u> eports <u>Window H</u> elp							
D1 📕 D2 📕 D3 📕 D4 🔜							
Study Explorer							
C Study							
📑 😽 New Report							
Iemplate: Kelect Template>							
Context Type:							
Context:							
Help OK Cancel							
Analyses Data Reports							
Ready							

Figure 8-49 New Report Dialog

All of the reporting features available in the Fragment Analysis module are located under the Reports tab of the Study Explorer. In addition, you can generate a new report using the **Reports** menu located on the menu bar.

The New Report window allows you to select the type of report you would like to generate from a list of available report templates (refer to the section: Use Report Templates) and saved data. You can access the New Report window from either the **Report** menu or the Study Explorer.

Create a New Report from the Report Menu

To create a new report from the **Report** menu:

- 1. Select **Report > New Report** to open the New Report window.
- 2. Select the type of report you would like to generate from the **Template** list. The selections available depend on the data contained in the open Study.
- 3. Select the saved data from which you want to generate a report from the **Context** menu.
- 4. Click **OK** to show the new report containing the selected information.

Create a New Report from the Study Explorer

To create a new report from the Study Explorer:

- 1. Select the **Reports** tab available at the bottom of the Study Explorer frame of the window.
- Right-click to highlight the type of report you would like to generate from the list of those available in the Reports tab and select New. The New Report window opens to show the Context Type for your selection.
- 3. Select the report template from the **Template** menu. The selections available depend on the **Context Type**.
- 4. Click **OK** to show the new report containing the selected information.

Use Report Templates

There are several types of elements in each report template:

- Header text for data, tables, or graphs (for example, Bin Scatter plot)
- Regular data objects (for example, Context.Locus.Name (Locus))
- Loop delimiters (for example, BeginLoop: Allele = Context.Locus.Alleles, EndLoop: Allele = Context.Locus.Alleles)

Table 8-4 Formatting Commands for Report Templates

ID	Allele.ID
Nominal Size (nt)	FormatNumber (Allele.TrueSize, 0)
Apparent Size (nt)	FormatNumber (Allele.Apparent- Size, 2)
Standard Deviation (nt)	FormatNumber (Sqr(Allele.Frag- mentSizeVariance))
Number of Data Points	Allele.Fragment-Count
Comments	Allele.Comment

- Table delimiters (for example, TableLoop: Allele = Context.Locus.Alleles)
- Graph objects (for example, Graph: Service.BinGraph (Context, Site, DisplayOptions))
- Graph placeholder pictures

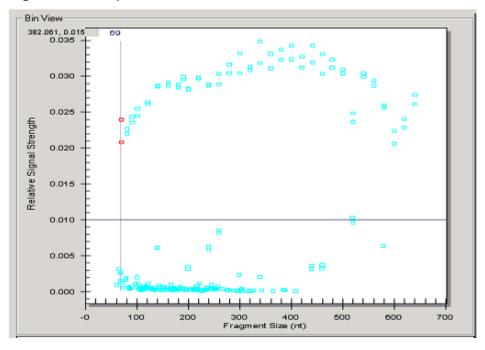


Figure 8-50 Graph Placeholder Pictures

You can edit or remove header text at will. Use the header text provided in the Report Templates to identify the data objects that follow.

You can remove, but should not edit, the data object descriptions, highlighted in gray, because they are specifically coded to extract data from the database. Do not overwrite the original report template .doc files. They contain a compilation of all available data objects for reporting.

Use loop delimiters or table iterations of similar data. While the data object names may appear squashed in the report template, if the data fits in the space provided, the tables should look normal in the report.

Graph objects and graph placeholders must remain together to show correctly on the graph.

Edit Graph Displays

For information about how to edit the five features of the graph displays, refer to the sections:

- X-Scale Type
- X-Axis
- Y-Axis
- Pane Count or Pane Width
- Dyes Shown on the Graph

Fragment Analysis Module

To edit the graph:

- 1. Right-click the graph placeholder and select Format Object.
- 2. Select the **Web** tab and create the following text:

```
<DisplayOptions
(Your commands will go here)
/>
```

X-Scale Type

The possible values for **XScaleType** are **Default**, **BySize**, and **ByTime**. **XScaleType** only applies to electropherogram view. **Default** allows the graph type to determine whether to sort by size or by time. If you do not specify the **XScaleType** attribute, it defaults to **Default**.

In the **DisplayOptions** section of the alternative text box, enter by size or time as follows:

```
<DisplayOptions
XScaleType="BySize"
/>
```

or

```
<DisplayOptions
XScaleType="ByTime"
/>
```

X-Axis

X-axis values specify either the size or the time, depending on the **XScaleType**. If either or both are not specified, they take on the minimum and maximum values from **Unzoom all graph**.

In the **DisplayOptions** section of the alternative text box, enter minimum and maximum values as follows:

```
<DisplayOptions
XScaleType="BySize"
MinX="100"
MaxX="300"
/>
```

Y-Axis

In the **DisplayOptions** section of the alternative text box, enter minimum and maximum values as follows:

```
<DisplayOptions
XScaleType="BySize"
MinX="100"
MaxX="300"
MinY="0"
MaxY="10000"
/>
```

If you do not specify either or both **MinY** and **MaxY**, they take on the minimum and maximum values from **Unzoom all graph**.

Pane Count or Pane Width

You may specify either PaneCount or PaneWidth:

- If you specify **PaneCount**, it represents the number of panes in which to break the graph. If you do not specify either **PaneCount** or **PaneWidth**, the system uses the default **PaneCount** of 1. **PaneCount** is an integer.
- If you specify PaneWidth, it represents either the size or time per pane, depending on the XScaleType. The graph is broken into ((MaxX MinX) / PaneWidth) full sized panes, as well as one additional pane if required for the remaining data. PaneWidth is a float.

In the **DisplayOptions** section of the **Alternative** text box, enter the desired number of panes to distribute the X-axis range of data over as follows:

```
<DisplayOptions
XScaleType="BySize"
MinX="100"
MaxX="300"
MinY="0"
MaxY="10000"
PaneCount="3"
/>
```

Dyes Shown on the Graph

SelectedDyes are dye selection options. It allows the electropherogram graph to select which dye to report. The Fragment Analysis view dye visibility does not affect this option. The possible values for **SelectedDyes** are **AII**, **1**, **2**, **3**, **4** or a combination of **1**, **2**, **3**, and **4**. If **AII** is selected, all four dyes show.

By default, the display for the four dyes is: 1 = D1, 2 = D2, 3 = D3, and 4 = D4.

In the **DisplayOptions** section of the **Alternative** field, enter the desired dyes as follows:

```
<DisplayOptions
XScaleType="BySize"
MinX="100"
MaxX="300"
MinY="0"
MaxY="10000"
PaneCount="3"
SelectedDyes="1,4"
/>
```

Export the Results

When exporting a fragment result in text format, you can include all properties. You can then open the exported file in any text editor or MS Excel to preview the file prior to printing.

To export results:

- 1. Open the Fragment Analysis module.
- 2. Select File > Export Results.

The Export Results dialog opens.

Figure	8-51	Export	Results	Dialog
--------	------	--------	---------	--------

Export Results Steps Results Options	Project: AFLP 06 TP2.20 Filter by date Start: 4 / 25/2006 Y End: 4 Available:	4 /25/2006		Selected: (8)	Fragment Resu	<u>ilts</u>
	Name DATS 93-D4.A01_06042513WU DATS 94-D4.B01_06042513WW DATS 96-D4.C01_06042513WW DATS 97-D4.D01_06042513WU DATS 97-D4.D01_06042513WL DATS 99-D4.E01_06042513WL DATS 99-D4.E01_06042513WL DATS 100-D4.F01_06042513KL DATS 100-D4.F01_06042513KS DATS 100-D4.F01_06042513KS DATS 102-D4.H01_06042513KB		ATS ATS ATS ATS ATS ATS ATS	Name DATS 93-D4.A01_060425122M DATS 94-D4.B01_06042512ZK DATS 94-D4.B01_06042512ZI DATS 97-D4.D01_06042512ZF DATS 99-D4.E01_06042512ZF DATS 99-D4.E01_06042512ZF DATS 100-D4.F01_06042512ZF DATS 100-D4.F01_06042512ZF DATS 100-D4.F01_06042512ZF DATS 100-D4.F01_06042512ZF DATS 100-D4.H01_06042512ZF		(DATS 9 (DATS 9 (DATS 9 (DATS 9 (DATS 9 (DATS 1 (DATS 1
н	lep			< Previous	Next >> Einish	Cancel

- 3. Select the results you want to export from a list of available results located in various projects:
 - a. Select the **Project** that contains the sample data that you want to export from the **Project** list.
 - b. In the **Available** list, select the samples to export, then click > to move the selection to the **Selected** list.

Note: The functionality of this window is very similar to the Select Raw Data window in the section: Select the Components of the New Study.

- 4. When you have finished making your selections, click **Next**. The Export Options dialog opens.
- 5. Define the export parameters and options, including the file name and location, using the **Options** tab of the Export Results dialog.

Export results as: C:\CEQ System\Export\{Result			
	Name}.txt	Save <u>A</u> s	
Elements	Options	٦	
✓ <u>H</u> eader	Remove CEQ Tracking Suffix		
🗖 <u>R</u> aw Data	Resolve Filename Conflicts		
Result Data			
Result Output			
Use the Save As button to change the	a evport file name, format, or directory	7	
	re}" assigns the actual result name to the exported file.		
Note: {Result Name} is not a valid file			
Note, (nesult name) is not a valid file i	name for Chivexport		
		_	

Figure 8-52 Options Tab of the Export Results Dialog

The Export Results - Options window provides the following fields and options:

Fragment Analysis Module

Option	Description
Export result as	Shows the selected export file, including its file path, file name, and extension (which identifies the file format). This is a read-only field.
	Note: To change this selection, click Save As and enter the required details to save the file using the application's format extension. Refer to the section: Text File Format or CEQ File Format.
Header	Select this option to include general information regarding the sample and run conditions with the exported results.
Raw Data	Select this option to include raw data with the exported results.
Result Data	Select this option to include dye traces and data points with the exported results.
Result Output	Select this option to include a fragment list with the exported results.
Remove CEQ Tracking Suffix	Select this option to remove the sample plate and date from the exported file name.
Resolve Filename Conflicts	Select this option to automatically append a unique sequence number to exported files of the same name.

6. Click **Finish** to complete the export application.

Text File Format

You can export the results file in a text file format. The data in this file are tab delimited and the export format has the extension txt.

Figure 8-53 Fragment Analysis Export Dialog

Export		? ×
Sample Elements I Header Raw Data Result Data Result Output	Save in: Export Export CTEST DATS 93-D4.txt DATS 94-D4.txt DATS 94-D4.txt DATS 94-D4.txt DATS 96-D4.txt DATS 97-D4.txt DATS 99-D4.txt	
Options Femove CEQ Tracking Suffix Resolve Filename Conflicts	File name: DEFAULT Save as type: Text (Tab Delimited) (".txt)	Export Cancel

To export the results as a text file from the Export Results - Options window:

- 1. Click Save As.
- 2. Use the folder navigation options next to the **Save in** field to locate and select the folder in which you want to save the export file.
- 3. Enter the desired file name in the **File name** field.

Note: Do not use special characters (*I* : * ? \ " < > |) in the file name.

- 4. In the Save as type field, select Text (Tab Delimited) (*.txt).
- 5. Click Save.

The system returns to the Export Results - Options dialog, showing the file path to the specified text file in the **Export results as** field.

- 6. If desired, modify the fields that apply to the text file:
 - a. Select Header and Result Data in the Elements section.
 - b. Select **Remove CEQ Tracking Suffix** to automatically remove the plate position coordinates (*.A01, *.B01, etc.) as well as the time/date stamp from the sample name. These are generated by the GenomeLab System software.
 - c. Select **Resolve Filename Conflicts** to enable the system to increment the sample names on export if it encounters samples with the matching names.
- 7. Click Export.
- 8. Navigate to the target folder and double-click the exported file to open it in Excel, Notepad, or any other text editor.

You can view the properties at the beginning of the file.

CEQ File Format

The GenomeLab GeXP system offers the CEQ (*.cq) export option from the Fragment Analysis module. You can select cq as an export file type for exporting fragment results from the Fragment Analysis module.

Figure 8-54 Fragment Result Export Dialog

Export		? ×
Sample Elements Header Raw Data Result Data Result Output Options	Save in: 🔁 Export 💌 🗭 🖻	* *
Remove CEQ Tracking Suffix Resolve Filename Conflicts	File name: DEFAULT Save as type: CEQ (".cq")	Export Cancel

To export fragment results in CEQ extension from the Export Results - Options window:

- 1. Click **Save As**. Use the folder navigation options next to the **Save in** field to locate and select the folder in which you want to save the export file.
- 2. Enter the desired file name in the **File name** field.

Note: Do not use special characters (*I* : * ? \ " < > |) in the file name.

- 3. Select **CEQ (*.cq*)** from the **Save as type** list.
- 4. Click Save.

The system returns to the Export Results - Options window, showing the file path to the specified CEQ file in the **Export results as** field.

- 5. If desired, modify the fields that apply to the CEQ file:
 - Select **Remove CEQ Tracking Suffix** to automatically remove the plate position coordinates (*.A01, *.B01, etc.) as well as the time/date stamp from the sample name. These are generated by the GenomeLab System software.
 - Select **Resolve Filename Conflicts** to enable the system to increment the sample names on export if it encounters samples with the matching names.
- 6. Click **Export** to export the result data and the result output.

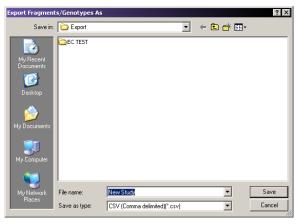
Export Fragment Lists or Genotypes from a Study

This feature allows you to export the opened Study's fragment list or genotype data. The pedigree and genotype data included in the export come from the results included in the opened Study.

To export the properties with a fragment result:

- 1. Open the Fragment Analysis module and open a Study.
- Select File > Export Fragment/Genotypes.
 The Export Fragments / Genotypes As dialog opens.

Figure 8-55 Exporting Fragment/Genotypes As Dialog



- 3. Use the folder navigation options next to the **Save in** field to locate and select the folder in which you want to save the export file.
- 4. Enter the desired file name.

Note: Do not use special characters (*I* : * ? \ " < > |) in the file name.

- 5. Select one of the four available export formats from the **Save as type** list:
 - CSV (Comma) *.csv
 - Linkage (Pedin) *.pre
 - Discovery Manager (DMPopulation) *.txt
 - Genotype Summary Report (tab delimited) *.gsr
- 6. Click **Save** to start the process and save the export file. The export status, along with a processing log, appears in a status window. This window indicates when the export procedure is complete and any export errors that may occur.

Transfer Fragment Data to the CSV Transformer Tool

Transfer fragment data from the GenomeLab GeXP system to the CSV Transformer Tool for use with normalization of gene expression results.

1. On the Main Menu, click the **FRAGMENTS** icon **V** to launch the Fragment Analysis module.

- 2. Open a set of analyzed GeXP data.
- 3. Select File > Transfer Fragments for GeXP.
- 4. Click Transfer Fragments for GeXP.
- 5. In the dialog that opens, name the file and browse to a transfer location.
- 6. Click **Save** to save the exported file as a csv file. A dialog opens, confirming the export process.
- Click **OK** to confirm the completion of the process and close the dialog. For additional information on using fragment data in the CSV Transformer Tool, refer to the section: Normalize the Exported Fragment Data.

Format Descriptions and Requirements for Exporting Fragment List and Genotypes

Export data along with their export requirements, by using one of the following file formats:

CSV (Comma) *.csv

Contains the export parameters of a Study's fragment list as selected, sorted, and ordered. Each line in the output file represents a fragment. The data in this export are comma delimited, suitable for viewing in most third-party spreadsheet applications. The export format has the *.csv extension.

Linkage (Pedin) *.pre

Contains the export parameters of a Study's pedigrees and genotypes. The Pedin file can be imported into a Linkage application's MakePed utility program. This file includes the pedigree and genotype information from each result in the Study. It supports up to two alleles per locus.

The export produces three files:

- *.pre = the Pedin file.
- *.log = the list of processing steps and errors encountered during the export.
- *.debug = the text file with column headings and comma separated data to aid in the correction of errors in the *.pre file.

The **Pedin** export requires a list of locus names, in a file named LocusList.txt. You must store this file in the GenomeLab GeXP system \export or \root directory. This text file must have only one locus name per line. The order of the loci included in this file determines the order of the loci in the Pedin file.

The **Pedin** export also requires that every result in the Study includes the pedigree information (at least the individual ID). It also requires that at least one result per individual has a complete pedigree (**Pedigree ID**, **Individual ID**, **Father ID**, **Mother ID**, and **Gender**).

Pedigree information is associated with samples through the sample plate editor, using the sample property sets **Pedigree** template. As an alternative, you can associate the pedigree information with results, using the single result view's property sets tab.

Discovery Manager (DMPopulation) *.txt

Contains the export parameters of a Study's pedigrees and genotypes. The DMPopulation file is designed to be imported into Genomica's Discovery Manager application. The DMPopulation file can also be imported into Discovery Manager as a **DMFastPopulation** file. The pedigree and genotype information from each result in the Study is included in the DMPopulation file.

The **DMPopulation** export produces two files:

- *.txt = the DMPopulation file.
- *.log = a list of processing steps and errors encountered during the export.

The **DMPopulation** export requires a list of locus names, in a file named LocusList.txt. This file must be stored in the GenomeLab GeXP system's \export or \root directory. This text file must have only one locus name per line. The order of the loci included in this file determines the order of the loci in the DMPopulation file.

The **DMPopulation** export also requires that pedigree information (at least the **Individual ID**) be included in every result in the Study. Additionally, at least one result per individual must have a complete pedigree: **Population ID (PopID)**, **Pedigree ID (KinID)**, **Individual ID (SubID)**, **Mother ID (MID)**, **Father ID (FID)**, and **Gender (Sex)**.

Pedigree information is associated with samples through the sample plate editor, using the sample property set's **Pedigree** template. As an alternative, you can associate the pedigree information with results, using the single result view's property set tab.

Genotype Summary Report (tab delimited) *.gsr

Accommodates **Subject IDs**, the occurrence of more than two alleles, and the absence of alleles. Use the Locus List Editor to customize the LocusList.txt file before exporting a Genotype Summary Report. Refer to the section: Create a Genotype Summary Report.

The following list describes the report layout:

- Column/Row individual headings are replaced with the heading of Subject ID.
- If the number of alleles is greater than two, the cell will indicate Too many.

When an allele is not found, the empty cell is replaced with Unknown Allele.

Figure 8-56 Genotype Summary Report

Create a Genotype Summary Report

The Locus List is required to create the Genotype Summary Report (GSV) and to perform some exports. Use the Edit Locus List dialog to customize the LocusList.txt file.

Press **F7** or select **View > Locus List Editor**. The Edit Locus List dialog opens.

Figure 8-57 Edit Locus List Dialog

쯋 Edit Locus List	
Study Loci	↑ ♥
Help OK	Cancel

Table 8-5 Edit Locus List Controls	Table	8-5 Edit	Locus List	Controls
------------------------------------	-------	----------	------------	----------

Item	Description
Loci in Database	Lists the loci from the database that have not yet been moved to the Selected Loci list.
Other Loci	Lists the loci to be removed from the LocusList.txt file.
Selected Loci	Lists the loci to be added to the LocusList.txt file.
**	Moves all loci from the Loci in Database and Other Loci list to the Selected Loci list.
*	Moves only the selected loci from the Loci in Database and Other Loci list to the Selected Loci list.

Item	Description
*	Moves all loci from the Selected Loci list to the Loci in Database or Other Loci list. If the loci exist in the database, moves the loci to the Loci in Database list.
44	Moves the selected loci from the Selected Loci list to the Loci in Database or Other Loci list. If the loci exist in the database, moves the loci to the Loci in Database list.
1	Use to rearrange the list order. Select an item and click the appropriate arrow to move it up or down the list.

Overview

The GenomeLab GeXP system uses a patented, highly-multiplexed PCR approach to efficiently examine the expression of multiple genes, with sensitivity and speed. Perform a multiplex gene expression analysis by completing the following procedures:

- Design a custom multiplex using the National Center for Biotechnology Information (NCBI) Primer-BLAST.
- Perform the reverse transcription (RT) reactions.
- Perform the PCR reactions.
- Run the PCR products on the GenomeLab GeXP system.
- Analyze the data using the Fragment Analysis module, CSV Transformer Tool, and GeXP Analysis Template.

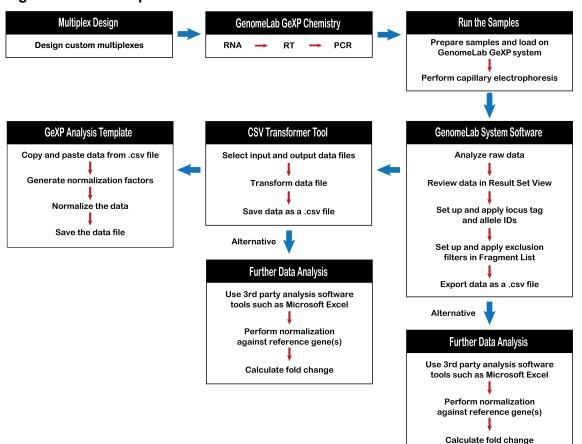


Figure 9-1 Gene Expression Workflow

Multiplex Primer Design Using NCBI Primer-BLAST

The NCBI Primer-BLAST tool is used to design an initial multiplex, using accession numbers of target and reference genes. The primer and amplicon information for the multiplex are saved in a Microsoft Excel file. The multiplex primer and amplicon sequences are evaluated using BLAST analysis. If necessary, individual primers can be redesigned. Finally, the primer and amplicon information for the multiplex in the Microsoft Excel file is updated.

Note: Add an appropriate universal tag sequence at the 5' end of the primers before ordering primers.

Pre-Design Considerations

1. Determine which genes to include in the multiplex for the study.

Gene Expression

2. Select a correct accession number for each of the targeting genes. If possible, always use reference sequence (RefSeq) (NM_XXXXX).

Since the sequence represented by the accession number will be used to generate the multiplex primers, certain considerations should be made when selecting accession numbers. A single gene can be represented in the GenBank database by multiple accession numbers such as those that refer to genomic sequences, partial sequences, mutations, mRNA, ESTs, alternate transcripts, splice variants, or pseudogenes.

Ensure the following when selecting an accession number:

Correct gene • I	Many genes have multiple names and aliases.
á	Different genes can have similar names (such as IL2, IL2 receptor alpha, IL2 receptor beta, and IL2 receptor gamma). Verify that the gene is from the species of interest.

Valid sequence	• Verify that the sequence contains only the letters A, T, G, C, or N.
	Verify that the sequence is for mRNA (cDNA).
	Avoid genomic DNA sequences with introns.
	 Verify that the sequence is still active by reviewing the accession number's revision history. Use the following page: www.ncbi.nlm.nih.gov/entrez/sutils/girevhist.cgi.
	• RefSeq accession numbers that start with <i>NM</i> _ are ideal choices for designing primers because they are from a curated, non-redundant database of known genes maintained by NCBI. The NM_ accession prefix denotes mRNA sequences.
	• Each RefSeq accession number correlates to an individual identified transcript variant. A gene may have more than one RefSeq identifier.
	 Be aware of and avoid pseudogenes by BLASTing the gene against the genome before primer design to avoid undesigned peaks (UDPs). See the BLAST tool on the NCBI website: www.ncbi.nlm.nih.gov/Blast.cgi.
	• Mutations or repeats can also influence the results of primer design.
	 Homologous genes, pseudogenes, or conserved domains should also be considered when choosing a gene sequence.

Note: Accession numbers can be found on the NCBI website at <u>www.ncbi.nlm.nih.gov</u>. Use the tools and information (such as Entrez Nucleotide, Entrez Gene, Homologene, Unigene, Blastn, or OMIM) to aid in identifying a valid accession number.

3. For genes with transcript variants, decide how many variants to detect.

Note: Be aware of transcript variants before primer design to avoid UDPs. See Tools on the NCBI website (http://www.ncbi.nlm.nih.gov/gene).

- 4. Select regions that do not have high homology to gene family members or regions without pseudogenes.
- 5. Choose reference genes.
 - Ideally, genes selected for normalization purposes should be expressed stably in all samples under all conditions.
 - To determine which genes are expressed stably, assay the reference genes in experimental samples with a custom multiplex.
 - To monitor the stability of gene expression between samples, normalize the peak areas of the reference genes, at minimum, to the KAN^r peak area in the CSV Transformer Tool and compare the resulting relative expression values between samples.
 - Select the reference genes with the most stable expression between samples as the normalization genes.
 - The greater the number of normalization genes used, the lower the chance of any one of those genes affecting the normalization factor. Therefore, if there is some fluctuation in expression of one reference gene, the effect will be diluted by the presence of the other reference genes.

Note: Several reference genes can be used as internal controls for normalizing the gene expression level across samples.

Note: The ability to select multiple reference genes and use the calculated geometric mean of expression for these genes to normalize expression in each well is a powerful tool in the XP-PCR process.

- 6. Design amplicons with sizes in the range of 105 nt to 350 nt (without universal tags).
- 7. Make sure that the spacing between amplicons in the same multiplex is at least 5 nucleotides.

Primer Design Using NCBI Primer-BLAST

- 1. Go to the website: http://www.ncbi.nlm.nih.gov/tools/primer-blast/.
- 2. In the PCR Template section, enter the accession number or FASTA sequence.

- 3. In the Primer Parameters section:
 - a. For PCR product size Min, enter 105. For FFPE samples, enter 63.
 - b. For PCR product size Max, enter 350. For FFPE samples, enter 150.
 - c. For **Primer melting temperatures**, accept the default settings. Ensure that the Max T_m difference between the forward and the reverse primers is no more than 5.
- 4. In the Exon/Intron selection section:
 - a. From the Exon junction span list, select Primer must span an exon-exon junction.
 - b. Select the Intron inclusion check box.
 - c. Modify Intron length range Min as needed.
- 5. In the Primer Pair Specificity Checking Parameters section:
 - a. Select the **Specificity check** check box.
 - b. In the **Database** list, select **Refseq mRNA**.
 - c. In the **Organism** field, type the first few letters of the appropriate organism and then click the organism in the list that shows.
- 6. Click Advanced parameters.
- 7. In the Primer Parameters section:
 - a. From the Salt correction formula list, select Schildkraut and Lifson 1965.
 - b. From the Table of thermodynamic parameters list, select Breslauer et al. 1986.
 - c. Select the SNP handling check box.
- 8. Click **Get Primers** to submit your search request. The NCBI website will return search results that contain information about the primer pair(s).

Assemble a Multiplex

- 1. Start with designing primers for genes that require targeting to a specific region in order to detect desired number of transcript variants or to avoid pseudogene or highly homologous region among gene family members. Set PCR product size range as 105 nt to 350 nt to generate multiple primer pairs. Select the best primer pair based on evaluation of primer and amplicon sequences. In a Microsoft Excel worksheet, record the gene name, the primer sequence, primer positions, the T_m for the forward primer, T_m for the reverse primer, and the amplicon size (without universal tags) for the chosen primer pair. Save this file with the name of the multiplex.
- 2. Include primers that were previously validated for a gene target or a reference gene. Record primer and amplicon information as in step 1.

3. For the remaining gene targets in the multiplex, design amplicons with smaller sizes first, then, work toward larger amplicon sizes. To ensure a desired amplicon size is assigned for a particular gene target, set the minimum and the maximum PCR product size at the same value.

For example, if 133 nt is the desired amplicon size, enter **133** both as the minimum PCR product size, and as the maximum PCR product size during primer design. Record primer and amplicon information as in step 1 for each gene target. Ensure the amplicons are at least 5 nt apart.

4. In the Microsoft Excel worksheet, create a new column named Amplicon size with Universal tags.

The amplicon size with universal tags is calculated by adding 37 nt to the amplicon size without universal tags.

- 5. In the Microsoft Excel worksheet, create a column named reverse primer with tag, copy and paste the reverse universal tag sequence at the 5' end of gene-specific reverse primer sequence.
- 6. In the Microsoft Excel worksheet, create a column named forward primer with tag, copy and paste the forward universal tag sequence at the 5' end of gene-specific forward primer sequence.
- 7. Click Save.

Note: Contact SCIEX Technical Support for sequences of universal tags.

Evaluate the Primer and Amplicon Sequences Using NCBI BLAST Searches

- 1. Perform BLAST with each primer sequence to ensure it does not have significant homology to amplicon sequences for other genes in the multiplex.
- 2. If a primer has significant homology to a region of an amplicon of another gene in the multiplex, redesign the primer.
- 3. Use NCBI BLAST with species-specific searches to determine if the amplicon for each primer set contains homologous regions and if so, where those regions lie. BLASTing against the RefRNA database is helpful for homology.
 - If the homologous regions are within the reverse primer sequence, redesign the primer to target a region of low homology, particularly at the 3' end of the primer.
 - If there is extensive homology between two genes within the same multiplex, be sure to design primers to unique regions of each gene in order to prevent cross amplification.
- 4. If primers were designed using primer 3, not using NCBI Primer-BLAST, evaluate primer and amplicon sequences by performing the following:

- a. Examine each primer for single nucleotide polymorphisms (SNPs) with BLAST-SNP, especially at the 3' end, which can lead to the preferential amplification of one allele over another. If a polymorphism is found, redesign the primer to a more conserved region.
- b. Examine the amplicon sequence, generated by each set of primers, for repeat sequences.
 - Repeat sequences can lead to Thermo-Start *Taq* DNA Polymerase slippage and result in stutter peaks.
 - If 5 or more consecutive single or di-nucleotide repeats or a series of such repeats in close proximity to each other are present, redesign the primers to target an amplicon without repeats.
- c. BLASTing against a genomic database will help determine if any pseudogene expression may be detected.

Any set of primers that has the potential to generate a UDP within the size range of multiplex should be redesigned to prevent the production of a UDP.

Once the primer and amplicon sequences have been reviewed, update the Microsoft Excel worksheet for the multiplex. Proceed with ordering and testing the multiplex primers. Order the primers with the appropriate universal tag sequence.

IMPORTANT Do not order the KAN^r primers. They are included in the GenomeLab GeXP Start Kit.

After the primer multiplex is designed, various RT and PCR reactions are performed with a reference RNA to evaluate the primers in the multiplex. Refer to detailed instructions on setting up RT and PCR reactions, and on optimizing multiplex in the *GenomeLab GeXP Genetic Analysis System Chemistry Protocol*. Once a multiplex is optimized, it is used to study gene expression in different samples.

Run Samples and Review Results

For various gene expression experiments, whether they are for evaluating primers or comparing gene expression levels in different RNA samples, RT-PCR reactions are prepared as described in the *GenomeLab GeXP Genetic Analysis System Chemistry Protocol*. Fluorescent dye-labeled DNA fragments are separated by capillary electrophoresis on the GenomeLab GeXP system. A new Study is created in the Fragment Analysis module. Raw data and analyzed data are reviewed in Result Set View window.

For more information, refer to the application note: *Multiple Gene Normalization and Alternative Export of GeXP Data* available at sciex.com.

Prepare the System Before the Sample Run Create a New Database and a New Project

- 1. On the Main Menu, click the DATABASE icon 📗
- 2. Create a new database for the interested multiplex or experiments, and make it the **Working Database**.
- 3. Create a new project in this database, and rename it as needed.
- 4. Close the Data Manager module.

Create a New Sample Plate

- 1. On the Main Menu, click the SET UP icon
- 2. Create a new sample plate for the sample run.
- 3. Name the samples.
- 4. Select **Frag-3** as the **Separation Method**.
- 5. Select Analysis Parameters. Use Default GeXP Analysis Parameters.
- 6. Save the sample plate to a new project. Refer to the section: Create a New Database and a New Project.

Prepare the GenomeLab GeXP System

- 1. Install the capillary array and gel cartridge. Make sure that the array serial number and gel lot number are entered into the GenomeLab GeXP system during installation.
- 2. Preheat the capillaries to 50 °C.
- 3. Perform a manifold purge with 0.4 mL gel three times and with gel capillary fill three times.
- 4. Perform an optical alignment.
- 5. Monitor the baseline. Clean the array window if the baseline for any color is above 6,000 counts.
- 6. Refresh the wetting tray with deionized water.

Start the Sample Run

- 1. Load sample and buffer plates.
- 2. Select the sample plate set up. Refer to the section: Create a New Sample Plate.
- 3. Ensure the sample position in the sample plate set up file matches the sample position on the actual sample plate and the buffer well position on the buffer plate.

4. Start the sample run.

Create a New Study

A *Study* is a set of multiple sample results together with the results of analyses performed on the group of sample results. A new Study can be created from either:

- Raw data
- Previously analyzed results
- 1. Select the **Raw Data** check box.
- 2. Click OK.
- 3. If using the List View, select the appropriate project name from the box next to **Project** and then highlight the raw data files in the **Available** table. Use > to move the selected files to the **Selected** table.
- 4. Click Next.

A minimum of 10 data files is required if the downstream analysis includes binning.

- 5. Select Analysis Parameters > Next. Use the DefaultGeXPAnalysisParameters.
- 6. Click **Analyze** to start the data analysis.

When the analysis is complete, the status column will indicate if the analysis succeeded or failed for each sample in the study.

- 7. Click Next.
- 8. Click **Finish** to complete the process of creating the new study. The data files in this study will be presented in a table in the Result Set View window.
- 9. Click **File > Save As** and save the study with a new name.
- 10. For creating a new study with previously analyzed results, perform the following steps:
 - a. Select the Analyzed Results check box.
 - b. Click OK
 - c. A dialog opens that allows data file selection from a database of samples that have been run in a particular project. The data file selection can be made either through the List View or Plate View tab.

The List View and Plate View tabs provide two methods of selecting the raw data. Each selection method contains the same sample information, presented in different ways.

• When creating a new study on a controller connected to a GenomeLab GeXP system that collected the raw data, Plate View is recommended due to the availability of the plate set-up information.

• If creating a new study on a laptop computer, it is a good idea to use List View, because the plate set-up information may not have been imported to the laptop from the controller that collected the raw data.

Review Data in the Result Set View Window

1. In the Result Set View window, double-click one of the data files to view the electropherogram of analyzed fragments.

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-STD.H05 090223187F	02/23/2009 18:46:00	Н	pass	DefaultGeXPAnalysisParameters						
-STD H04 0902231877	02/23/2009 18:45:56	Н	pass	DefaultGeXPAnalysisParameters						
-STD H03 090223186Z	02/23/2009 18:45:54	Н	pass	DefaultGeXPAnalysisParameters						
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-STD.G12 0902231898	02/23/2009 18:46:20	G	pass	DefaultGeXPAnalysisParameters						
STD.G11 090223186Z	02/23/2009 18:46:16	G	pass	DefaultGeXPAnalysisParameters						
-STD.G10_090223188P	02/23/2009 18:46:14	G	pass	DefaultGeXPAnalysisParameters						
STD.G09 090223188C	02/23/2009 18:46:10	G	pass	DefaultGeXPAnalysisParameters						
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L STD, GOT_090223186G	<i>UCL29 2003</i> (64046									

Figure 9-2 Result Set View Window Data Files

- 2. Check whether all size standard peaks are called correctly.
- 3. Check whether all designed gene peaks in the multiplex are present and called correctly.
- 4. Deselect **D1**, **D2**, and **D3** traces and show only **D4** trace for a simple view of the data.
- 5. Zoom-in inside the Fragment Data window to view all the peaks generated from the GeXP multiplex.

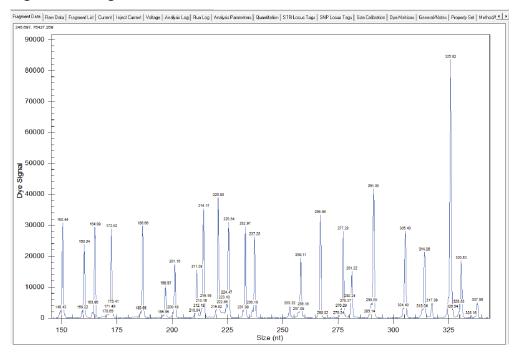


Figure 9-3 Fragment Data Window Gene Peaks

- 6. Click the **Raw Data** tab and review the raw data trace to ensure there are no separation anomalies, such as split peaks or poor resolution.
- 7. Click the **Current** tab to review the separation current trace. If there is a current dip or crash, this data file should be excluded from further analysis.
- Click Inject Current to review the injection current trace. The injection current values should be in the range of 1 μA to 5 μA. Some fluctuation is normal.
- 9. Remove data files that are unsuitable for the study by right clicking on the specific file and then clicking **Exclude Results**.

Note: Data files that contain the following situations should be removed:

- Over-range peaks that are split into two called peaks.
- Data with abnormal current.
- Data with separation anomalies such as split peaks or poor resolution of fragments.
- Negative controls: NTC and RTminus.

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L-STD.603_090223186U		2/23/2009 18:45:52	6	patt	DefaultEx/PAnalysisParameters					
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Figure 9-4 Examples of Data Files to Exclude

Set Up Locus Tag and Allele IDs

The fragment results created through default GenomeLab System software analysis parameters in the Fragment Analysis module contain information on fragment size, peak height, and peak area. Labeling each gene peak with the gene name makes the results more meaningful to scientists working on gene expression experiments. It also facilitates sharing results with coworkers and collaborators. Labeling a gene peak with its corresponding gene name can be achieved by setting up locus tag and allele IDs through a process named *binning*. For each multiplex, setting up locus tag and allele IDs only needs to be done once on a GenomeLab GeXP system. After that, the allele IDs can be applied automatically to the new data generated with the same multiplex by selecting the locus tag as an STR locus tag during analysis.

Perform the following steps to setup the locus tag and allele IDs:

1. Select Analysis > New Binning Analysis.

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sults	New Binning Analysis New Peak Ratio Analysis.	_					Exclusion Filter Set	
-	Run LOH Analysis	result	date	capillary	analysis outcome	analysis parameters	New Besult Filter Set 3	 App)
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L-STD H04_090223187		02/23/2009		H	pass	DefaultGeXPAnalysisParameters		
L-STD H03_0902231862		02/23/2009		H	pass	DefaultGo/PAnalysisPatameters		
L-STD H02_090223186		02/23/2009		H	pass	DefaultGeVPAnalysisParameters DefaultGeVPAnalysisParameters	1	
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L-STD G10_0902231888		02/23/2009		G	Dass	DefaultGeVPAnalysisPatameters		
L-STD.609_0902231880		02/23/2009		G	pass	DefaultGo/PAnalysisParameters		
L-STD 608 0902231883		02/23/2009		G	pass	DefaultGeVPAnalysisPatameters		
L-STD.607_0902231871		02/23/2009		G	pass	DefaultGe\PAnalysisParameters		
L-STD 606_0902231877		02/23/2009		G	pass	DefaultGeVPAnalysisParameters		
L-STD.G05_0902231870		02/23/2009		G	pass	DefaultGeXPAnalysisParameters		
L-STD 604_0902231873		02/23/2009		G	pass	Defaultic/PAnalysisParameters		
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inmary A cluded 0	8 C		0	E 0	F 0	6 H Total 10 6 16 2 1 3	•	

Figure 9-5 New Binning Analysis Option

The New Binning Analysis window with default bin parameters opens.

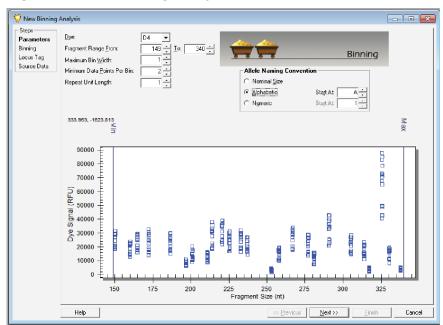


Figure 9-6 New Binning Analysis Window with Default Bin Parameters

2. Modify the binning parameters. The following settings are recommended:

- **Dye**: D4
- Fragment Range: 149 to 340 (fragments size ranging from 150-338 nt)
- Maximum Bin Width: 1
- Maximum Data Points Per Bin: 2
- Repeat Unit Length of Allele: 1
- Allele Naming Convention: Alphabetic
- 3. Click Next.

The new parameters will be applied to the scatter plot and the bins will be assigned as specified. The results of the binning analysis will show in the New Binning Analysis window.

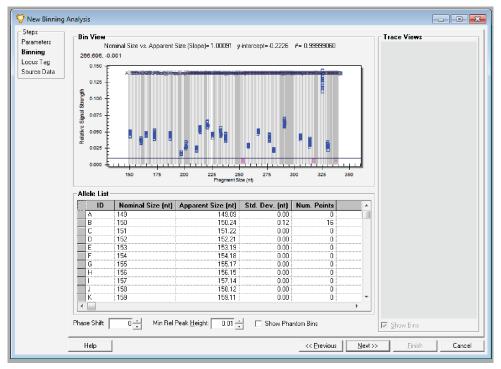


Figure 9-7 New Binning Analysis Window

The New Binning Analysis window is composed of the following three areas representing the selected results:

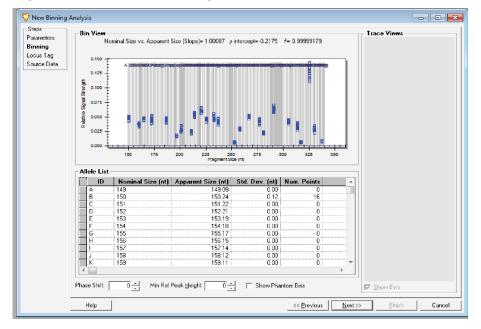
- Bin View: A scatter plot showing the Fragment Size (X-axis) vs. the Relative Signal Strength (Y-axis).
- Trace View: Traces to be shown are selected by clicking on a point in the Bin View.
- Allele List: Developed and carried over from previous views. When the analysis is completed, corrections to the allele list can be made.

4. Adjust the **Minimal Relative Peak Height (Min Ref Peak Height)** to exclude any data points generated from small noise peaks.

Note: Excluded small peaks are shown as pink-colored dots at the bottom of the Bin View.

5. If too many small peaks are excluded, reduce the **Min Ref Peak Height** from **0.01** to **0.00**.

Figure 9-8 Min Ref Peak Height Reduced to 0.00



- 6. Double-click **Num. Points** in the Allele List table to sort the alleles by number of data points in a bin. Bins with zero values in the **Num. Points** column are considered empty bins.
- 7. Select or highlight all alleles with empty bins by clicking on the gray boxes on the left side of the allele names. Then right-click the highlighted area, and select **Remove Allele(s)** to delete alleles with empty bins.

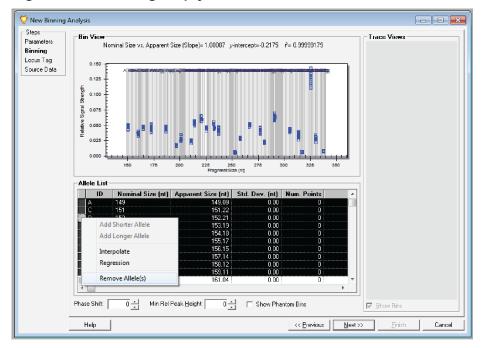


Figure 9-9 Removing Empty Bins

If a peak had a significant shoulder, or -A peak, then there will be a number of data points in the bin before the actual peak. These -A peaks should be deleted as well.

- 8. Select **Sort by Nominal Size (nt)** to put the alleles list back to the order of fragment size.
- 9. Rename the allele ID with the corresponding gene name for each gene peak. Ensure the gene name is no more than 8 characters.

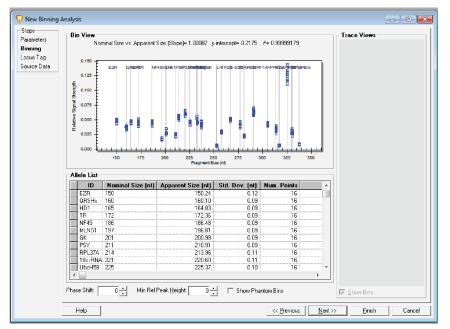


Figure 9-10 Rename Allele IDs during Binning Process

10. After renaming all allele IDs corresponding to all gene peaks in the multiplex, remove extra alleles.

11. Click Next.

The **Locus Tag** section of theNew Binning Analysis window will allow you to create the locus tag and locus name.

Figure 9-11 Locus Tag Section

inning	Locus Tag: Project: Locus Ta	Human Ref Default g Allele ID Criteri	•]	▼ Date la	st modified;		
	- Repeat unit	e: Human Ref	D4 • 149 and 3 1 - nt 1 -	340 nt	Ancillary Inform Primer set name: GenBank Accessi Repeat unit seque Ploidy: Primer Label C Eorward C Reverge G Ngne	on:	5' to 3' 5' to 3'
	Allele List						
	ID	Nominal Size (nt)		Std. Dev. (nt)	Num. Points	Comment	-
, and the second s	ID EZR	150	150.24	0.12	16	Comment	
	ID EZR QRSHs	150 160	150.24 160.10	0.12	16 16	Comment	
	ID EZR QRSHs HD1	150 160 165	150.24 160.10 164.83	0.12 0.09 0.09	16 16 16	Comment	
	EZR QRSHs HD1 TR	150 160 165 172	150.24 160.10 164.83 172.36	0.12 0.09 0.09 0.09	16 16 16 16	Comment	
	ID EZR QRSHs HD1 TR NF45	150 160 165 172 186	150.24 150.10 164.83 172.36 186.48	0.12 0.09 0.09 0.09 0.09 0.09	16 16 16 16 16 16	Comment	
	ID EZR QRSHs HD1 TR NF45 MLN51	150 160 165 172 186 197	150.24 160.10 164.83 172.36 186.48 196.81	0.12 0.09 0.09 0.09 0.09 0.09 0.09 0.09	16 16 16 16 16 16 16 16	Comment	
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- 12. Select the **Locus Tag** tab and set the parameters as follows:
 - Locus Name: Locus Tag and Locus Name should be the same.
 - **Project**: The project from which the data for this study was selected
 - Dye: 4
 - Fragment length range between: 149 nt and 340 nt
 - Repeat unit length: 1
 - # of repeats in shortest Allele: 1
 - **Ploidy**: 2 (default)
 - Primer Label: None (default)
 - Allele List: In Column ID, type a gene name for each applicable size. If the gene is a housekeeping gene, then type HK followed by a space at the beginning of the gene name.
- 13. Select the Allele ID Criteria tab.

Locus Tag	: Human Ref					
Project	Default		▼ Date la	ist modified:		
Locus				in moanoa.		
- Stutter	Definition			-Spurious Peak [Detection	
🗆 Searc	h for Stutter			Detect spurious	peaks	
Shuth	er detection window width	1 repeats			t for spurious peaks	1 + 2
					the shares have	
Maxi	mum relative stutter peak. h	neight 1 🚽 %				
	etect stutter shorter than a	alelle				
	letect stutter longer than a	llele				
	etect stutter longer than a	llele				
		llele		. 1 D-1		
	etect stutter longer than a			+ A Detection		
Confide			+/· nt	Apparent size in	cludes +A	
- Confide Sys	nce Interval	dence interval 0.21		Apparent size in Detect +/- A		
- Confide Sys	ence Interval	dence interval 0.21		Apparent size in		
⊂ Confide Sys ▼ Ove	ance Interval Item generated allele confi erwrite system confidence i	dence interval 0.21		Apparent size in Detect +/- A		
Confide Sys Confide Sys Confide Confide System Confide Confid	ence Interval stem generated allele confi arwrite system confidence i	dence interval 0.21 interval 0	15 +/- nt	Apparent size in Detect +/- A Use +A peak to	call Allele	
Confide Sys V Ove	nce Interval Rem generated allele confi erwrite system confidence i st Nominal Size [nt]	dence interval 0.21 interval 0	1.5 +/- nt Std. Dev. (nt)	Apparent size in Detect +/- A Use +A peak to Num. Points	call Allele	omment
Confide Sys V Ovi Allele Lis EZR	ince Interval dem generated allele confi erwrite system confidence i at Nominal Size [nt] 150	dence interval 0.21 Interval 0 Apparent Size [nt] 150.24	1.5 +/- nt Std. Dev. (nt) 0.12	Apparent size in Detect +/- A Use +A peak to Num. Points 16	call Allele	omment
Confide Sys I Ow Allele Lis EZR ORSH	Arm generated allele confi enwrite system confidence i st Nominal Size (nt) 150 50 160	dence interval 0.21 interval 0 Apparent Size [nb] 150.24 150.10	1.5 +/- nt Std. Dev. (nt) 0.12 0.09	Apparent size in Detect +/- A Use +A peak to Num. Points 16 16	call Allele	omment
Confide Sys V Ovi Allele Lis EZR	ince Interval dem generated allele confi erwrite system confidence i at Nominal Size [nt] 150	dence interval 0.21 Interval 0 Apparent Size [nt] 150.24	1.5 +/- nt Std. Dev. (nt) 0.12	Apparent size in Detect +/- A Use +A peak to Num. Points 16	call Allele	omment
Confide Sys Confide Sys Const D EZR ORSH	Ince Interval Item generated allele confli Item confidence i Item Nominal Size (nt) 150 6 160 165	dence interval 0.21 interval 0 Apparent Size [nt] 150.24 160.10 154.83	15 +/- nt Std. Dev. (nt) 0.09 0.09	Apparent size in Detect +/- A Use +A peak to Num. Points 16 16 16	call Allele	omment
Confide Syg I Ow Allele Lis EZR ORSH HDI	Ann generated allele confi enverte system confidence i at Nominal Size [nt] 150 165 165 172 188	dence interval 0.21 interval 0 Apparent Size [nt] 150.24 160.10 164.83 112.36	1.5 +/- nt Std. Dev. [nt] 0.12 0.09 0.09 0.09	Apparent size in Detect +/- A Use +A peak to Num. Points 16 16 16	call Allele	omment
Confide Sys I O vv Allele Lis E2R ORSH HD1 TR NF45	Ann generated allele confi enverte system confidence i at Nominal Size [nt] 150 165 165 172 188	dence interval 0.21 interval 0 Apparent Size [nt] 150.24 160.10 164.83 172.36 186.48	5 +/- nt Std. Dev. [nt] 0.12 0.09 0.09 0.09 0.09 0.09 0.09	Apparent size in Detect +/- A Use +A peak to Num. Points 16 16 16 16 16 16 16	call Allele	omment

Figure 9-12 Parameters for Allele ID Criteria

- 14. Set up the parameters as follows:
 - For Stutter Definition, Spurious Peak Detection, and +A Detection make no modifications.
 - For **Confidence Interval**, select the **Overview system confidence interval** check box and set the value to **0.5**.

At this point, the allele list can be either exported or printed. To export the allele list, right-click the **ID** header in the Allele List table, and select **Export Grid** and save it as a csv file.

To print the allele list, right-click the **ID** header in the Allele List table, and select **Print Grid**.

Figure 9-13 Review Source Data

	result	result date	capillary	analysis outcome	analysis parameter
ita 🕴	L-STD.H07_090223187X	02/23/2009 18:46:04	Н	pass	DefaultGeXPAnalysisParameters
	L-STD.H06_090223187M	02/23/2009 18:46:02	н	pass	DefaultGeXPAnalysisParameters
	L-STD.H05_090223187F	02/23/2009 18:46:00		pass	DefaultGeXPAnalysisParameters
16	L-STD.H04_0902231877	02/23/2009 18:45:56	H	pass	DefaultGeXPAnalysisParameters
	L-STD.H03_090223186Z	02/23/2009 18:45:54		pass	DefaultGeXPAnalysisParameters
16	L-STD.H02_090223186N	02/23/2009 18:45:50		pass	DefaultGeXPAnalysisParameters
	L-STD.G11_0902231862	02/23/2009 18:46:16		pass	DefaultGeXPAnalysisParameters
	L-STD.G09_090223188C	02/23/2009 18:46:10		pass	DefaultGeXPAnalysisParameters
10	L-STD.G08_0902231882	02/23/2009 18:46:06	G	pass	DefaultGeXPAnalysisParameters
	L-STD.G07_090223187T	02/23/2009 18:46:04	G	pass	DefaultGeXPAnalysisParameters
	L-STD.G06_090223187N	02/23/2009 18:46:02	6	pass	DefaultGeXPAnalysisParameters
15	L-STD.G05_090223187G	02/23/2009 18:46:00		pass	DefaultGeXPAnalysisParameters
	L-STD.G04_0902231873	02/23/2009 18:45:56		pass	DefaultGeXPAnalysisParameters
16	L-STD.G03_090223186U	02/23/2009 18:45:52		pass	DefaultGeXPAnalysisParameters
	L-STD.G02_0902231860	02/23/2009 18:45:50	G	pass	DefaultGeXPAnalysisParameters
					DefaultGeXPAnalysisParameters
	L-STD.G01_090223186G	02/23/2009 18:45:48	6	pass	DeraultureArAnalysisParameters
	L S 1 D. GUT_USU2231866	j 02/23/2009 18 45 46		pass	Deraulices-ranaystic araneters
		X	6	pass	Deraultussennasster anameters
	<	X			Derauludov-Panayon Parameters
	<	17			
E	4	"" C D E 0 0	0 0		S H Total 10 6 15 0 0 0
	< Summary A B Included 0 0	™ C D E 0 0	0 F		а <u>н Total</u> 10 6 тв

15. Click Next. The source data will be listed in the Results table.

The **Summary** area at the bottom of this table lists all of the samples included in the bin analysis. You may view and compare individual samples manually to include or exclude individual samples, or groups of samples, from the analysis. You can also reanalyze selected samples.

- 16. Click **Finish** to complete the binning process.
- 17. Open the Results Set View window.
- 18. Highlight all files in this study. Right-click highlighted area. Select **Reanalyze Results**, and then select **Using Additional/Edited Locus Tags**.

	TR Locus Tags	 <u> </u>	SNP Locus Tags	
Available: Name Test 4 Test 3 Penta E(2) Penta E(1) Penta E(1) Penta D(2) Penta D(1) Penta D(1) Penta D(1) Penta D New Locus Tag Locus 1 HuRef1 HuRef1 GeXP2 Edit Locus	Date 0/5/201412032 0/25/201412032 0/25/201412000 6/11/201442722 6/11/201442722 6/11/201442722 6/11/201442722 6/11/201442722 6/11/201442722 6/11/201442722 6/11/201442500	Selected:	Date	

Figure 9-14 Select Allele Identification Type and STR Locus Tag

- 19. Select **STR** as the **Allele Identification Type**. This will activate the STR Locus Tags tab.
- 20. On the STR Locus Tags tab, make the appropriate selection for the STR locus tag from the **Available** list. Move it to the **Selected** area using the > button.
- 21. Click **Next** to continue.

suits				Egolus	ion Filter Set		
result L-STD.H07_090223187X L-STD.H06_090223187M	V Reanalyze Data						• App
4.4 for leng, doccoming of the leng docco	Analyse Data	Image: 1 Pre-sett L 410 0.01 000221900 L 410 0.01 000221902 L 410 0.01 000221902 L 410 0.01 000221902 L 410 0.01 000221902 L 410 0.01 000221902	Date 2222005 6.46 IH 2222005 6.45 IH 2222005 6.45 IH 2222005 6.45 IH 2222005 6.45 IH 2222005 6.45 IH 2222005 6.45 IH 2222005 6.46 IH 22220005 6.46 IH		Date Analyzed Длав Аладузеd флафон флафон ()	• • •	

Figure 9-15 List of Sample Files Selected for Reanalysis

22. Click Analyze.

As the system proceeds with the reanalysis, the progress bar will be active and the lower dialog will record and show the analysis log for each sample as it is reanalyzed.

Figure 9-16 Reanalysis Is Complete

lts				Exclu	usion Filter Set	
	Reanalyze Data					
	Steps					1
	Locus Tags			Time s	tarted: 4/25/2014 5:45:00 PM	
	Analyze Data					
TD.H04_0902231877		Result	Date	Status	Date Analyzed 🔺	
TD.H03_090223186Z		L-STD.G09_090223188C	2/23/2009 6:46:10 PM pass		4/25/2014 5:45:01 PM	
TD.H02_090223196N		L-STD.G10_090223188P	2/23/2009 6:46:14 PM pass		4/25/2014 5:45:01 PM	
TD.H01_090223186F		L-STD.G11_0902231982	2/23/2009 6:46:16 PM pass		4/25/2014 5:45:01 PM	
TD.G12_0902231698		L-STD.G12_0902231898	2/23/2009 6:46:20 PM pass		4/25/2014 5:45:02 FM	
TD.G11_090223188Z		L-STD.H01_090223186F	2/23/2009 6:45:48 PM pass		4/25/2014 5:45:02 PM	
TD.G10_090223188P		L-STD.H02_090223186N	2/23/2009 6:45:50 PM pass		4/25/2014 5:45:02 PM	
TD.G09_090223188C		L-STD.H03_090223186Z	2/23/2009 6:45:54 PM pass		4/25/2014 5:45:02 PM	
TD.G08_0902231882		L-STD.H04_0902231877	2/23/2009 6:45:56 PM pass		4/25/2014 5:45:02 FM	
TD.G07_090223187T		L-STD.H05_090223187F	2/23/2009 6:46:00 PM pass		4/25/2014 5:45:05 PM	
TD.G06_090223187N		L-STD.H06_090223187M	2/23/2009 6:46:02 PM pass		4/25/2014 5:45:05 PM	
TD.G05_090223187G TD.G04_0902231873		L-STD.H07_090223187X	2/23/2009 6:46:04 PM pass		4/25/2014 5:45:05 PM	
D.G03 0902231873		Dye 3: QuantStd at 0.00+/- 0.00	0 min Amb - 0.000 -			
D.G02 0902231860		Dive 4: QuantStd at 0.00 +/- 0.00	0 min Amt = 0.000 g		^	
TD G01 0902231866		exec_FreLoad[] completed successfully	Similarit - 6.600 g			
10.001_0302231860		Operation PreLoad succeeded				
		Identify Alleles: Start				
		Identity Alleles: Processing locus: Human	n Ref			
		Identify Alleles: 71 fragments in locus: He Identify Alleles: End	uman Het			
		Operation AlleleID succeeded				
					E	
					-	
					*	
		<			F	
		Total Results: 19 Analyzed:	19 Passed: 19 Faile	ed 0		
						1
		Help	_	<< Brevious N	ext >> Einish Cancel	1
						_
	#I					
an	#1					
		n r r	G H Tatal	_		
A B	C	D E F	G H Total			
ied 0	C 0	0 0 0	10 6 1	16		
A B led 0 ded 0	C 0 0	0 0 0	10 6 1 2 1	3		
A B led 0 ded 0	C 0	0 0 0	10 6 1			

Gene Expression

When the reanalysis is complete, the **Status** column will indicate pass or fail for each sample data selected for reanalysis.

23. Click **Finish** to return to the Result Set View window.

When reanalysis is completed, the initial selected results are removed from the study and replaced with the reanalyzed results.

To view the reanalyzed sample data, highlight a sample data file in the sample list and double-click it to open the data file.

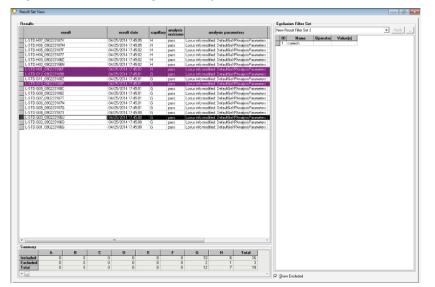


Figure 9-17 Selecting Reanalyzed Sample File for Review

Review the electropherogram of the selected sample file to ensure that correct allele ID (gene name) and locus tag (multiplex name) are tagged for each gene peak in the multiplex.

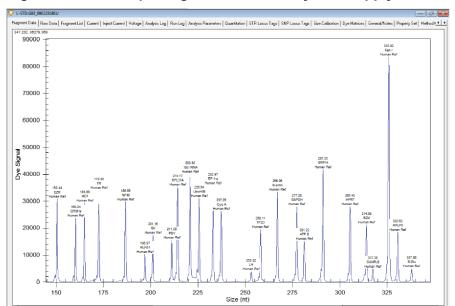


Figure 9-18 Electropherogram after Reanalysis to Apply Allele IDs and Locus Tag

Apply Exclusion Filters and Export Fragment Results

After gene peaks are labeled with locus tag and allele IDs, there may be some untagged shoulder peaks or small noise peaks present. These untagged peaks can be excluded from further data analysis by applying an exclusion filter in Fragment List view. The fragment results can then be exported for downstream gene expression analysis.

Apply an Exclusion Filter

Use the following exclusion filter in the Study-Data-Fragments List view to exclude untagged fragments from the sample results:

 Table 9-1 Exclusion Filter Table

ID	Name	Operator	Value
1	Allele ID	=	

Return to the Result Set View window to review the analyzed data.

Export Fragment Results

1. Select **File > Transfer Fragments for GeXP** to export fragment results.

Close Study Manage Studies					
Save Study					
					·
				Exclusion Filter Set	
Save Study As	result date	capillary analys		New Result Filter Set 3	 Apply
Add Raw Data to Study		capinary outcom	ne	1D Name Operator Value(s)	
Add Result Data to Study	04/25/2014 17:45:05	H pass	Locus into modified. DefaultGeXPAnalysisParameters	1 (select)	
Add Kesult Data to study	04/25/2014 17:45:05 04/25/2014 17:45:02	H pass H pass	Locus into modified: DefaultGeXPAnalysisParameters Locus into modified: DefaultGeXPAnalysisParameters		
Export Results	04/25/2014 17:45:02	H pass H pass	Locus info modified: DefaultGeVPAnalysisParameters Locus info modified: DefaultGeVPAnalysisParameters		
Export Fragments/Genotypes	04/25/2014 17:45:02	H pass	Locus into modified. DefaultGeXPAnalysisParameters		
Transfer Fragments for GeXP	04/25/2014 17:45:02	H pass	Locus into modified: DefaultGeXPAnalysisParameters		
	04/25/2014 17:45:02	H pass	Locus into modified: DefaukGeX(PAnalysisParameters		
Preferences	04/25/2014 17:45:01	G pass	Locus into modified. DefaultGeXPAnalysisParameters		
Print Screen		G pass	Locus info modified: DefaultGeXPAnalysisParameters		
Exit	04/25/2014 17:45:01	G pass G pass	Locus into modified: Defaul/GeX/PAnalysisParameters Locus info modified: Defaul/GeX/PAnalysisParameters		
ESR L-STD/608_0902231882	04/25/2014 17:45:01 04/25/2014 17:45:01	G pass G pass	Locus into modified. DefaultGeXPAnalysisParameters Locus info modified: DefaultGeXPAnalysisParameters		
L-STD 607_090223187T	04/25/2014 17:45:01	G pass	Locus into modified: DefaultGeXPAnalysisParameters		
L-STD.606_090223187N	04/25/2014 17:45:01	G pass	Locus into modified DefaultGeXPAnalysisParameters		
L-STD. 605_0902231876	04/25/2014 17:45:01	G pass	Locus into modified: DefaukGeXPAnalysisParameters		
L-STD.604_0902231873	04/25/2014 17:45:00	G pass	Locus info modified: DefaultGeXPAnalysisParameters		
L-STD.G03_090223186U	04/25/2014 17:45:00	G pass	Locus into modified: DefaultGeXPAnalysisParameters		
L-STD 602_0902231860 L-STD 601_0902231866	04/25/2014 17:45:00 04/25/2014 17:45:00	G pass G pass	Locus into modified DefaultGeXPAnalysisParameters Locus into modified DefaultGeXPAnalysisParameters		
E-510.001_0302231060	04/25/2014 17:45:00	u pass	Locus into modified. Defaulture/invitativessinarameters		
۷			,		
			,		
rinchuladd 0 0 0		E F	, 6 H Total 0 10 6 16		

Figure 9-19 Exporting Fragment Results for Gene Expression Analysis

2. In the Transfer Fragment for GeXP As dialog, type a file name (study name) for the fragment results in a designated direct path.

ulta				Exclusion Filter Set		
result	result date o	apillary analysis analy	sis parameters	lew Result Filter Set 3		-
STD.H07_090223187X STD.H06_090223187M STD.H05_090223187F	04/25/2014 17:45:05 H 04/25/2014 17:45:05 H 04/25/2014 17:45:05 H	pass Locus info modified: I pass Locus info modified: I pass Locus info modified: I	DefaultGeXPAnalysisParameters DefaultGeXPAnalysisParameters DefaultGeXPAnalysisParameters	ID Name 1 cselect	Operator Value(s)	
STD.H04_0902231877 STD.H03_0902231882 STD.H02_0902231882 STD.H02_090223186H STD.H01_090223185F STD.G12_0902231838	V Transfer fragments for Ge		v 49 Search Expant	 9		
STD 611 0902231882	Organize 🔻 New folde	er -	311	- 0		
110 010 000221888 110 040 000221882 110 040 000221882 110 040 00022188 110 040 00022188 110 040 00022188 110 040 000221880 110 040 000221880 110 040 000221880	Computer Co	Name Parama Ref. GoldP.cov Parama HuMe, GoldP.cov	4/25/2014 5:50 PM	Type <u>Microsoft Ex</u> Microsoft Ex		
	🕞 Local Disk (Q:) 🔫					
	File name: Huma			•		
	Save as type: GeXP	(*.csv)		•		
	Hide Folders		Save	ancel		
nay -			,			
A B aded 0 0 udedd 0 0 al 0 0	C D E 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	F G 0 0 10 0 0 2 0 0 12	H Total 6 16 1 3 7 18	Show Excluded		

Figure 9-20 Transfer Fragment for GeXP As Dialog

- 3. From the list next to **Save as type**, select **csv** as the export file name extension.
- 4. Click Save.

The status of the data file exporting, along with a processing log, opens in a status window.

The Transfer Fragments for GeXP dialog indicates when the export procedure is complete and whether any errors occur.

8				Eze	lusion Filter S	et		
result	result date	capillary analysis	analysis parameters	Neo	v Result Filter Set	3		 Apply
ID.H07_090223187X	04/25/2014 17:45:05	H pass	Locus info modified: DefaultGeXPAnalysisParameters	- 0	D Name	Operator	Value(s)	
TD.H06_090223187M	04/25/2014 17:45:05	H pass	Locus info modified: DefaultGeXPAnalysisParameters	- 1	(select)			
ID.H05_090223187F	04/25/2014 17:45:02	H pass	Locus info modified: DefaultGeXPAnalysisParameters					
D.H04_0902231877	04/25/2014 17:45:02	H pass	Locus info modified: DefaultGeXPAnalysisParameters					
TD.H03_090223186Z	04/25/20			- 1111				
ID.H02_090223186N	04/25/20 💙 Transfer	fragments for GeXP						
D.H01_090223186F	04/25/20					1		
10.G12_0902231898 10.G11_0902231862	04/25/20 04/25/20							
D.G10_090223186P	04/25/20	GeXP Transfer S	Status:					
D.G09 090223188C	04/25/20							
D.G08_0902231882	04/25/20	Transfer compl						
D.G07_090223187T	04/25/20	ranster compl	1818.					
D.G06_090223187N	04/25/20							
D.G05_090223187G	04/25/20							
D.G04_0902231873 D.G03_0902231860	04/25/20	Cancel		0K				
D.G02_0902231860	04/25/20							
10.G01 090223186G	04/25/20							
wy								
A B	C D E		G H Total					
ed 0 0 jed 0 0	0 0	0 0						
	0 0	0 0						

Figure 9-21 Transfer Fragments for GeXP Dialog

5. If no error occurs, click **OK** to complete the export process.

Normalize the Exported Fragment Data

After exporting the fragment data, it can be normalized using the GeXP Analysis Template. Before normalizing the data, it must be converted to the correct format using the CSV Transformer Tool.

Both tools are available on the GenomeLab System software DVD. If the DVD is not available, then contact SCIEX support at: sciex.com/request-support.

Convert the Exported Fragment Data

- 1. In File Explorer, double-click **CVS Transformer**.
- 2. Click Transfer Format and then select GeXP Normalization Format.

- 3. Click **Output By** and then select **Peak Area**.
- 4. Click **Select Input Files** and then select the file containing the data to be transformed. If required, additional files can be selected.
- 5. Click Select Output Folder and then select the location for the transformed file.
- 6. In the **Size difference range** field, type the difference between the lengths of the most closely spaced fragments in the multiplex.

CSVTransformer - Version 2.0.3	Х		
GeXP Normalization Format	Output By		
Input File Folder and List	Output File Folder and List		
C:\Growth Study Results\	C:\Growth Study Results\		
Test.csv	Test_xfer.csv		
Select Input Files	Select Output Folder		
Suffix string for output files:	Apply New Suffix		
Allow Overwrite Output Files			
Consolidate different sizes in same allele			
Size difference range: 2 nt	Transform Exit		

Figure 9-22 CSV Transformer Tool

7. (Optional) Type a different suffix in the **Suffix string for output files** field and then click **Apply New Suffix**.

The file name in the Output File Folder and List list updates with the new suffix.

8. Click **Transform** and then click **OK** in the confirmation message. The gene name, sizes, sample names, and peak areas of the fragments are extracted and saved to the output file.

Perform Gene Normalization

- 1. In Excel, open the .csv file created by the CSV Transformer Tool.
- 2. If neccesary, for each housekeeping gene, type HK followed by a space at the beginning of the gene name in the **Locus ID** field.

This may apply to a single gene, multiple genes, or every gene in the multiplex.

Note: To avoid editing the gene names in each exported file, add the HK to the gene names before exporting the fragment data from the GenomeLab System software. Refer to step12 in Set Up Locus Tag and Allele IDs.

- 3. In Excel, open the GeXP Analysis Template and then click the **Raw Data** tab.
- 4. Copy the data from the transformed .csv file, and then paste the data in the B1 cell in GeXP Analysis Template.

Note: Do not copy the entire worksheet. Copy only the part of the worksheet with data.

Figure 9-23 Location to Paste Data in the GeXP Analysis Template

C1	Ŧ	: × ✓ f _x			
	A.	В	С	D	E
1	Normalization				
2	Factor	Generate Normalization Factor			
3		Generate Normalization Factor			
4					
5					
6					
7					
8					
9					
10					

5. Click **Generate Normalization Factor**, and then click **OK** in the two dialogs. Column A in the worksheet is filled with the normalization factors for the data.

B3	1 🔻	: × ✓ fx r54.F02_150416	6110G				
	Α	В	с	D	E	F	G
1	Normalization		GENE A	GENE B	HK GENE C	GENE D	GENE E
2	Factor	Generate Normalization Factor	140	145	148	152	160
3		Generate Normalization Factor					
4	5944	N9.A08_2102151109	8566	489	1506	3693	2642
5	5402.666667	N9.A05_210215110A	7163	444	1098	3119	2126
6	3351.333333	N9.A02_210215110A	4299	212	623	1949	1340
7	4702	N8.H07_210215110A	9529	5770	2272	9753	6421
8	9325	N8.H04_210215110A	20463	13212	5441	22049	14419
9	6154	N7.G07_210215110B	12106	516	1639	6437	1603
10	8929.333333	N7.G04_210215110B	14493	618	2004	8256	1979
11	4069.333333	N7.G01_210215110B	7603	402	1146	4334	962
12	16059.66667	N60.E09_210215110B	25156	1567	5477	17363	7502
13	38127.66667	N60.E06_210215110C	49534	3022	12807	35386	15669

Figure 9-24 Normalization Factors

- 6. To normalize the data:
 - a. Click the Normalized Data tab.
 - b. Click Insert Names and then click OK in the two dialogs.
 - c. Click **Normalize Data** and then click **OK** in the two dialogs. The normalized data is shown in the worksheet.

Figure 9-25 Normalized Results

K2	K2 ▼ : × ✓ fx						
	A	В	С	D	E	F	G
1	1 Insert Names		GENE A	GENE B	HK GENE C	GENE D	GENE E
2		Normalize Data					
3							
4	N9		1.441117093	0.082267833	0.253364738	0.621298789	0.44448183
5	N9		1.325826752	0.082181639	0.203232971	0.577307502	0.393509378
6	N9		1.282773026	0.063258405	0.185896161	0.581559578	0.399840859
7	N8		2.026584432	1.227137388	0.483198639	2.074223735	1.365589111
8	N8		2.194423592	1.416836461	0.583485255	2.364504021	1.546273458
9	N7		1.967175821	0.083847904	0.266330842	1.04598635	0.260480988
10	N7		1.623077497	0.069210094	0.224428849	0.924593101	0.221629088
11	N7		1.868365007	0.09878768	0.281618611	1.065039318	0.236402359

7. Click File > Save As, type a name for the worksheet, and then click Save

Note: The GeXP Analysis Template file is in xls (Excel 97-2003) format and the file contains macros. Saving the file in xlsx (Excel Workbook) format deletes the macros from the file. To retain the macros, save the file in xlsm (Excel Macro-Enabled Workbook) format.

Data Manager Module

Overview

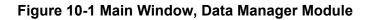
Use the Data Manager module to create, save, and modify databases.

These databases can contain any of the following data types:

- Filter sets
- Fragment analysis parameters
- Fragment results
- Methods
- Optical scan data
- Sample data
- Sample plates
- Sample plate results
- Sequence analysis parameters
- Sequence results
- SNP locus tags
- Standards
- STR locus tags

Main Window

The main window for the Data Manager module has the following sections.



А _В	_c		D	
📴 Data Manager				- 6
File Edit View Tools Window Help	/			
🗉 🌺 GENOMELAB DBASE	Name	Туре	Modified	Project
🖨 🖻 CEQ	1 - D11S1984 - 0.1pmol, 0	Sample Data	4-20-2001 11:35:52	D11S1984
🖻 🛱 Default	1 - D11S1984 - 0.1pmol, 0	Sample Data	4-20-2001 11:35:52	Default
E CEOBAK1	1 - D22S683 - 0.1pmol.A0	Sample Data	4-25-2001 17:23:53	D22S683
E CEQBAK2	1 - D3S2387 - 0.25pmol.A	Sample Data	4-18-2001 18:01:31	0352387
🖨 🐻 FAII-B	1 - D9S2157 - 0.1pmol.A0	Sample Data	5-2-2001 09:33:22	09S2157
⊕- ²⁷ D11S1984	1 - D9S938 - 0.2pmol - 0.2	Sample Data	5-17-2001 13:46:44	D9S938
🖨 🛱 D14S599	1 - DXS7132 - 2pmol.A03	Sample Data	4-3-2001 18:44:22	DXS7132
🖶 🛱 D1S1679	1 - GATA193A07 - 0.25pm	Sample Data	4-3-2001 20:40:16	GATA193A07
🖶 🛱 D22S683	1-D1S1679 at 0.25pmol.A0	Sample Data	5-7-2001 10:25:34	D1S1679
👜 📛 D3S2387	1-pull 0.25, primer 9 (599)	Sample Data	4-26-2001 16:18:11	D14S599
📴 🛱 D9S2157	10 - D11S1984 - 0.1pmol,	Sample Data	4-20-2001 12:36:30	D11S1984
🖨 🛱 D95938	10 - D22S883 - 0.1pmol.B	Sample Data	4-25-2001 18:24:30	D22S683
🖶 📛 Default	10 - D3S2387 - 0.25pmol	Sample Data	4-18-2001 19:00:02	D3S2387
📴 📛 DXS7132	ID - D9S2157 - 0.1pmol.B	Sample Data	5-2-2001 09:33:26	D9S2157
📴 📛 GATA193A07	10 - D9S938 - 0.2pmol - 0	Sample Data	5-17-2001 15:20:04	D9S938
👜 📛 Test project	10 - DXS7132 - 2pmol.B04	Sample Data	4-3-2001 19:42:17	DXS7132
B-B USERTEMPLATE	10 - GATA193A07 - 0.25p	Sample Data	4-3-2001 21:38:12	GATA193A07
	12 - D22S883 - 0.1pmol.D	Sample Data	4-25-2001 18:24:33	D22S683
	12 - D3S2387 - 0.25pmol	Sample Data	4-18-2001 19:00:05	D3S2387
V.,	12 - D9S2157 - 0.1pmol.D	Sample Data	5-2-2001 09:33:28	D9S2157
	17 nacase n 200001 n	Samela Data	6 17 2001 16:20 0R	nacase
Ready			Fik	er Off NUM

ltem	Description
A	Title Bar: Shows the module name.
В	Menu Bar: Provides access to all menus. Refer to the section: Menu Bar.
С	Toolbar: Contains the icons that execute pre-defined functions. Refer to the section: Toolbar.
D	Display Area: Lists the files contained in the selected node.
E	Status Bar: Shows information concerning the current selection.

Menu Bar

The following example shows the menu bar for the Data Manager module.

Figure 10-2 Data Manager Module Menu Bar

<u>File Edit View Tools Window Help</u>

File Menu

Click **File** to show the following menu.

Figure 10-3 File Menu, Data Manager Module

Eile	
New	Ctrl+N
New Data <u>b</u> ase	Ctrl+B
Open	Ctrl+O
Print	Ctrl+P
Print Setup	
Print Screen	•
Delete	
<u>R</u> ename	
Properties	
Import	
<u>E</u> xport	
Set As Working Database	
E <u>x</u> it	

Option	Description			
New	Create a new project or standard in a database.			
New Database	Create a new database.			
Open	Open data files, including: Edit Fragment Results, Optical Scan Data, Sample Data, Sequence Results, Sample Plate Results, and Standards.			
Print	Print the selected item.			
Print Setup	Define printer properties.			
Print Screen	Open a sub-menu with options to print an image of the computer desktop or application window.			
Delete	Delete items, projects, and databases.			
	Note: The working database cannot be deleted.			
Rename	Rename items, projects, and databases.			
Properties	Show the current database and modification date as well as other sample data collection information concerning the selected item in the same format as sequence result properties. Refer to the section: Sequence Result Properties.			

Option	Description
Import	 Import a file in one of the following formats: Standard chromatogram format versions 2.10 and 3.00 (*.scf)
	Electropherogram sample data format (*.esd)
	• CEQ (*.cq*)
	Note: You must select a project in which to import the items.
Export	 Export a file in one of the following formats: Standard chromatogram format versions 2.10 and 3.00 (*.scf)
	Tab-delimited ASCII Text (*.txt)
	SEQ sequence text (*.seq)
	FASTA and Quality (*.fasta)
	PHRED and SCF (*.scf.phd.1)
	• CEQ (*.cq*)
	Electropherogram sample data format (*.esd)
	For more information about export file types in the Data Manager module, refer to the section: Data Manager Module Export File Types.
Set as Working Database	Define the currently selected database as the default database.
Exit	Close the module.

Edit Menu

Click **Edit** to show the following menu.

Figure 10-4 Edit Menu, Data Manager Module

Edit	
Cu <u>t</u>	Ctrl+X
⊆ору	Ctrl+C
Paste	Ctrl+∀
Eind	Ctrl+F
Find <u>N</u> ext	F3
Select <u>A</u> ll	Ctrl+A

Option	Description
Cut	Copy the selected item to the clipboard and removes it from its current location.
Сору	Copy the selected item to the clipboard without removing it from its current location.
Paste	Insert copied or cut items from the clipboard into the selected location.
Find	Open a Find dialog, which you can use to locate a specific item, project, or database.
Find Next	Search for the next occurrence of the item or folder defined in the Find dialog.
Select All	Select all items under the selected item.

View Menu

Click **View** to show the following menu.

Note: A check mark next to an option indicates that the option is enabled.

Figure 10-5 View Menu, Data Manager Module



Option	Description			
Toolbar	oggle to show or hide the toolbar.			
Status Bar	Toggle to show or hide the status bar.			
Refresh	Rebuild the window display to reflect the most recent changes.			
Filter By	Filter the list (on the right side window) by the dates modified.			
Database	Show all items in the selected database.			
Sample Run History	Show a list of items that were run in a project during specified dates.			

Tools Menu

Click **Tools** to show the following menu.

Figure 10-6 Tools Menu, Data Manager Module

Tools Backup Database Restore Database Shrink Database Associate Default Dye Spectra Convert Individual ID to Subject

Associate Default Dye Spectra Convert Individual ID to Subject ID Administrative Tools

Option	Description			
Backup Database	Create a backup copy of the selected database.			
Restore Database	Restore a previously backed up copy of a database to the Data Manager module.			
Shrink Database	Decrease the size of the currently selected database.			
	Note: If the 21 CFR Part 11 tools are in use, then this function is only available to users with Write access.			
Associate	Associate previously saved data with a new default dye spectra.			
Default Dye Spectra	Note: If the 21 CFR Part 11 tools are in use, then this function is only available to users with Write access.			
Convert Individual ID to	Convert all sample data records that use Individual IDs to records that use Subject IDs.			
Subject ID	Note: If the 21 CFR Part 11 tools are in use, then this function is only available to users with Write access.			
Administrative Add the CEQUSERS group to the SQL login. Only members of the Tools > Add CEQUSERS group are given access the CEQ databases. CeqUsers Group to SQL Login Add the CEQUSERS group are given access the CEQ databases.				

Window Menu

Click **Window** to show the following menu.

Figure 10-7 Window Menu, Data Manager Module

W	indow
	New Window
	Cascade
	Tile
	Arrange Icons
	Close
~	1
	2

Option	Description	
New Window	Open a new display window.	
Cascade	Cascade the open windows.	
Tile	Tile the windows in a horizontal orientation.	
Arrange Icons	Automatically arrange the icons.	
Close All	Close all currently active windows.	
√1	Bring the selected window (#1) to the foreground.	

Help Menu

Click **Help** to show the following menu.

Figure 10-8 Help Menu

He	elp
	Help <u>T</u> opics
	<u>U</u> sing Help
	About GenomeLab System
	SCIEX Home Page

Option	Description		
Help Topics	Select this option to show the Help topics. By default, the Contents show in the navigation pane on the left and the introductory topic shows in the topic pane on the right. Use the Contents to select and print specific Help topics. In the navigation pane, use the Search and Index tabs to search for information by topic, index entry, and/or keyword.		
Using Help	ing Help Select this option to open the Contents window on how to use the Window Help system.		

Option	escription			
About GenomeLab System	Select this option to access software and system information.			
SCIEX Home Page	Select this option to access the SCIEX Home Page on the Internet.			

Toolbar

The Data Manager module provides one toolbar with icons that correspond to common menu items.

Figure 10-9 Toolbar, Data Manager Module

	<u>⊜</u> ×≊∎⊒ ?№
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lcon	Description
£	Up One Level: Move the directory structure tree up one level.
D	New: Create a new project or database.
B	Open: Open a standard.
*	Cut: Copy the selected item to the clipboard and removes it from its current location.
	Copy: Copy the selected item to the clipboard without removing it from its current location.
	Paste: Insert copied or cut items from the clipboard into the selected location.
4	Print: Print a report of the selected item.
×	Delete: Delete selected items, projects, and databases.
	Properties: Shows the current database and modification date and other information concerning the selected data file.
B	Backup Database: Create a backup of the database.

lcon	Description
Ē	Restore: Restore the database with the backup.
8	Help Topics: Open the Help System. By default, the Contents show in the navigation pane on the left and the introductory topic shows in the topic pane on the right. Use the Contents to select and print specific Help topics. In the navigation pane, use the Search and Index tabs to search for information by topic, index entry, and/or keyword.
▶?	Context-Sensitive Help: Click this icon and then on a menu item to open the Help topic related to the selection.

Using the Data Manager Module

Store Methods and Parameters

The **USERTEMPLATE** database stores newly created methods and parameters. This database is created during software installation. These methods and parameters are then included in all subsequently created databases. Use the **Cut**, **Copy**, and **Paste** functions to add methods and parameters to the **USERTEMPLATE**.

Although you cannot set the **USERTEMPLATE** database as the working database, you can delete it. If this database is deleted, no template will be used for subsequent databases. If you need a new template, create a new one named **USERTEMPLATE**.

Create a Database

- 1. On the Main Menu, click the **DATABASE** icon
- In the Data Manager main window, right-click the GENOMELAB DBASE node and select New Database.

The New Database dialog opens.

3. Enter a name for the database and, if desired, select Set As Working Database.

🛅 Data Manager				_ 8 ×
File Edit View Tools Window Help		New Database 🗙		
		Enter Name for New Database:		
GENOMEL AD DD ACC New Database Ctrl+B		MyNewDatabase		Size
Berger Carley	CE CE	i got no montang batabasa		MB
In the Default of the second			5 N 15	MB
	E FA) MB
🛱 🖾 FAII-B	🖻 US	E	5 N	4B
👜 🛱 D11S1984				
🗣 🛱 D148599				
📴 🛱 D1S1679				
■ 📴 D22S683				
📴 🛱 D3S2387				
🕸 🛱 D9S2157				
🟚 🛱 D9S938				
🗊 🛱 Default				
📴 🛱 DXS7132				
📴 📛 GATA193A07				
🖻 🖑 Test project				

Figure 10-10 Setting Up New Working Database in the Data Manager Module

4. Click OK.

Set the Working Database

Program the database that contains all of the projects and the location where data is stored until another database is set as the working database.

- 1. Close all modules except for the Data Manager module.
- 2. In the Data Manager module, select the database to be used as the working database.
- 3. Select File > Set as Working Database.

The name of the working database shows in bold.

Note: Database size is limited to approximately 1,700 megabytes. However, working with smaller databases to improve performance is recommended.

Create and Name a Project

To create and name a project:

- 1. In the Data Manager window, select and highlight the database where the project will reside, then click **File > New**.
- 2. Highlight the new project and click **File > Rename**.
- 3. Enter a descriptive name for this project and press Enter.

```
Note: Do not use special characters (I : * ? \ " < > |) in the file name.
```

4. Close the Data Manager module.

Delete a Project or Database

Note: If the 21 CFR Part 11 tools are in use, then this function is only available to users with Write access.

- 1. In the Data Manager window, select and highlight the folder or database to be deleted.
- Select File > Delete.
 A Data Manager dialog opens, prompting you to confirm deletion.
- 3. Click **Yes** to delete the folder or database.

Note: The working database cannot be deleted.

IMPORTANT Deleting a folder deletes all data contained within that folder.

Rename a Project or Database

- 1. In the Data Manager window, select and highlight the project or database.
- 2. Select File > Rename.
- 3. Enter the new name for the database and press Enter.

Note: Do not use special characters (*I* : * ? \ " < > |) in the file name.

Check the Database Size

To check the size of each database:

- 1. On the Main Menu, click the DATABASE icon
- 2. Left-click the **GENOMELAB DBASE** node at the top of the left pane. The size for each database shows in the right pane.

Figure 10-11 Checking the Database Size

🧮 Data Manager		
File Edit View Tools Window Help	New Database	
	Enter Name for New Database:	
		33 MB 5 MB 15 MB 150 MB 5 MB 5 MB
B OS/132 B OS/133 B OS/133 B OS/132 B OS/		

Note: If you recently removed data from the database, the size shown may not be accurate. Refer to the section: Decrease the Database Size.

Decrease the Database Size

Note: If the 21 CFR Part 11 tools are in use, then this function is only available to users with Write access.

Manually decrease the size of the database to a new (smaller) size. After removing studies or results from the database, the size of the database does not immediately decrease in size.

Note: Always decrease the database size before backing it up. The database size cannot be decreased during database back up.

- 1. Open the Data Manager module.
- 2. Select and highlight the database that will be reduced.
- 3. Select **Tools > Shrink Database**.

Back Up a Database

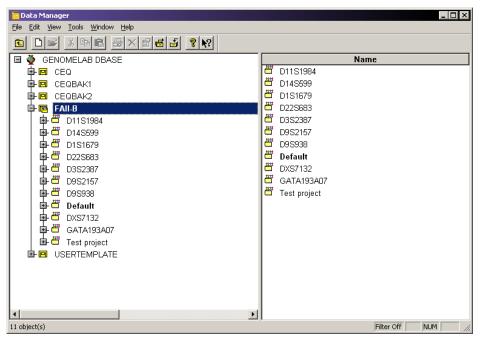
Periodic backups are essential to decrease the risk of losing data. Save backups to any folder on the local drive.

IMPORTANT If a database is deleted or damaged, only the data created since the last backup will be lost.

Note: Shrink the database before backing up any database. Refer to the section: Decrease the Database Size.

- 1. Open the Data Manager module.
- 2. Left-click the **GENOMELAB DBASE** node at the top of the left pane.
- 3. Select the database to be backed up.

Figure 10-12 Select Database to Back Up



- 4. Select Tools > Backup Database.
- 5. Browse to the location on the local disk where the file will be saved.

Figure 10-13 Backup Database Dialog



6. Accept the file name or rename it as necessary.

Note: Since databases are backed up frequently, you might want to add the date as part of the name to prevent overwriting valuable backups and to ensure accurate database recovery.

7. Click Save.

Restore the Database

Recover a database that was previously saved using the backup procedure.

Note: If you are restoring a database that has the same name as one already residing in the Data Manager module, you must either restore it with a new name or delete the existing database before restoring it. This option is not recommended.

- 1. Open the Data Manager module.
- 2. Select Tools > Restore Database.
- 3. Browse to the location on the local disk where the backup file was saved.

Figure 10-14 Restore Database Dialog

Restore Database	? ×
Look in: 🗀 Dbase 💌	← 🗈 📸 Ⅲ-
CEQ_2006_4_29.BAK	
File name: CEQ_2006_4_29.BAK	Restore
Files of type: Database Backup Files (*.bak)	Cancel
Database To Restore: CEQ_2006_4_29	

4. Select and highlight the file to restore and click **Restore**.

Note: If a database with the same name exists, a warning dialog will open.

Figure 10-15 Data Manager Warning Message

Data Ma	nager 🗙
♪	'Database To Restore' name already exists. Please enter a new database name.
	ОК

- 5. If this occurs, click **OK** and either:
 - Delete the database in the Data Manager module. This option is not recommended.
 - Rename the database being restored. To do this, enter a new name in the **Database To Restore** field.

Convert Individual IDs to Subject IDs

Note: If the 21 CFR Part 11 tools are in use, then this function is only available to users with Write access.

Converts all sample data records with Individual IDs to records that use Subject IDs.

Before you begin, confirm that the present database is the Working Database. Refer to the section: Set the Working Database.

The **Subject ID** attribute applies to the sequence and fragment records that were generated from the converted sample data record. Performing this action removes the **Individual ID** column and adds a new **Subject ID** column to all existing studies within the database.

1. Open the Data Manager module.

Note: The working database cannot be changed while other modules are open.

2. Select and highlight the database to convert and ensure that it is the Working Database.

Note: This option is available only for the working database.

 Select Tools > Convert Individual ID to Subject ID. After the IDs are converted, the status bar shows the message Conversion Completed Successfully.

Create New Size Standards

- 1. Open the Data Manager module.
- 2. Select the **Default** or **Project** folder.
- 3. Open the **Standards** sub-folder in the **Default** or **Project** folder.
- 4. Select **File > New** to create a new standard item.
- 5. Double-click **New Standard Item** to begin defining the individual size standard fragments.
- 6. Select the appropriate dye label attached to the size standard fragments.
- 7. Input the sizes of each standard fragment with one fragment per line.
- 8. Select **Save As** to save the new size standard.

Export Database Files

- 1. Open the Data Manager module.
- 2. Open a sequence result.
- Select File > Export. The Export dialog opens.
- 4. Browse to the target folder, then select and highlight the file name.
- 5. Select the desired format in the Save as type field.

Figure 10-16 Export Dialog

Export				
Sample Elements Header Raw Data	Save in: 🗀	Export 💌] 🕁 🖻	I 💣 🎟 -
Result Data Result Output Guality Parameters Alignment Results Alignment Accuracy				
Options Remove CEQ Tracking Suffix Resolve Filename Conflicts Apply Trimming	File name: Save as type:	SEQ TEST_2006_04_01 SCF (Ver 3.00) (*.scf) SCF (Ver 3.00) (*.scf)	_	Export Cancel
		SCF (Ver 2.10) (".scf) Text (Tab Delimited) (".txt) SEQ (Sequence Text only) (".seq) FASTA (FASTA and QUAL) (".fasta) PHRED (PHRED and SCF) (".scf.phd.1 ESD (".sed) CEQ (".cq"))	

6. Click Export.

Data Manager Module Export File Types

The following table describes the available export file types in the Data Manager module.

Format	Description
SCF (.scf)	Use the standard chromatogram format (SCF) to store analyzed DNA sequence data for a single sample. The format describes both public and private data sections. The well-defined public data section contains the trace data points, called sequence, positions of each called base within the trace data, and call scores for each called base. It also includes a public comment section. The private section may store other information that most software packages ignore.
	The GenomeLab GeXP system uses the private data section to store sample data (including raw data along with current and voltage data). You can store sample data from either sequencing or fragment analysis samples. The notes and property sets for the sample are stored in the comment section. The GenomeLab System software supports SCF versions 2.1 and 3.0. (You would use this format, for instance, to export analyzed sequence data into Lasergene's SeqMan from DNASTAR). Third-party packages cannot currently view raw data, current, or voltage. However, custom applications can be written to view the data.
	Use the SCF format to transfer data from instrument to instrument, or to import data from third-party software into the Sequence Analysis module. Using SCF to exchange data between instruments removes information, such as sample plate name and capillary array serial number. To maintain exact copies of data including header information, use the CEQ format and import and export these data using the Data Manager module.
Tab-Delimited Text (.txt)	The tab-delimited format is valid for raw data, sequence results and fragment results. Select the Raw Data check box to include raw data, as well as current and voltage data. Select the Results Data and Results Output check boxes to include the sequence results.
	The format expresses the data points as text values that can be viewed in any text editor or in Excel.

Table 10-1 Data Manager Module Export Options

Format	Description
PHRED (.scf.phd.1)	The PHRED format produces two files: a PHRED file and an SCF file. A PHRED file is a text file composed of a SEQUENCE section containing a COMMENT and DNA section. The SEQUENCE section also contains the name of the analyzed result that was stored.
	The COMMENT section contains the file name of the associated SCF file (CHROMAT_FILE) that was produced along with the PHRED file. The values for ABI_THUMBPRINT and PHRED_VERSION are fixed text N/A . The values for CALL_METHOD and QUALITY_LEVELS are fixed as CEQ SEQUENCING and 99 , respectively for the GenomeLab GeXP system. The time stamp is the current time at the time of export.
	The DNA section is composed of the base-call (always lowercase), PHRED quality value, and the peak index (data point) of the analyzed data contained within the associated SCF file. Values are separated by spaces.
	Selecting the PHRED format exports the result data, result output (base sequence), and quality parameters automatically if the sample has been analyzed with a sequence analysis parameter set. If the sample has been analyzed with a fragment analysis parameter set, nothing is exported.
ESD (.esd)	The electropherogram sample data (ESD) format exports raw data from the GenomeLab GeXP system to a third-party software package for analysis. This option exports only raw data and does not include any other information.
SEQ (.seq)	The SEQ format only applies to the sequence results portion (such as text bases) of analyzed sequence data. This format is used, for instance, to export data into Lasergene's SeqMan from DNASTAR.
	Selecting the SEQ format exports the result output (base sequence) automatically if the sample has been analyzed with a sequence analysis parameter set. If the sample has been analyzed with a fragment analysis parameter set, nothing is exported.

Table 10-1 Data Manager Module Export Options (continued)

Format	Description
FASTA (.fasta)	The FASTA format only applies to the sequence results portion (such as text bases) of analyzed data, and contains a comment line that is used to identify the data. Along with the text file, a quality values file is also generated (*.FASTA.QUAL) which lists the quality value for each base. (Quality values correspond to -10*log10[error rate]). The range for the quality values is 1 to 99. Edited and inserted bases have a value of 99. This format is used, for instance, to export data into PHRAP.
	For instance, in PHRED, a quality value of 10 means 1 error in 10. A quality value of 20 means an error rate of 1 error in 100. A quality value of 30 means an error rate of 1 error in 1,000. A quality value of 40 means an error rate of 1 error in 10,000, etc. Normally PHRED would export these quality values into PHRAP. In the case of exporting from the GenomeLab GeXP system, we bypass PHRED and export directly into PHRAP.
	Selecting the FASTA format exports the result output (base sequence) and the quality parameters automatically if the sample has been analyzed with a sequence analysis parameter set. If the sample has been analyzed with a fragment analysis parameter set, nothing is exported.

Table 10-1 Data Manager Module Export Options (continued)

Format	Description		
CEQ (.cq*)	Selecting the CEQ format, the format native to our database, exports sample data, sequence or fragment results, sample plate results, sequence or fragment analysis parameters, sample plates, methods, locus tags (fragment data only), standards (fragment data only), and optical scan data. The sample elements are automatically selected in the Export dialog, which would export all of the associated information listed above. This format essentially takes a snapshot of the file and can retain all of the information belonging to that file.		
	This format is available only to import data into the Data Manager module. This format can be used to export data from the Sequence Analysis, Fragment Analysis, and Data Manager modules.		
	When exporting as CEQ data, the various data types are exported to files with the following extensions:		
	Sequence analysis parameter = CQA		
	Sequence investigator = CQC		
	 Fragment size standard = CQD 		
	 Fragment analysis parameter = CQF 		
	 Galvo scan cata = CQG 		
	Fragment locus tags = CQL		
	• Method = CQM		
	SNP locus tag = CQN		
	Sequence result = CQR		
	Sample data = CQS		
	Sample table = CQT		
	Fragment result = CQU		
	 Sample table result = CQX 		
Remove CEQ Tracking Suffix	You can automatically remove the plate position coordinates (*.A01, *.B02, etc.) and the time/date stamp from the sample name which is normally generated by the GenomeLab System software on export. To do so, select the Remove CEQ Tracking Suffix check box.		

Table 10-1 Data Manager Module Export Options (continued)

Format	Description
Resolve Filename Conflicts	If the system encounters the same sample name at the destination, it usually overwrites the name (if you choose to remove the plate position coordinates and the time/date stamp which ensures a unique name). If you select the Resolve Filename Conflicts check box, the system increments the sample names on export when it encounters duplicate names. For example, if the system encounters the duplicate sample name of <i>Fred</i> on export, it renames the file exported to <i>Fred_1</i> .
Apply Trimming	Selecting this option removes the trims from the sequence before you export it and replaces internal trims with the letter <i>X</i> .

Table 10-1 Data Manager Module Export Options (continued)

Format	Description
CRV (*.crv)	The CRV export option from the Data Manager module exports the four colors (dyes) as four separate files. When exporting batches, the resulting four files contain separate dye colors for all the samples.
	You can select *.CRV as an export file type for exporting fragment results from the Fragment Analysis module.
	Individual files are created representing individual colors:
	 {FileName}_D1.crv D1 contains dye 1 - red
	 {FileName}_D2.crv D2 contains dye 2 - black
	 {FileName}_D3.crv D3 contains dye 3 - green
	 {FileName}_D4.crv D4 contains dye 4 - blue
	These are default dye colors. You can reassign the dye colors at any time.
	Batch CRV Exporting
	More than one result can be exported in a batch. If, for instance, more than one result is exported in a batch, the export generates five files, one for each dye and one to assist in determining the trace order.
	{FileName}_D1.crv D1 contains all dye 1 results in the batch
	 {FileName}_D2.crv D2 contains all dye 2 results in the batch
	 {FileName}_D3.crv D3 contains all dye 3 results in the batch
	 {FileName}_D4.crv D4 contains all dye 4 results in the batch
	 {FileName}_CRV_TRACE_ORDER.txt created for batch export files to assist in determining trace order in the CRV files

Table 10-1 Data Manager Module Export Options (continued)

Import Database Files

To import files into the database:

- 1. Open the Data Manager module.
- 2. In the Data Manager window, browse and select the project where the imported file will reside.
- 3. Select **File** > **Import**. The Import dialog opens.

- 4. Browse to locate the folder where the file resides.
- 5. Select the import format from the **Files of type** list.
- 6. Select the file name.
- 7. Click **Import**.

Generate a Sample Run History

- 1. Select **View > Sample Run History**. The Sample Run History dialog opens.
- 2. Select a **Project** name from the list.
- 3. Select **Enable** and enter a filtering start and end date.
- 4. Click Compute.
- 5. View the total number of runs during the specified dates in the **Total Sum of Samples Run** field.
- 6. If desired, print a report of all samples run during the specified dates by clicking **Print**.

Administrative Tools

The System Administrator can access the database without logging onto the computer as an Administrator. Before using the Administrative Tools, an Administrator must first set up a group and assign to it the users to have access to the GenomeLab GeXP system databases.

Users and Groups

Adding Users and Groups is done outside of the GenomeLab System software. After creating the **CEQUSERS** group and adding users to it, you must activate the group within the Data Manager module.

Add Users

1. Select Start > Settings > Control Panel > Administrative Tools > Computer Management.

The Computer Management console opens.

📙 Computer Management			
📃 Eile Action View Window H	elp		_ 8 ×
← → 🗈 🖬 🕄 🖧 😫			
Computer Management (Local) Computer Management (Local) System Tools Shared Folders Cocal Users and Groups Cocal Users Cocal Users Cocal Users Cocal Users Cocal Users Cocal Users Cocal Users Cocal Users Cocal Users Cocal Users Cocal Users Cocal Users Cocal Users Cocal Users Cocal Users Cocal Users Cocal Users Cocal Users Cocal Users Cocal Users Cocal Users Cocal Users Co	Name	Full Name ASP.NET Machine Account Remote Desktop Help Assi CN=Microsoft Corporation	Description Built-in account for administering th Account used for running the ASP.I Built-in account for guest access to Account for Providing Remote Assis This is a vendor's account for the H
	•		

Figure 10-17 Computer Management Console - Selecting Users

- 2. In the left pane, click the + symbol next to the **System Tools** item to expand its contents.
- 3. Click the + symbol next to the Local Users and Groups item to expand its contents.
- 4. Right-click **Users** and select **New User**. The New User dialog opens.

Figure 10-18 New User Dialog

New User	? ×
User name:	estUser1
Eull name:	
Description:	
Password:	
Confirm password:	
🔽 User <u>m</u> ust chang	ge password at next logon
🔲 Uger cannot cha	inge password
Pass <u>w</u> ord never	expires
🔲 Account is disa <u>b</u>	led
	Cr <u>e</u> ate Cl <u>o</u> se

- 5. Enter a new **Username**. This step is required.
- 6. Edit the other fields as necessary.
- 7. Click Create.

Add Groups

To add a new group from the Windows desktop:

- Select Start > Settings > Control Panel > Administrative Tools > Computer Management. The Computer Management console opens.
- 2. In the left pane, click the + symbol to next to the **System Tools** item to expand its contents.
- 3. Click the + symbol next to the Local Users and Groups item to expand its contents.
- 4. Right-click **Groups** and select **New Group**. The New Group dialog opens.
- 5. Enter **CEQUSERS** in the new **Group name** field and, if necessary, enter a description in the **Description** field.

New Group			? ×
<u>G</u> roup name:	CEQUSERS		
Description:			
Members:			
			- 1
<u>A</u> dd	<u>R</u> emove		
		<u>C</u> reate Cl <u>o</u> se	,

- Click Add to add created users. Refer to the section: Add Users. The Select Users or Groups dialog opens. The local computer appears as the default location in the Look in field. If it does not, use the list and select the local computer.
- 7. Scroll down the **Name** list and select the user names created. If you created more than one user, click the first name, press and hold the **Ctrl** key, and then select any additional names to add to the group.
- 8. Click Add > OK to add the selected users to the new group.

Figure 10-20 Select Users Dialog

Select Users		? ×
Select this object type:		
Users or Built-in security principals		<u>O</u> bject Types
Erom this location:		
EC-WINXP-TEST		Locations
Enter the object names to select (<u>examples</u>):		
EC-WINXP-TEST\TestUser1		<u>C</u> heck Names
<u>A</u> dvanced	ОК	Cancel

9. Click **Create** to save the new group.

Figure 10-21 New Group Dialog

New Group			? ×
<u>G</u> roup name:	CEQUSERS	 	
Description:			
<u>M</u> embers:			
TestUser1			
<u>Add</u>	<u>R</u> emove		
		<u>C</u> reate	Cl <u>o</u> se

Activate the CEQUSERS Group

After creating the **CEQUSERS** group and adding users to the group, you must activate the group within the Data Manager module. This is done only once and users can be added or removed at any time thereafter.

To activate the **CEQUSERS** group:

- 1. Open the Data Manager module.
- 2. Select **Tools > Administrative Tools > Add CeqUsers to SQL Login**. All users added to the group **CEQUSERS** now have access to the database.

The GeXP Home Page provides access to the 21 CFR Part 11 tools.

Figure 11-1 GeXP Home Page

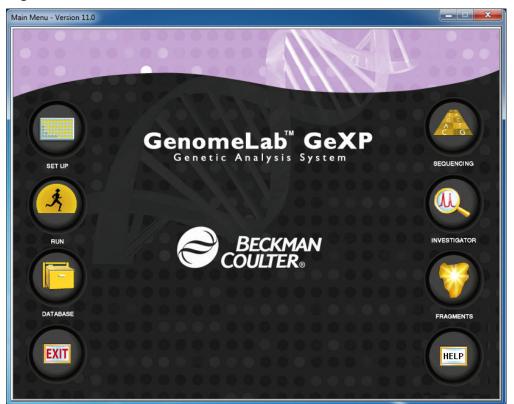
🟠 GeXP Home Page			– 🗆 X
SCIEX			Exit
	APPLICA	TIONS	
GeXP Main	User	Sign Off a	Audit Trail
Menu	Management	Report	
LUGGED IN USER	: Local, Administrator		Logout

Тооі	Description
GeXP Main Menu	Provides access to the software modules
User Management	Provides the tools to create and manage user accounts
Sign Off a Report	Enables users with sign-off authority to sign a report
Audit Trail	Provides a log of the main operations that have occurred in the GenomeLab System software and the ability to save events from the log in an Excel file

GeXP Main Menu

On the GeXP Home Page, click GeXP Main Menu.

Figure 11-2 Main Menu



To open a module, click its icon. Users with Write access can use all of the functions in the software modules to create, edit, and analyze methods and data. Users with Read access can view methods and data but cannot create or edit them.

Note: For a description of the software modules, refer to the section: Software Module Descriptions.

After opening a software module, the Main Menu collapses to the Windows taskbar or as a shortcut toolbar. Refer to the section: Main Menu Collapse Options.

User Management

The User Management tool is used by the software Administrator to create and manage user accounts. The Administrator can:

- Add, edit, and delete user accounts
- Add, edit, and delete roles
- Assign Read or Write access to roles

• Give users the authority to sign reports

Other users can edit the first name, last name, password, and digital signature for their own user account.

Add a User

- 1. Log on as the software Administrator.
- 2. On the GeXP Home Page, click User Management.

User Managemen	t		- 🗆 🗙
Users Roles			
Total Number of Users : 10 + Add			
User Name	Role	Digital Signature	Sign-Off Authority
admin	Administrator	No	No
Jane	Chemist	No	No
suneelm	Analyst	No	Yes
naveen	Analyst	Yes	Yes
mario	Analyst	No	Yes
janejo	Analyst	No	Yes
herman	TechWriter	Yes	Yes
bea	TechWriter	No	Yes
Fperez	Service	No	Yes
Greg	Manager	No	Yes

Figure 11-3 User Management Application, Users Tab

3. On the Users tab, click **Add**. The Add User dialog opens.

Figure 11-4 Add User Dialog

ser			×
First Name		User Role	
Last Name		Digital Signature (optional)	
User Name			
Password	Show		
Confirm Password	Show		
Add]	Cancel	
	Last Name User Name Password Confirm Password	First Name Last Name User Name Password Show Confirm Password Show	First Name User Role Last Name Digital Signature (optional) Select pfx file to uplo; Browse User Name Isign-Off Authority (optional) Password Show Confirm Password Show

- 4. Type the first and last name of the user, a user name, and a password.
- 5. Make a selection from the User Role list.
- 6. (Optional) To attach a digital ID to the user account, click **Browse** and then select the applicable file.

Note: If a user signs a report without a digital ID, then the user name cannot be authenticated and will not show in the **Signature Panel**. For instructions to create a digital ID, refer to the section: Create a Digital ID in Adobe Reader.

- 7. (Optional) To give the user the ability to sign reports, select the **Sign-Off Authority** check box.
- 8. Click Add.

The **Digital Signature** and **Sign-Off Authority** columns show if a digital ID is attached to a user account and if a user has sign-off authority.

Edit a User

The software Administrator can change any information in the user account. Other users can change their own first name, last name, password, and digital signature.

1. On the GeXP Home Page, click **User Management**.

User Management			– 🗆 X
Users Roles			
Total Number of Us	+ Add		
User Name	Role	Digital Signature	Sign-Off Authority
admin	Administrator	No	No
Jane	Chemist	No	No
suneelm	Analyst	No	Yes
naveen	Analyst	Yes	Yes
mario	Analyst	No	Yes
janejo	Analyst	No	Yes
herman	TechWriter	Yes	Yes
bea	TechWriter	No	Yes
Fperez	Service	No	Yes
Greg	Manager	No	Yes

Figure 11-5 User Management Application, Users Tab

2. On the Users tab, double-click the user.

Figure 11-6 Edit User Dialog

Edit User	-		×
First Name			
Jane	Show Password		
Last Name			
Johnson	Change Password		
User Name	Digital Signature (Optional)		
janejo	Select pfx file to upload	Browse	
User Role			
Analyst -	Sign-Off Authority		
Update	Delete		

3. Modify the user information as required, and then click **Update**. For users other than the software Administrator, a Warning dialog opens.

To accept the changes and close the GenomeLab System software, click Yes.

- 4. Do the following to change the password:
 - a. On the Users tab, double-click the user.
 - b. Click Change Password.

Figure 11-7 Change Password Dialog



- c. Type a new password and then type it again to confirm it.
- d. Click Update.

Delete a User

- 1. Log on as the software Administrator.
- 2. On the GeXP Home Page, click User Management.

User Managemen	t		- 🗆
Jsers Roles			
Total Number of	Users : 10		+ Add
User Name	Role	Digital Signature	Sign-Off Authority
admin	Administrator	No	No
Jane	Chemist	No	No
suneelm	Analyst	No	Yes
naveen	Analyst	Yes	Yes
mario	Analyst	No	Yes
anejo	Analyst	No	Yes
herman	TechWriter	Yes	Yes
bea	TechWriter	No	Yes
Fperez	Service	No	Yes
Greg	Manager	No	Yes

Figure 11-8 User Management Application, Users Tab

3. On the Users tab, double-click the user.

Note: The admin (software Administrator) user account cannot be deleted.

Figure 11-9 Edit User Dialog

Edit User	– 🗆 X
First Name Jane	Show Password
Last Name	
Johnson	Change Password
User Name	Digital Signature (Optional)
janejo	Select pfx file to upload Browse
User Role	
Analyst -	Sign-Off Authority
Update	Delete

- 4. Click Delete.
- 5. In the Confirmation dialog that opens, click **Yes**.

Add a Role

- 1. Log on as the software Administrator.
- 2. On the GeXP Home Page, click **User Management**.

User Management	t	-		Х
Users Roles				
Total Number of I	Roles : 7		+ /	٨dd
Role Name	Access level			
Administrator	Write			
Chemist	Read			
Service	Read			
Analyst	Write			
Manager	Read			
TechWriter	Read			
Support	Read			

3. Click the **Roles** tab, and then click **Add**.



Add Role	-		×
Role Name			
Access Level			
			•
Add		Cancel	
Add		Jancer	

- 4. In the **Role Name** field, type a name for the role.
- 5. In the Access Level field, select Read or Write, and then click Add.

Edit a Role

- 1. Log on as the software Administrator.
- 2. On the GeXP Home Page, click User Management.

Figure 11-12 User Management Application, Roles Tab

💩 User Management	:	-		Х
Users Roles				
Total Number of I	Roles : 7		+ /	Add
Role Name	Access level			
Administrator	Write			
Chemist	Read			
Service	Read			
Analyst	Write			
Manager	Read			
TechWriter	Read			
Support	Read			

3. Click the Roles tab, and then double-click the role to be changed.

Figure 11-13 Edit Role Dialog

Edit Role	_		\times
Role Name			
Analyst			
Access Level			
Write			•
Update		Delete	

4. Make the required changes, and then click **Update**. The changes are applied to all user accounts with this role.

Delete a Role

- 1. Log on as the software Administrator.
- 2. On the GeXP Home Page, click **User Management**.

User Management	1	-		×
Users Roles				
Total Number of I	Roles : 7		+ 4	٨dd
Role Name	Access level			
Administrator	Write			
Chemist	Read			
Service	Read			
Analyst	Write			
Manager	Read			
TechWriter	Read			
Support	Read			

3. Click the Roles tab, and then double-click the role to be deleted.

Figure 11-15 Edit Role Dialog

Edit Role	-		\times
Role Name			
Analyst			
Access Level			
Write			-
		Delete	
Update		Delete	

- 4. Click Delete.
- 5. In the Confirmation dialog that opens, click Yes.
- 6. In the Warning dialog that opens, click **Yes**.

Create a Digital ID in Adobe Reader

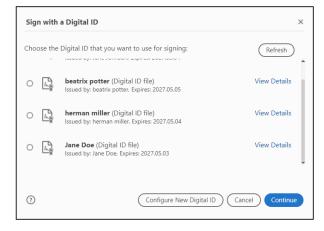
Use this procedure to create a digital ID and enable the use of an authenticated signature.

Note: Actual onscreen selections may vary depending on the version of Adobe Reader in use.

- 1. In Adobe Reader version 2021.005.20048 or later, open a pdf file. Any pdf file can be used to create a digital ID.
- 2. Click **Tools > Certificates**.
- 3. Click **Digitally Sign**, and then click **OK**.
- 4. Press and hold the left mouse button, and then drag the cursor to draw an area for the signature.

The Sign with a Digital ID dialog opens.

Figure 11-16 Sign with a Digital ID Dialog



5. Click Configure New Digital ID.

Figure 11-17 Configure a Digital ID for signing Dialog

A Digital ID is required to create a digital signature. The most secure Digital ID are issued by trusted Certificate authorities and are based on secure devices like smart	Select the type of Digital ID:		
	0	ā	Use a Signature Creation Device Configure a smart card or token connected to your computer
card or token. Some are based on files. You can also create a new Digital ID, but they provide a low level of identity	0	L.	Use a Digital ID from a file Import an existing Digital ID that you have obtained as a file
assurance.	0		Create a new Digital ID Create your self-signed Digital ID

6. Click **Create a new Digital ID**, and then click **Continue**.

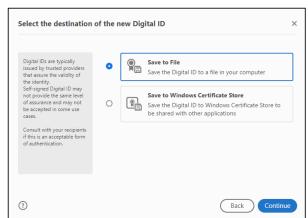


Figure 11-18 Select the destination of the new Digital ID Dialog

7. Click Save to File, and then click Continue.

Figure 11-19 Create a self-signed Digital ID Dialog

Enter the identity information to be used for	Name	Enter Name	
information to be used for creating the self-signed Digital ID.	Organizational Unit	Enter Organizational Unit	
Digital IDs that are self-	Organization Name	Enter Organization Name	
signed by individuals do not provide the assurance that the identity information is	Email Address	Enter Email	
valid. For this reason they may not be accepted in	Country/Region	US - UNITED STATES	~
some use cases.	Key Algorithm	2048-bit RSA	~
	Use Digital ID for	Digital Signatures	~

8. Type a name and an email address, and then click **Continue**.

Figure 11-20 Save the self-signed Digital ID to a file Dialog

Save the self-signed D	igital ID to a file >
Add a password to protect the private key of the Digital ID. You will need this password again to use the Digital ID for signing. Save the Digital ID file in a known location so that you can copy it or back it up.	Your Digital ID will be saved at the following location : C:\Users\Administrator\AppData\Roaming\Adobe\Acrc Browse Apply a password to protect the Digital ID: Confirm the password:
0	Back Save

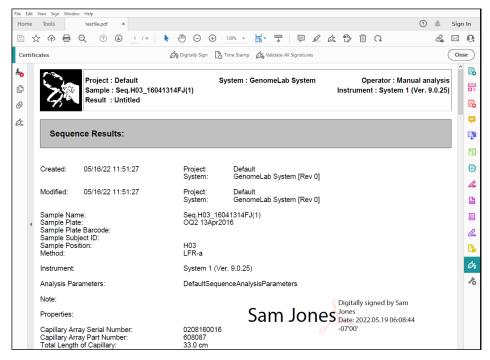
- 9. Type a file path or click **Browse** to find a location to save the file.
- 10. Type the password that was used to log on to the GenomeLab GeXP system, and then type it again to confirm it.
- 11. Select a location to save the signed file, and then click **Save**.
- 12. In the Sign with a Digital ID dialog, click the applicable digital ID file, and then click **Continue**.

Figure 11-21 Sign as Dialog

Sign as "Sam Jones"	×
Appearance Standard Text	~ Create
Sam Jones	Digitally signed by Sam Jones Date: 2022.05.19 05:43:21 -07'00'
Lock document after signing	View Certificate Details
Review document content that may affect si	igning Review
Enter the Digital ID PIN or Password	Back Sign

13. Type the password used to log on to the GenomeLab GeXP system, and then click **Sign**. The digital signature shows in the area that was created for it.

Figure 11-22 Digital Signature



Sign Off a Report

For this function to be available to the user, the software Administrator must add sign-off authority to the user account.

Note: If a user signs a report without a digital ID, then the user name cannot be authenticated and will not show in the **Signature Panel**. For instructions to create a digital ID, refer to the section: Create a Digital ID in Adobe Reader.

1. On the GeXP Home Page, click **Sign Off a Report**.

Figure 11-23 Signed Reports Dialog

Record	Report Name	Signed By	Sign-Off Date and Time
SEQ.A02_21012016GT	C:\Users\Administrator\Desktop\	greg	3/11/2022 9:12:58 AM
SEQ.A02_21012016GT	C:\Users\Administrator\Desktop\	Lisa	4/14/2022 1:34:31 PM
Frag.A02_22041805AR	C:\Users\Administrator\Desktop\	suneel	4/19/2022 11:18:23 AM
SEQ.A02_21012016GT	C:\Users\Administrator\Desktop\	suneel	4/19/2022 11:24:29 AM
Frag.A02_1604131388(1)_2202	C:\Users\Administrator\Desktop\	naveen	4/21/2022 10:32:11 PM
SEQ.A02_21012016GT	C:\Users\Administrator\Desktop\	naveen	4/21/2022 10:33:02 PM
SEQ.A02_21012016GT	C:\Users\Administrator\Desktop\	naveen	4/21/2022 10:33:20 PM
Frag.A02_1604131388(1)_2202	C:\Users\Administrator\Desktop\	suneel	4/22/2022 6:26:59 AM
Frag.A02_1604131388(1)_2202	C:\Users\Administrator\Desktop\	suneelm	4/22/2022 12:12:31 PM
Frag.A02_1604131388(1)_2202	C:\Users\Administrator\Desktop\	suneelm	4/22/2022 12:14:00 PM
Frag.A02_1604131388(1)_2202	C:\Users\Administrator\Desktop\	naveen	5/3/2022 7:57:38 AM
Frag.A02_22041805AR	C:\Users\Administrator\Desktop\	janej	5/4/2022 6:02:56 AM
SEQ.A02_21012016GT	C:\Users\Administrator\Desktop\	heman	5/4/2022 7:36:41 AM
Frag.A02_1604131388(1)_2202	C:\Users\Administrator\Desktop\	Herman	5/4/2022 8:01:03 AM
Frag.A02_1604131388(1)_2202	C:\Users\Administrator\Desktop\	Herman	5/4/2022 8:06:41 AM
SEQ.A02_21012016GT	C:\Users\Administrator\Desktop\	heman	5/5/2022 5:47:49 AM
Frag A02 1604131388(1) 2202	C:\Users\Administrator\Deskton\	berman	5/5/2022 6:37:03 AM

- 2. Do one of these actions to open a report:
 - Double-click a record in the list.
 - Click Select Report to Sign, find and select the applicable file, and then click Open.

Note: The default location for reports is C:\CEQ System\Reports.

Figure 11-24 Report Preview Dialog

Report Preview						-	
🖺 🔶 🖡		1 / 5	▶ ⊕ ⊕		Q: Share	? Sig	n In
Z	Project : Default Sample : SEQ.A02_210 Result : SEQ.A02_210		System : Genon		Oper : GenomeLab System	rator : Fabian (Ver. 11.0.24)	
	nce Results: SEQ.A02	-					
		Project:	Default				
Created:	01/21/21 11:34:12	System:		System [Rev 0]			
Created: Modified:	01/21/21 11:34:12		GenomeLab Default	System [Rev 0] System [Rev 0]			
Modified: Sample Na Sample Pla	01/21/21 11:34:12 me: tte: tte Barcode: bject ID:	System: Project: System:	GenomeLab Default				*

3. Review the report, and then click **Apply Signature**.

Figure 11-25 Sign Off the Report Dialog

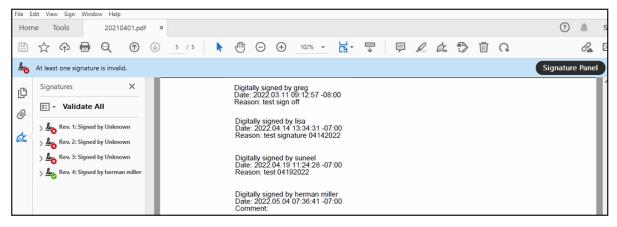
Sign Off the Repo	rt	×
User Name]
Password		Show
Comment		
	OK Cancel	

- 4. Type the user name and password that were used to log on to the GenomeLab GeXP system.
- 5. (Optional) Type comments related to the report.
- 6. Click OK.
- 7. Select a location to save the signed report.
- 8. To see the users who have signed the report, click **Signature Panel**.

A panel opens on the left. A green check mark shows next to users who have a digital ID, which is verified with password authentication. A red X shows next to users who have signed the report but do not have a digital ID. The name of the user does not show.

To see all of the information for the users who have signed the report, go to the end of the document.

Figure 11-26 Signature Panel



Audit Trail

On the GeXP Home Page, click Audit Trail.

Figure	11-27	Audit	Trail	Window
iguio		Addit	man	

Aud	it Trail			- 0	_
			Export to Excel	Refresh	
intry No.	User Name	Event	Date and Time	Record ID	
53	admin	admin modified janejo credentials	5/19/2022 5:20 AM		
52	admin	admin Logged into application	5/19/2022 5:12 AM		
51	admin	admin Logged out of application	5/18/2022 12:49 PM		
50	admin	admin Logged into application	5/18/2022 12:30 PM		
49	mario	mario Logged out of application	5/16/2022 3:44 PM		
48	mario	mario Logged into application	5/16/2022 3:42 PM		
47	admin	admin Logged out of application	5/16/2022 3:40 PM		
46	admin	admin Logged into application	5/16/2022 3:33 PM		
45	mario	mario Logged out of application	5/16/2022 3:32 PM		
44	mario	mario Logged into application	5/16/2022 3:31 PM		
43	mario	mario Logged out of application	5/16/2022 3:31 PM		
542	mario	mario modified mario credentials	5/16/2022 3:31 PM		
41	mario	mario Logged into application	5/16/2022 3:28 PM		
540	mario	mario Logged out of application	5/16/2022 3:28 PM		
39	mario	mario modified mario credentials	5/16/2022 3:28 PM		
538	mario	mario Logged into application	5/16/2022 3:27 PM		
537	mario	mario Logged out of application	5/16/2022 3:27 PM		
536	mario	mario Logged into application	5/16/2022 2:50 PM		
535	mario	mario Logged out of application	5/16/2022 2:50 PM		
534	mario	mario Logged into application	5/16/2022 2:49 PM		
533	mario	mario Logged out of application	5/16/2022 2:49 PM		
532	mario	mario modified mario credentials	5/16/2022 2:49 PM		
531	mario	mario Logged into application	5/16/2022 2:46 PM		
530	mario	mario Logged out of application	5/16/2022 2:46 PM		

To sort the events in chronological order, click the arrow in the **Date and Time** column header.

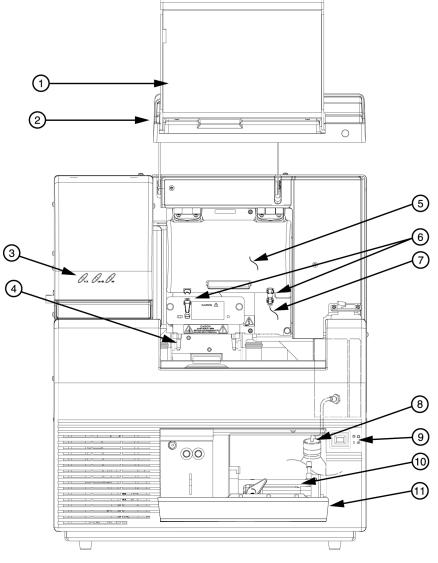
To save an event or range of events as an Excel file, click the event or select a range, and then click **Export to Excel**. Browse to the location to save the file, type a file name, and then click **Save**.

To show the most recent events, click **Refresh**.

This chapter provides routine maintenance, replenishment, and biological waste disposal procedures. It provides a list of the consumable materials used in the system. It also includes some diagnostic procedures performed as needed.

Routine Maintenance

Note: Use Figure 12-1 to locate hardware components referenced in this section.





901632L.AI

ltem	Description
1	Sample access cover (extended)
2	Capillary access cover (extended)
3	Status indicators
4	Plate holders/sample transport
5	Capillary temperature control cover

Item	Description
6	Rubber latches
7	Manifold access cover
8	Gel waste bottle
9	Power switch
10	Gel pump
11	Gel pump/gel cartridge access cover

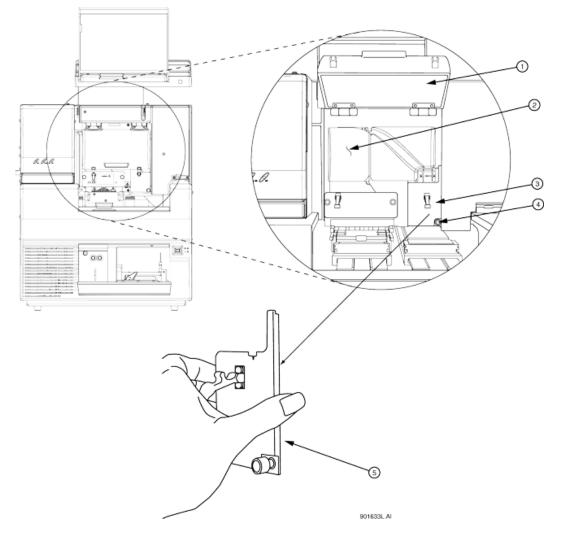
Clean the Capillary Array

Note: The capillary array windows must kept free of any contaminants. Otherwise, high backgrounds and/or drifting baselines will occur. All background levels should be below 6,000 counts.

Water used during this procedure must be fresh, distilled, deionized water (18 Mohm/cm water).

- 1. From the Run module menu bar, select **Replenish > Release Capillary Array**.
- 2. Wait for the Remove Capillary Array dialog to open.
- 3. Open the sample access cover and lift it to the vertical locking position.
- 4. Open the capillary access cover and lift it to the vertical locking position.
- 5. Unlatch the two rubber latches holding the capillary temperature control cover and lift it to the vertical locking position.
- 6. Loosen the manifold access cover captive screw, remove the cover, and set it aside.



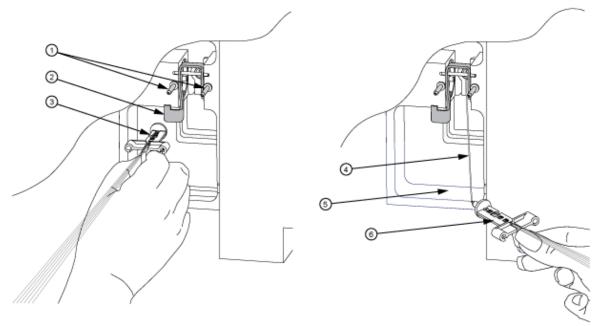


Info	Description
1	Capillary temperature control cover (open)
2	Plenum assembly
3	Manifold access cover
4	Captive screw
5	Manifold access cover (removed)

- 7. Lift the eject lever to release the array fitting.
- 8. Grasp the array fitting tab and then:

- a. Pull the fitting approximately 1 inch away from the manifold.
- b. Touch the tip of the fitting to the bottom of the optics base plate.
- c. Hold this position and wait 5 seconds for the gel strand to dry.
- d. Pull the fitting away from the instrument.
- e. Use a tissue to wipe gel strands off of the instrument.

Figure 12-3 Removing/Replacing the Array Fitting



Item	Description
1	Guide pins
2	Eject lever
3	Array fitting
4	Gel strand
5	Optics base plate (bottom)
6	Array fitting

9. With the array fitting in hand, blow dust and debris off of the windows with compressed gas (Texwipe Microduster III AccTech).

10. Using a water-moistened swab (Texwipe Alpha Mini Swab), gently wipe the detection window by stroking in one direction only.

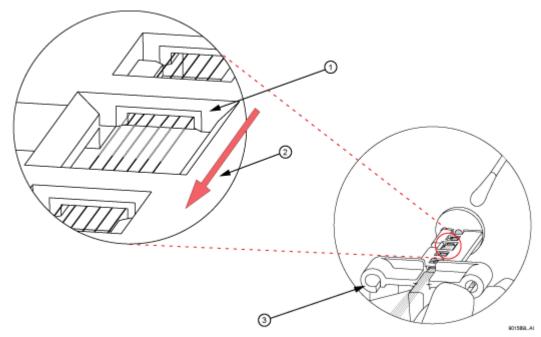


Figure 12-4 Cleaning the Detection Window

ltem	Description
1	Detection window
2	Stroke in one direction only!
3	Array fitting

- 11. With a new water-moistened swab, repeat wiping on the other side of the window.
- 12. With a dry swab, gently wipe the windows to remove excess water, again repeating on the backside with a new, dry swab.
- 13. Blow compressed gas on the windows to remove all excess water.

Note: Make sure not to invert the compressed gas bottle. Otherwise, propellant will contaminate the capillary windows.

- 14. If dried gel or other debris remains on the window, repeat this procedure.
- 15. In the Remove Capillary Array dialog, select Clean Capillaries and click OK.

Remove Capillary Array	x		
Go You may now open sample/capillaries access doors to change capillaries.			
To replace the capillary array immediately, select "Replace capillary array."	OK		
To install a manifold plug, select "Install manifold plug."	Cancel		
To clean the capillary windows, select "Clean capillaries."	Help		
Remove Capillary Array Options Replace capillary array Install manifold plug C Clean capillaries			
Capillaries exposed to air:			
Time Remaining min: sec: 14 14			

Figure 12-5 Remove Capillary Array Dialog

- 16. While holding the array fitting tab, align the array fitting with the manifold opening and guide pins. Push the fitting into the manifold until it is completely seated against the bases of the guide pins.
- 17. Replace the manifold access cover and tighten the captive screw.
- 18. Lower the capillary temperature control cover and secure the two rubber latches.
- 19. Lower the capillary access cover and sample access cover to their closed/locked positions.
- 20. In the Install Capillary Array dialog, click **Done**.

Figure 12-6 Install Capillary Array Dialog

Install Capillary Array				
Please enter the serial number for the capillary array. If you wish to reset the number of runs for the capillary or number of days it has been on the instrument, enter the new values.				
Cancel				Cancel
Click on Done when you have installed the capillary array and have closed both the capillary access cover and the sample access cover.			Set to New	
Capillaries exposed to a	air:	Time Rema min: 14	aining sec: 11	Help
Capillary Array				
Part Number:	608087	•	Total Length:	33.00 cm
Serial Number:			Length to Detector:	30.00 cm
Date Installed:	04/27/2006		Internal Diameter:	75.00 μm
Time Installed:	16:45:20			
Number of Runs:	0	÷		
Days on Instrument:	13.1	÷		

Note: After cleaning the detection windows, perform the optical alignment procedure and monitor the baseline. If background levels are above 6,000 counts, repeat the cleaning process.

Replace the Gel Waste Bottle for Dual-Rail System

Note: This procedure assumes that you are replacing a used (full) waste bottle with an empty waste bottle.

Use the Run module to ensure the GenomeLab GeXP system is ready and follow these procedures to change the bottle.

 From the Run module menu bar, select **Replenish > Empty Bottle**. The Empty Waste Bottle dialog shows a warning as the GenomeLab GeXP system prepares for removal.

Figure 12-7 Empty Waste Bottle Dialog - Stop



2. Wait until the dialog shows the green text message GO. At this time, it is safe to open the sample access door. Leave this dialog open.

Figure 12-8 Empty Waste Bottle Dialog - Go



- 3. Remove the cap from the new (empty) waste bottle.
- 4. Open the sample access cover and lift to the vertical locking position.
- 5. With the gel waste bottle 80% to 90% full, remove the cap and pull the bottle out of the GenomeLab GeXP system.
- 6. Place the cap from the new bottle over the full waste bottle and secure.
- 7. Thread the new bottle onto the cap attached to the GenomeLab GeXP system and set the bottle into position.
- 8. Close the sample access cover.
- 9. Dispose of the full waste bottle according to proper procedures. Refer to the section: Dispose of the Gel Waste Bottle.

10. In the Empty Waste Bottle dialog, click **Done**.

Replace the Gel Waste Bottle for Single-Rail System

Note: This procedure assumes that you are replacing a used (full) waste bottle with an empty waste bottle. Replacing the gel waste bottle on the single-rail system is performed through the **Replenish > Replace Wetting Tray** function.

Use the Run module to ensure the GenomeLab GeXP system is ready and follow these procedures to change the bottle.

- 1. Remove the cap from the new (empty) waste bottle.
- 2. From the Run module menu bar, select **Replenish > Replace Wetting Tray**.
- 3. Click **OK** when prompted to verify this action.
- 4. Wait until the dialog shows the green text message GO.
- 5. Open the sample access cover and lift to the vertical locking position.
- 6. With the gel waste bottle 80% to 90% full, remove the cap and pull the bottle out of the GenomeLab GeXP system.
- 7. Place the cap from the new bottle over the full waste bottle and secure.
- 8. Thread the new bottle onto the cap attached to the GenomeLab GeXP system and set the bottle into position.
- 9. Close the sample access cover.
- 10. In the Replace Wetting Tray dialog, click **Done**.
- 11. Dispose of the full waste bottle according to proper procedures. Refer to the section: Dispose of the Gel Waste Bottle.

Replace the Wetting Tray Remove the Wetting Tray

- 1. From the Run module menu bar, select **Replenish > Replace Wetting Tray**.
- 2. Click **OK** when prompted to verify this action.
- 3. Wait until the dialog shows the green text message GO.
- 4. Open the sample access cover and lift to the vertical locking position.
- 5. Remove the sample plate and set it aside.
- 6. Rotate the wetting tray retainers outwards to release the wetting tray.
- 7. Lift the wetting tray vertically.

Clean the Wetting Tray

- 1. Remove the wetting tray lid assembly from the wetting tray reservoir.
- 2. Rinse the wetting tray lid and reservoir with deionized water and dispose of the rinse into a hazardous liquid organic waste container.
- 3. Dry the lid assembly and reservoir.
- 4. To fill with deionized water:
 - a. Remove the lid of the wetting tray and fill it with deionized water to the indicator line.
 - b. Replace the wetting tray lid.

Note: You should process no more than one 96-well plate for each wetting tray without replenishing the wetting tray.

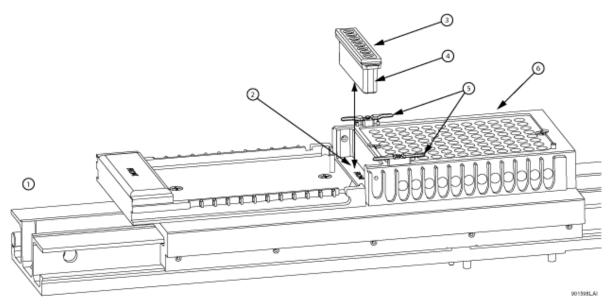
Note: Periodically check the liquid level in the wetting tray. The liquid level should never be allowed to rise into the eight recesses of the wetting tray lid, nor drop below the fill level indicator line (9 mL minimum). The top surface of the wetting tray lid must remain clean and dry under any and all circumstances.

Install the Wetting Tray

- 1. From the Run module menu bar, select **Replenish > Replace Wetting Tray**.
- 2. Click **OK** when prompted to verify this action.
- 3. Open the sample access cover and lift to the vertical locking position.
- 4. Rotate the wetting tray retainers outward. Insert the wetting tray into the receptacle between the sample and buffer plates and then gently press it down into the well.
- 5. Rotate the wetting tray retainers inwards to lock the wetting tray in place.

Note: If a sample plate is installed in the system, you must remove it before installing the wetting tray to allow movement of the wetting tray retainers.

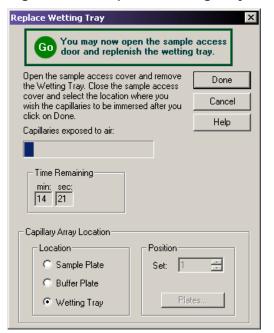
Figure 12-9 Replacing the Wetting Tray



ltem	Description
1	Front location
2	Wetting tray well
3	Wetting tray lid
4	Wetting tray
5	Wetting tray retainers
6	Sample plate holder

- 6. Close the sample access cover.
- 7. In the Replace Wetting Tray dialog, click **Done**.

Figure 12-10 Replace Wetting Tray Dialog



Direct Control and Replenishment

The following procedures provide system replenishment using the Run module's Direct Control functions. To access these functions, use the Run module's **Direct Control** menu, the **Replenish** menu, and the selectable areas of the Direct Control main window.

Note: To locate hardware components, refer to the figure: Figure 12-1.

Refer to the sections:

- View Capillary Information
- Load the Sample Plate and Buffer Plate for Dual-Rail System
- Load the Sample Plate and Buffer Plate for Single-Rail System
- Set the Capillary Temperature
- Denature Samples
- Inject Samples
- Perform a Separation
- Replenish the Capillaries with Gel
- Purge the Manifold

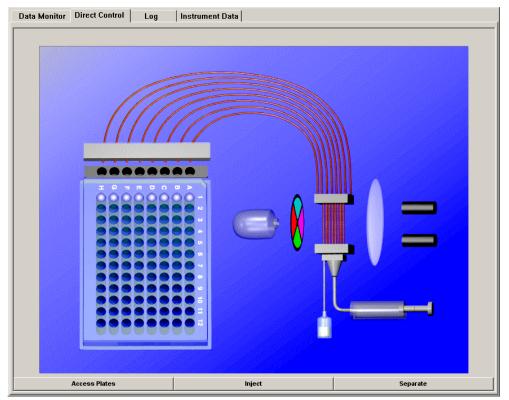
Maintenance and Diagnostics

- Perform an Optical Alignment
- View Gel Information
- View or Change Buffer Information
- Remove and Replace the Capillary Array
- Remove and Replace a Gel Cartridge/Gel Pump Plug for Dual-Rail System
- Remove and Replace a Gel Cartridge/Gel Pump Plug for Single-Rail System
- Remove the Manifold Plug

Access the Direct Control Window

- On the Main Menu, click the RUN icon The Run module main window opens.
- 2. Select the **Direct Control** tab. The Direct Control window opens.

Figure 12-11 Direct Control Window



Biological Waste Disposal

WARNING! Biohazard. Be careful when removing used chemicals and biological samples from the instrument. The GenomeLab GeXP Genetic Analysis System has been designed to minimize exposure to hazardous chemicals and biological waste. The information below provides the minimum protocols to use when handling hazardous chemicals and biological waste.

Dispose of Sample Loading Solution from the Sample Plate Multi-Channel Pipettor



WARNING! Biohazard. When performing this procedure, use an exhaust ventilation unit that meets TLV requirements.



WARNING! Biohazard. Dispose of Sample Loading Solution in accordance with all applicable federal, state, and local environmental regulations concerning hazardous liquid waste.

- 1. Aspirate 40 μ L of Sample Loading Solution from the thermo-cycling plate.
- 2. Dispense the Sample Loading Solution into a hazardous liquid organic waste container.
- 3. After all wells are clear, allow the empty plate to sit in the ventilation unit for 24 hours.

Bulk Disposal



WARNING! Biohazard. When performing this procedure, use an exhaust ventilation unit that meets TLV requirements.



WARNING! Biohazard. Dispose of Sample Loading Solution in accordance with all applicable federal, state, and local environmental regulations concerning hazardous liquid waste.

- 1. Using a 1 L side arm flask as a trap, connect the trap to a vacuum.
- 2. Attach a pipette to the trap using chemical resistant tubing.
- 3. Aspirate the Sample Loading Solution from each well and dispose of it in a hazardous liquid organic waste container.
- 4. After complete removal of all Sample Loading Solution from the plates, allow the empty plate to sit in the ventilation unit for 24 hours.
- 5. Dispose of the plate in a solid waste container.

Dispose of Buffer/Gel Mixture from the Buffer Plate Multi-Channel Pipettor



WARNING! Biohazard. When performing this procedure, use an exhaust ventilation unit that meets TLV requirements.



WARNING! Biohazard. Dispose of buffer/gel mixture in accordance with all applicable federal, state and local environmental regulations concerning hazardous liquid waste.

- 1. Aspirate 400 µL of buffer/gel mixture from the 96-well plate.
- 2. Dispose the buffer/gel mixture into a hazardous liquid organic waste container.
- 3. Rinse the plate wells with water and dispose of the rinse in the liquid waste container.
- 4. After all wells are clear, allow the empty plate to sit in the ventilation unit for 24 hours.
- 5. Dispose of the plate in a solid waste container.

Bulk Disposal



WARNING! Biohazard. When performing this procedure, use an exhaust ventilation unit that meets TLV requirements.

WARNING! Biohazard. Dispose of buffer/gel mixture in accordance with all applicable federal, state and local environmental regulations concerning hazardous liquid waste.

- 1. Using a 1 L side arm flask as a trap, connect the trap to a vacuum.
- 2. Attach a pipette to the trap using chemical resistant tubing.
- 3. Aspirate the buffer/gel mixture from each well and dispose of it in a hazardous liquid organic waste container.
- 4. After complete removal of all buffer/gel mixture from the plates, allow the empty plate to sit in the ventilation unit for 24 hours.
- 5. Dispose of the plate in a solid waste container.

Dispose of the Capillary Array



WARNING! Biohazard. After removing the expended capillary array from the instrument, dispose of it in a solid hazardous waste container.

Dispose of the Gel Cartridge



WARNING! Biohazard. After removing the expended gel cartridge from the instrument, use a lab spatula or wooden dowel to push any remaining gel into a liquid hazardous waste container. Dispose of the empty cartridge in a solid hazardous waste container.

Dispose of Deionized Water/Gel Mixture from the Wetting Tray



WARNING! Biohazard. When performing this procedure, use an exhaust ventilation unit that meets TLV requirements.



WARNING! Biohazard. Dispose of deionized water/gel mixture in accordance with all applicable federal, state, and local environmental regulations concerning hazardous liquid waste.

- 1. Pour the deionized water/gel mixture into a hazardous liquid organic waste container.
- 2. Rinse the wetting tray with water and dispose of the rinse in the liquid waste container.

Dispose of the Gel Waste Bottle



WARNING! Biohazard. When performing this procedure, use an exhaust ventilation unit that meets TLV requirements.



WARNING! Biohazard. Dispose of buffer/gel mixture in accordance with all applicable federal, state and local environmental regulations concerning hazardous liquid waste.

Diagnostics

This section provides some common diagnostic procedures you may perform as needed on the GenomeLab GeXP system.

Re-Initialize the System

To re-initialize the system, in the Run module, select **Run > Reset**.

Home the Plates or Gel Pump

To re-establish the position of the plates or gel pump:

 From the Run module menu bar, select Run > Diagnostics. The Diagnostics dialog opens.

Maintenance and Diagnostics

- 2. Select the **Home Plate** or **Gel Pump** check box.
- 3. Click OK.
- 4. Monitor the Tests dialog and verify that the final results is Test passed.
- 5. Click **Close**.

View PC Settings

To view the computer settings:

- 1. From the Run module menu bar, select **Run > Diagnostics**. The Diagnostics dialog opens.
- Click PC Settings. The PC Communication Settings dialog opens.
- 3. View the settings used to communicate with the GenomeLab GeXP system, then exit the dialog.

View Instrument Status

To view the instrument status:

- From the Run module menu bar, select Run > Diagnostics. The Diagnostics dialog opens.
- 2. Click Status.
- 3. View or change the settings in the Run Control Monitor dialog.
- 4. Click OK.
- 5. The dual-rail system has a barcode reader. To read the barcode on sample plates:
 - a. In the Device/System Values section, click Select Item.
 - b. In the Available Information list, select Left Barcode and Right Barcode.
 - c. Move them to the **Display Information** list, and then click **OK**.
 - d. Select **Direct Control > Access Plates** to load the sample plates with barcode.

The barcode will show in the Run Control Monitor dialog in the Device/System Values section.

View Optical Scan Data

To view the optical scan data in either the Sequence Analysis or Fragment Analysis module:

 In the desired analysis module, select File > Open. The Open dialog opens.

- 2. Select the Optical Scan Data tab, highlight the desired sample name, then click OK.
- 3. Verify that the Optical Scan window opens.

Monitor the Baseline

To ensure that the optics are working correctly and that the capillaries are clean, view the baseline after installing a new capillary array, filling with gel for the first time, or performing an optical alignment. The baseline trace should be low and relatively flat.

Note: You must perform the optical alignment procedures prior to monitoring the baseline. Refer to the section: Perform an Optical Alignment.

To monitor the baseline:

1. From the Run module menu bar, select **Run > Monitor Baseline**.

The Monitor Baseline dialog opens.

Figure 12-12 Monitor Baseline Dialog

1onitor Baseline 🛛 🔀		
Enable Monitor Baseline Baseline Parameters Autosave	OK Cancel	
View Injection Instrument Data Name: BASELINE Project: Default	Help	

Note: If you select the **Autosave** check box, the system stores baseline data under the Sample Data node (for the selected project) in the Data Manager module. If you do not manually end the Monitor Baseline function, it collects baseline data until you run a sample plate or 6 hours have elapsed from the start of the baseline monitoring process.

- 2. Select the **Enable Monitor Baseline** check box.
- 3. Click OK.
- 4. To view the baseline trace, select the **Data Monitor** tab.
 - a. Access the desired analysis module.
 - b. Select **File > Open**.
 - c. Select the Sample Data tab.
 - d. Select the desired baseline data.

Maintenance and Diagnostics

- 5. After reviewing the baseline trace, disable Monitor Baseline.
 - a. Select **Run > Monitor Baseline**. The Monitor Baseline dialog opens.
 - b. Select the **Enable Monitor Baseline** check box to clear the check mark.
 - c. Click **OK**.

The following information must be noted and the relevant safety measures taken. Refer to the respective safety data sheets for more information. The safety data sheets are available upon request or can be downloaded from our website, at sciex.com/tech-regulatory.

Hazard classification according to HCS 2012.

DNA Size Standard 400



RT Buffer, 5×

WARNING! May be harmful if swallowed. Causes minor skin irritation.

Sample Loading Solution



Other Reagents

These components are not classified as hazardous:

- DNase/RNase-free distilled water
- KAN^r RNA with RI
- Mineral oil
- PCR Buffer 5×
- Reverse transcriptase
- Separation Buffer
- Separation gel
- Thermo-Start Taq DNA Polymerase

For reagents from other vendors, read the Safety Data Sheet from the vendor before use.

Instrument Specifications

Dimensions (H × W × D)	92.7 cm x 66.7 cm x 57.2 cm
	36.5 in. × 26.3 in. × 22.5 in.
Weight	82 kg (181 lb)
Electrical	Power requirement: 100 VAC to 240 VAC, 5.0 A, 50 Hz or 60 Hz
	The supply voltage must not exceed 10% of nominal.
	Fuses: 6.3 A time delay; 20 mm (2 each); 200 VAC to 240 VAC
	Installation (overvoltage) category: Category II
Working environment	Altitude: ≦2,000 m (6,562 ft) above sea level
	Humidity: <85% (non-condensing) at 30 °C (86 °F)
	Temperature 15 °C to 30 °C (59 °F to 86 °F) recommended
Maximum heat dissipation	400 W (1365 BTU/hr) under steady-state conditions
Maximum sound pressure	73 dB
	Maximum pressure at 1 m: 67 dB

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SCIEX Support

SCIEX and its representatives maintain a staff of fully-trained service and technical specialists located throughout the world. They can answer questions about the system or any technical issues that might arise. For more information, visit the SCIEX website at sciex.com or contact us in one of the following ways:

- sciex.com/contact-us
- sciex.com/request-support

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