CEQ™ 8000

Genetic Analysis System

User's Guide

Table of Contents

Foreword

About this Guide

This guide is intended for use with the CEQ™ 8000 Genetic Analysis System. It is divided into the following sections:

- *"Foreword*," this section, describes the purpose of this guide, provides a list of its contents, discusses the use of the symbols used in the document as well as providing service contact information.
- *"System Overview,"* discusses the purpose and functional description of the system, provides an overview of the three main components (chemistry, hardware and software) comprising the system and details the safety features relevant to the system.
- *"Program Description,"* provides descriptions of the main windows, menus, dialog boxes and software commands used in controlling the system.
- *"Operating the System,"* contains step-by-step procedures for all common tasks necessary to prepare and use the system on a daily basis. It also provides procedures to perform the tasks involved in analyzing/managing samples and sample data.
- *"Routine Maintenance,"* provides routine maintenance procedures and biological waste disposal procedures. It also lists the consumable materials used in the system.

Symbols Used in this Guide

The following information describes the symbols used in this document. Beckman Coulter recommends that you review this information before using the CEQ System.

WARNING **When the "warning" icon accompanies text, it indicates that a potential hazard to your personal safety exists if information stated within the "Warning" paragraph is not adhered to or if procedures are executed incorrectly.**

CAUTION **Paragraphs marked by the "caution" symbol indicate that there is a potential danger of equipment damage, software program failure or that a loss of data may occur if information stated within the "Caution" paragraph is not adhered to or if procedures are executed incorrectly.**

Note *Paragraphs marked by the "notepad" icon contain supplemental or explanatory information concerning the current topic or procedural step.*

Getting Help

If you encounter a problem that is not discussed in this guide and you need technical support, contact your local dealer, the provider of this product, or contact Beckman Coulter directly using the information below.

| Note | *Whenever you call your local dealer or Beckman Coulter, be sure to have your registration material, instrument serial number and software version number available. For future reference, record this information here.* |

Instrument Serial Number:	
Software Version:	
Firmware Version:	
Dealer Name:	
Dealer Phone Number:	

Mail	Beckman Instruments, Incorporated 4300 North Harbor Boulevard Fullerton, CA 92834-3100
Product Support	1-800-854-8067
Sales	1-800-742-2345
Service	1-800-551-1150
Telex	678413
FAX	l-800-643-4366
Internet	http://www.beckmancoulter.com

System Overview

This section describes the purpose and function of the system, provides an overview of the three main components (chemistry, hardware and software) comprising the system and details the safety features relevant to the system.

Purpose of this System

The purpose of the Capillary Electrophoretic (CE) Genetic Analysis System (CEQ) is two-fold:

1. To determine the nucleotide sequence of any given DNA sample.
2. To estimate sizes of DNA fragments.

The system is comprised of three main components: chemistry, hardware and software.

Functional Description

The CEQ Genetic Analysis System is fully automated and capable of determining the base sequence and fragment length of DNA samples that have been prepared with Beckman Coulter, Inc. dye-labeled 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 dye-labeled primers.

The CEQ 8000 accepts up to 96 four-color samples in a microplate. Each row of eight samples (sample set), containing labeled DNA fragments, is automatically denatured and then separated by capillary electrophoresis. The replaceable medium (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 a full microplate (96 samples).

Detection is by laser-induced fluorescence in four spectral channels. The four-channel raw data sets generated by each of the eight capillaries are automatically processed to produce high quality base sequences or fragment lists after separation.

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

Hardware

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

Figure 1 shows the CEQ 8000 instrument. The user accessible hardware components of the CEQ instrument are described below.

Figure 1: Hardware Overview for the CEQ 8000

Sample Access Cover (extended)

Capillary Access Cover (extended)

Capillary Temperature Control Cover

Rubber Latches

Manifold Access Cover

Status Indicators

Plate Holders/ Sample Transport

Gel Waste Bottle

Power Switch

Gel Pump

Gel Pump/Gel Cartridge Access Cover
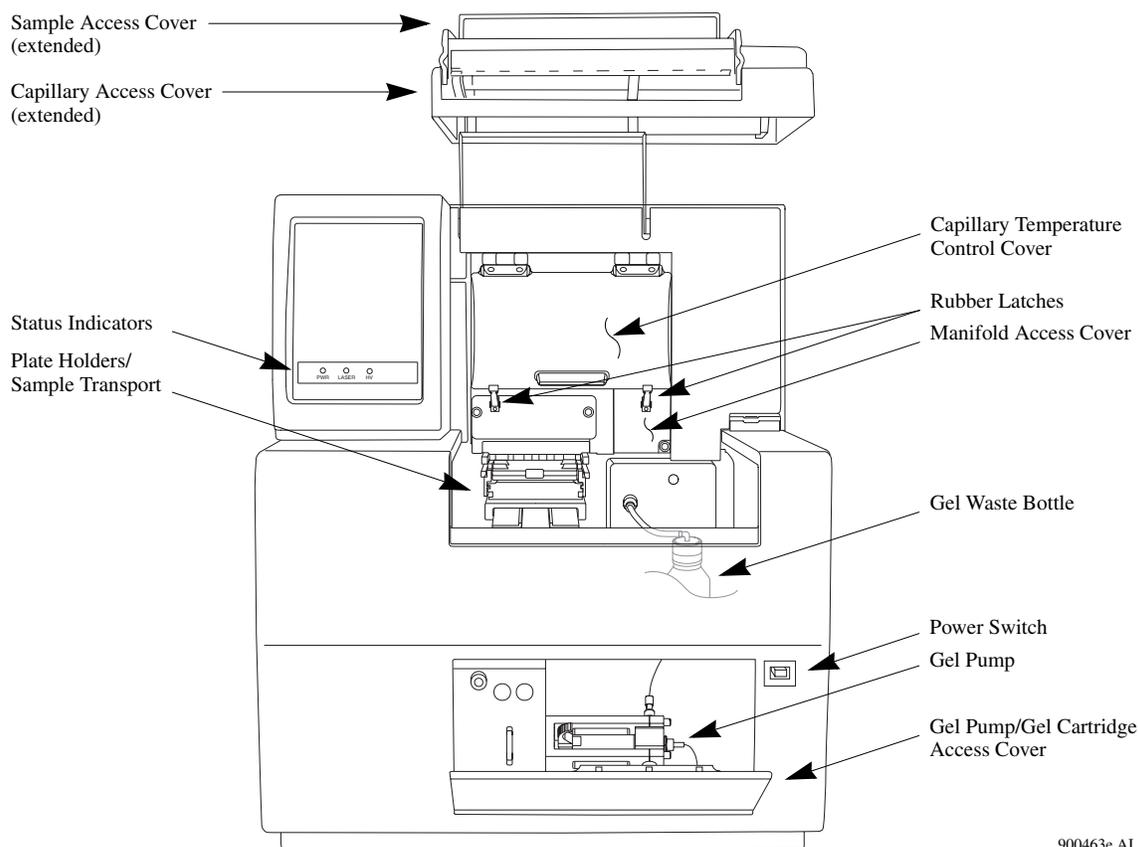
900463e.AI

Sample Access Cover

The Sample Access Cover provides access to the Buffer Plate, Wetting Tray and Sample Plate.

Capillary Access Cover

The capillary access cover provides access to the capillary array via the Capillary Temperature Control Cover.

Capillary Temperature Control Cover

This cover provides an enclosed environment for the Capillary Temperature Control and allows access to the capillary array.

Capillary Array

The capillary array used to produce raw data for sequencing and fragment analysis is 33 centimeters in length, and has an i.d. of 75 micrometers. A unique Short Plenum Assembly is used with the 33 cm capillary array.

The capillary array (Figure 2) has three components: Electrode Block (inlet), eight capillaries and the Array Fitting (outlet).

- The Electrode Block is the DNA sample inlet side of the array. It holds eight hollow, stainless-steel electrodes. Each stainless-steel electrode holds a capillary in the center. These electrodes are designed such that they can be immersed into an entire row (8 wells) of a 96-well microplate (8-rows x 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.
- The Array Fitting contains the Detection Window and is the outlet side of the array. The Detection Window of the fitting is used to expose 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, e.g., during shipping and storage, the Manifold Plug is inserted into the Manifold (Array Fitting outlet) to prevent any gel (inside of the CEQ) from drying.*

Figure 2: Capillary Array

Electrode Block (inlet)

Capillaries (8)

Array Fitting (outlet)
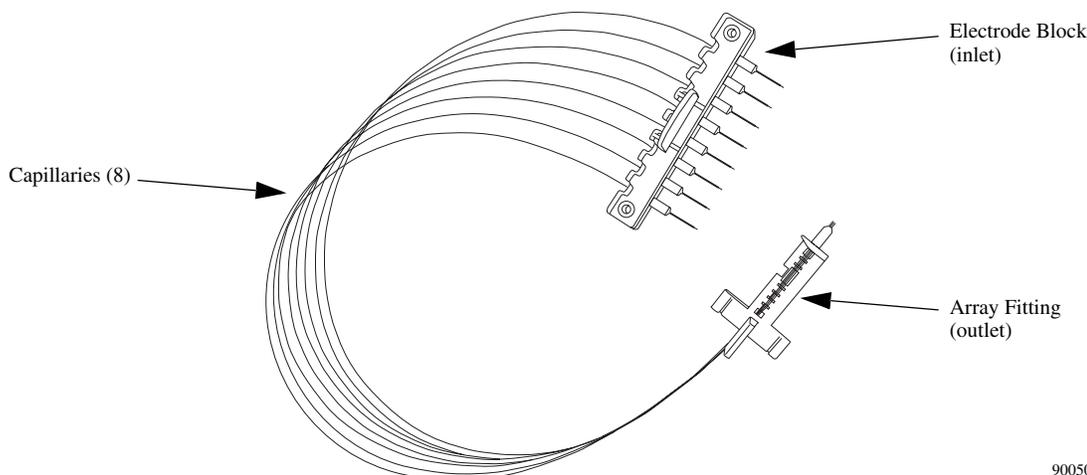
900505e.A1

Plenum

The CEQ System is supplied with a Capillary Heater Plenum suitable for use with a 33cm long capillary array. This "Short" plenum assembly has a capillary array Routing Notch and a Conical Depression on the back as shown in Figure 3.

> **Note** *The "Short" plenum assembly is not suitable for use with 53cm long capillary arrays. If 53cm long capillaries are used, a "Long" plenum assembly must be used which does not have the routing notch or conical depression shown in Figure 3. This "Long" plenum can be purchased as a separate item and is not supplied with the CEQ System.*

> *Note* **Refer to the caution label on the front of the plenum assembly for positive identification**

Figure 3:   Plenum Assembly (Back View)

Routing Notch

Conical Depression

900696L.AI
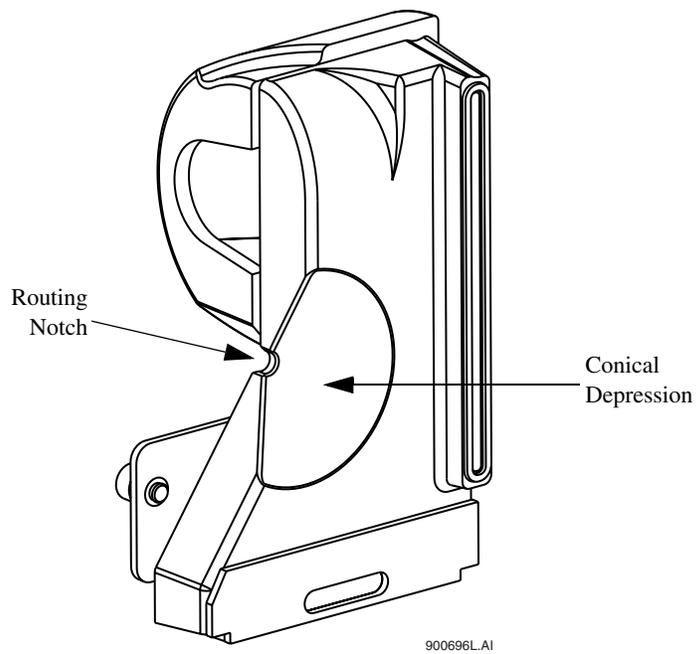
Sample Transport and Plate Holders

The Sample Transport (Figure 4) contains the Plate Holders which are used to hold the Sample Plate, Buffer Plate w/Buffer Evaporation Cover and Wetting Tray.

Figure 4:   Sample Transport and Plate Holders

900500L.A1

Plate Holders

part of Sample Transport
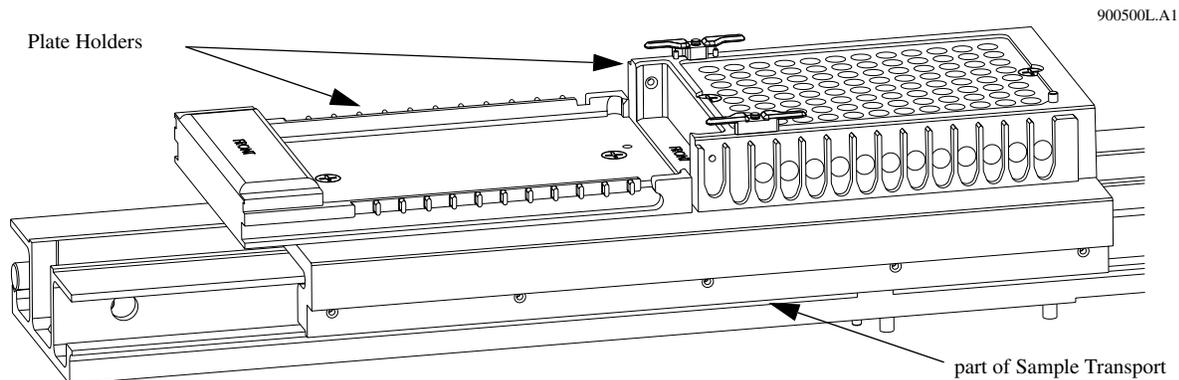
Sample Plate

The Sample Plate (Figure 5) is used to hold the sample(s) for separation. The plate is a V-bottom, thermal cycler-compatible, polypropylene plate containing 96 wells (8-rows x 12-columns). The wells have a 200μL volume capacity.

Figure 5:   Sample Plate

900497e.AI
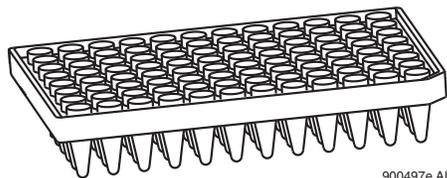
Buffer Plate with Buffer Evaporation Cover

The Buffer Plate (Figure 6) is used to hold the DNA separation buffer used during a sample run. The plate is a flat-bottom, polystyrene, non-sterile plate containing 96 wells (8-rows x 12-columns). An evaporation cover, placed over the Buffer Plate, is used to maintain the proper level of buffer (250-300 μL) in the Buffer Plate by preventing evaporation of the buffer. The cover slips over the Buffer Plate position of the Sample Transport. As the CEQ advances through the Sample Plate during a run, the evaporation cover is pushed back just far enough to expose the next row of buffer wells to be used for the next sample set run.

Figure 6:   Buffer Evaporation Cover and Buffer Plate

Buffer Evaporation Cover

Buffer Plate

900496e.AI
900498e.A1
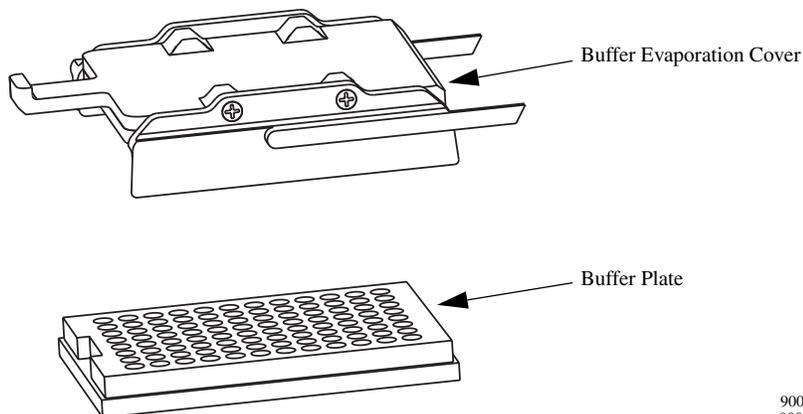
Wetting Tray

The Wetting Tray (Figure 7) 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 CEQ 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 purged from the capillaries.

> **!** **CAUTION** **No more than one 96-well plate should be processed without replenishing the Wetting Tray. For information on Cleaning and Filling the Wetting Tray, see page 356.**

Figure 7:   Wetting Tray

900499e.AI
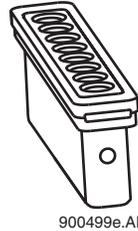
Gel Waste Bottle

The Gel Waste Bottle (Figure 8) 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. Periodic observation of the gel level in the waste bottle is recommended to prevent overflow.

Figure 8:   Gel Waste Bottle

901514L.AI
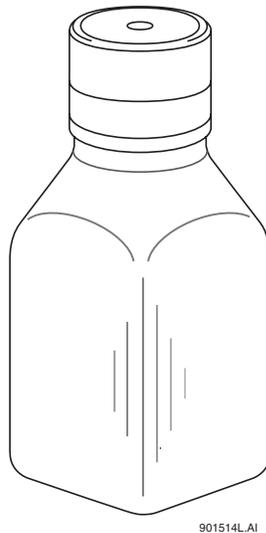
Power Switch

This push-button switch, when in the **ON** position, provides power for the CEQ.

Gel Pump/Gel Cartridge Access Cover

Provides access to the Gel Pump and Gel Cartridge.

Gel Pump, Gel Cartridge and Gel Pump Plug

Gel Pump (Figure 9)   Used to replenish the capillaries with fresh gel (from the cartridge) after each sample set and each 96-well plate runs.

Gel Cartridge   Contains the fresh DNA separation gel. The separation gel is used for both Sequence and Fragment Analysis. When full, the cartridge contains a sufficient amount of gel for 12 runs (96 templates). (See Table 75 on page 362.)

Gel Pump Plug   When the gel cartridge is not installed, e.g., during shipping and storage, the Gel Pump Plug is inserted into the pump barrel to prevent gel from drying in the system.

Figure 9:   Gel Pump
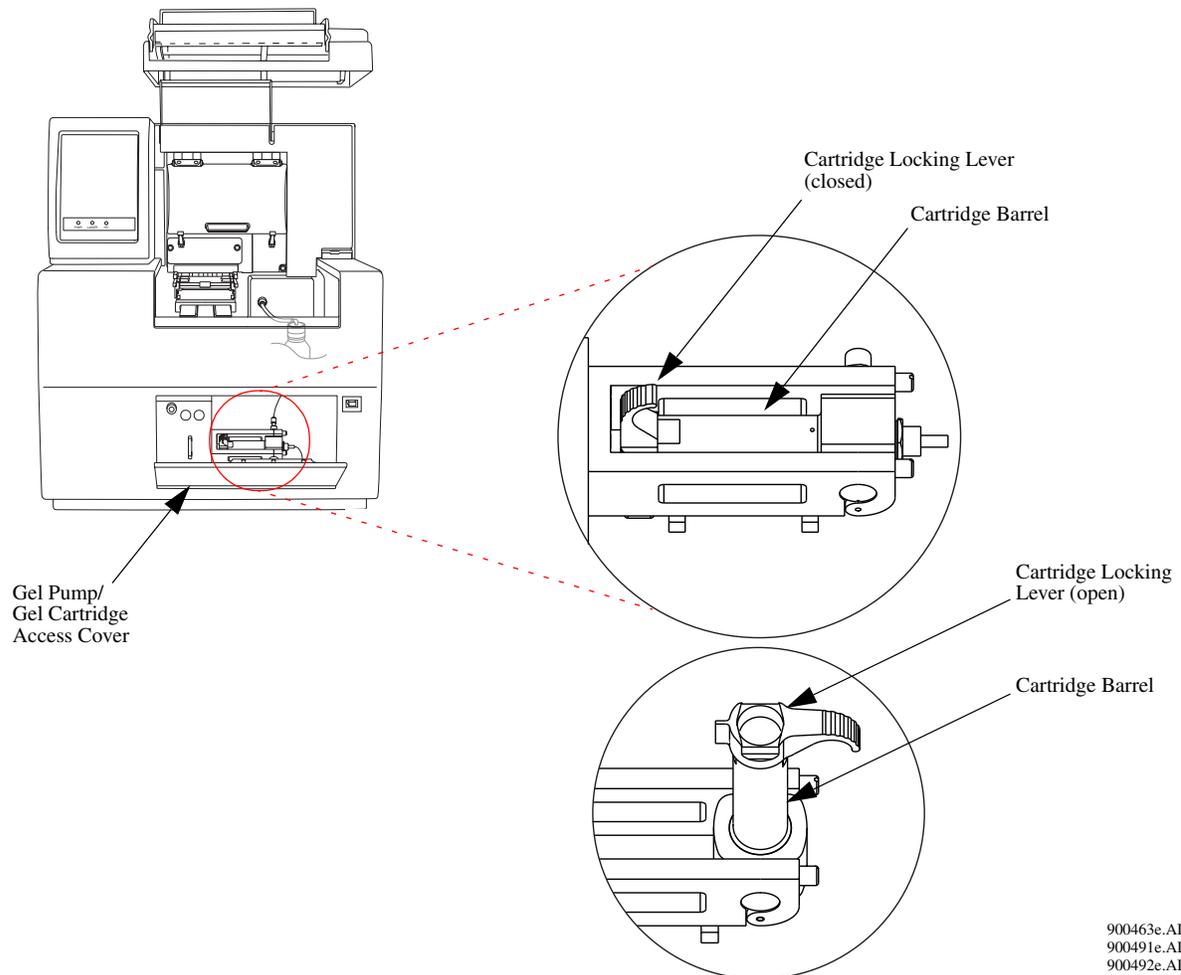
Status Indicator Lights

There are three status indicators: PWR (power), LASER and HV (high voltage).

PWR	Green when power is supplied to the CEQ. Off when no power is supplied.
LASER	Green when the lasers are turned on during a run. Off when the lasers are turned off.
HV	Green when the high voltage is applied during the injection or separation. Off when the high voltage is turned off.
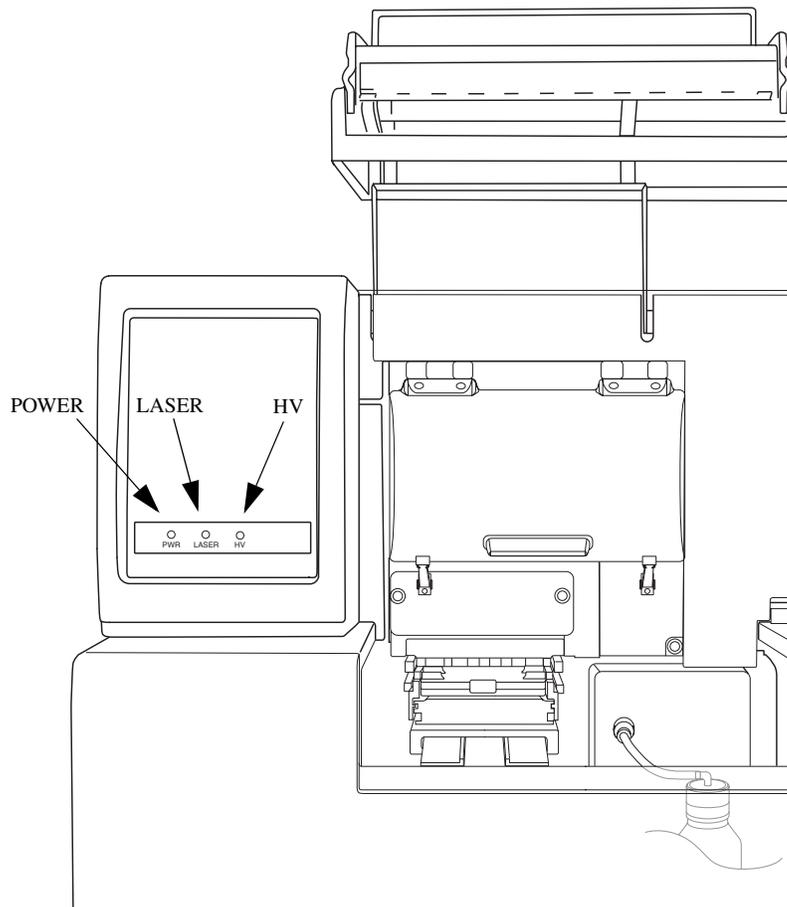
Software

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

User Interface

The Main Menu is shown in Figure 10. This window provides access to all modules of the CEQ Software. The software modules are described in Table 1.

Figure 10: Main Menu

Table 1:   Software Module Descriptions

Module	Description
	Sample Setup Module Use this module to create, save and modify sample plates. Sample plates are used to assign methods to control sample sets and determine the sequence of methods that will be used to produce data.
	Run Module Use this module to run sample plates and control individual functions of the instrument.
	Sequence Analysis Module Use the Sequence Analysis module to view, analyze, compare, manipulate and print base sequence data produced by sample runs.
	CEQuence Investigator Module This icon accesses the CEQuence Investigator module. This module is used to compare sequence(s) to create a consensus that will then be compared against a reference.
	Fragment Analysis Module Use the Fragment Analysis module to view, analyze, compare, manipulate and print fragment data produced by sample runs.
	Data Manager Module Use this module to modify and print database items.
	Exit When selected, the Exit icon closes any active modules and shuts down the CEQ System software.

Safety Information

This section provides safety information and instructions for the hardware and accessories of the system. It is broken down into the following subsections:

- Symbols
- Safety Features
- Chemical and Biological Safety
- Electrical Safety
- Moving Parts Safety
- Laser Safety
- Electrostatic Discharge

Safety Symbols

The symbols displayed below and on the instrument are reminders that all safety instructions should be read and understood before installation, operation, maintenance or repair to this instrument is attempted.

When symbols are displayed in this manual, pay particular attention to the safety information associated with the symbol.

WARNING **WARNING**

> This icon accompanies text and/or other international symbols dealing with hazards to personnel. When present, it indicates that a potential hazard to your personal safety exists if information stated within the "**WARNING**" paragraph is not adhered to or procedures are executed incorrectly.

CAUTION

> This icon accompanies text and/or other international symbols dealing with potential damage to equipment. When present, it indicates that there is a potential danger of equipment damage, software program failure or that a loss of data may occur if information stated within the "**CAUTION**" paragraph is not adhered to or procedures are executed incorrectly.

HIGH VOLTAGE

> Paragraphs marked by this symbol indicate that a potential hazard to your personal safety exists from a high voltage source. In this document, the "**WARNING**" icon will accompany this symbol.

BIOHAZARD

Paragraphs marked by this symbol indicate that a potential hazard to your personal safety exists from a biological source. In this document, the "**WARNING**" icon will accompany this symbol.

LASER LIGHT

Paragraphs marked by this symbol indicate that a potential hazard to your personal safety exists from a laser source. In this document, the "**WARNING**" icon will accompany this symbol.

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. In this document, the "**WARNING**" icon will accompany this symbol.

HOT SURFACE

Paragraphs marked by this symbol indicate that a potential hazard to your personal safety exists from heated faces or other appendages on the outside or inside of the equipment. In this document, the "**WARNING**" icon will accompany this symbol.

CONFORMITE EUROPEENE

Equipment displaying the $C\!\!\in$ mark has been tested and is in compliance with all applicable European safety directives.

Protective Earth or Ground Terminal

This symbol identifies the location of the protective earth or ground terminal lug 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.

ON Position of Principal Power Switch

This symbol graphically represents the equipment main power push-button switch when it is in the on position.

Chemical and Biological Safety

WARNING **Normal operation of the system can involve the use of solvents and reagents that are toxic, flammable or biologically harmful.**

- **Observe all precautionary information printed on the original solution containers.**
- **Operate the system in the appropriate environment.**
- **Take all necessary precautions when using pathology or toxic materials to prevent the generation of aerosols.**
- **Observe all applicable precautionary procedures when using flammable solvents in or near the instrument.**
- **Wear appropriate laboratory attire, e.g., safety glasses, gloves, lab coat and breathing apparatus, when working with hazardous materials.**
- **Dispose of all waste solutions in a proper manner.**

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.

WARNING **A high voltage power supply is used with this instrument. Safety interlocks disable high-voltage output while the capillary access cover is open and remove the risk of shock while performing routine instrument functions. However, removal of any panel may expose an individual to the possibility of severe electrical shock and/or mechanical injury. For this reason, any service requiring removal of a panel or otherwise overriding or disabling safety interlocks must be done by Beckman Coulter personnel only.**

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

WARNING **The CEQ 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 Beckman Coulter Field Engineers.

During normal operation of the system, laser light is not accessible to the user. Therefore, the overall laser classification of the CEQ System is "Class 1," i.e., "lasers which are safe under reasonably foreseeable conditions of operation."

To prevent users from potentially harmful laser light, observe all safety warnings (see Figure 11 for label locations) and NEVER REMOVE THE OUTER CASING OF THE LASER ASSEMBLY.

Figure 11: Laser Label Locations

Capillary Access Cover
(extended)

Laser Assembly Cover
(cosmetic panel removed)

CAUTION
LASER LIGHT ACCESSIBLE WHEN COVER IS OPEN OR REMOVED. AVOID EXPOSURE

*approximate location

AVOID EXPOSURE
LASER LIGHT IS EMITTED FROM THIS APERATURE.

*approximate location

rear

CLASS 1 LASER PRODUCT
THIS PRODUCT CONFORMS
TO APPLICABLE REQUIRE-
MENTS OF 21 CFR 1040 AT
THE DATE OF MANUFAC-
TURE.

270-726024-A   Printed in U.S.A.

*approximate location

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900468e.AI

EMI Effect on CEQ™ (2000 Series / 8000 Series) Performance and Recommended Mitigations

Under the test conditions specified by the European normative electromagnetic compatibility standard EN 61326-1, the CEQ instrument 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 base calling accuracy. In some cases, significant noise will appear in the measured results. Will return to normal performance once exposure is removed.	• Re-run sample. • Move location of the product by several meters. • Change the orientation of the equipment by 90 degrees. • Avoid using transmitters or cellular phones within 1 meter of the equipment. • Review the temperatures in the run log for any anomalies and re-run as necessary.
	May cause temporary loss of capillary temperature control resulting in lower than expected capillary temperature. Affect will be documented in the run log. Will return to normal performance once exposure is removed.	
	May cause temporary loss of capillary temperature control resulting in run not being initiated because temperature cannot reach desired level.	• Apply mitigation techniques above. • Disabling the temperature limit feature in the Method Edit screen (Capillary Temperature box) will allow the run to be performed without desired temperature being reached.

Program Description

The following sections provide detailed descriptions of the main windows, menus, dialog boxes and software commands, in each module, that are used in controlling the system. The module titles are:

- Sample Setup Module
- Run Module
- Sequence Analysis Module
- CEQuence Investigator Module
- Fragment Analysis Module
- Data Manager Module

Additional instruction on the use of this software/system is located in *Operating the System* and subsequent sections and in the online help.

Sample Setup Module

The Sample Setup module is used to create, save and modify methods and sample plates. Sample plates are the means by which samples are named and organized. Methods consist of sequential events needed to perform a DNA separation. 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." You can also edit method parameters to modify or create new methods. Selecting the Sample Setup *icon* from the Main Menu (Figure 12) executes this module.

Figure 12: Main Menu, Sample Setup Icon
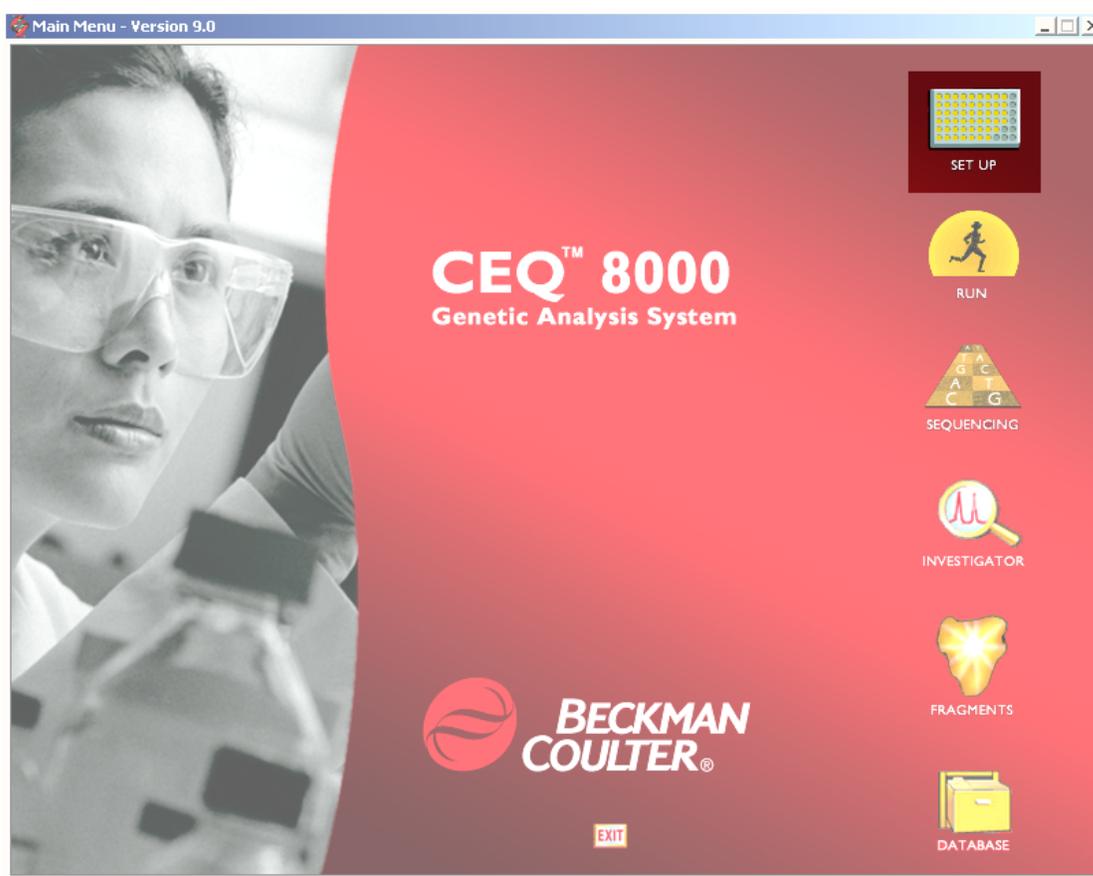
Main Window

The main window of this module is shown in Figure 13 and described in Table 2.

Figure 13: Sample Setup Module, Main Window

Table 2: Sample Setup Module, Main Window Descriptions

Item	Description
A	The ***Title Bar*** showing the module name ("*Sample Setup*") and the active sample plate ("*DefaultSamplePlate*").
B	***Menu Bar*** (See ***"Menu Bar Options"*** on page 22.)
C	***Toolbar*** (See ***"Toolbar Icons"*** on page 26.)
D	View by toggles the plate view from Sample Name to Subject ID view. (See ***"View by Sample Name/Subject ID"*** on page 127.)
E	Sample Name to Subject ID fields. When a cell is selected, its Sample Name and Subject ID will appear in these fields. (See ***"Naming Samples and Assigning Subject IDs"*** on page 128.)
F	The ***Plate Status*** indicator identifies if the plate is empty, has been edited or saved.
G	***Sample Plate*** containing 96 cells that corresponds to a 96-well sample plate. (Sample plate ***Columns*** are numbered 1 through 12 and ***Rows*** are labeled A through H. This naming convention uniquely identifies each sample location.)
H	***Sample Name*** of the currently active cell.
I	Currently active (selected) ***Method***. This drop-down is used to assign a pre-defined method to the corresponding samples in the column above. • The **Frag** methods are used for fragment analysis. • The **LFR** methods are used for sequencing. • The **SNP-1** method is used for SNP analysis.

Table 2:   Sample Setup Module, Main Window Descriptions

Item	Description
J	*Notes Window* Used to enter explanatory text concerning the selected sample(s). The text box will display "(--multiple selection--)" when multiple samples *with different descriptions* are selected. **Figure 14: Notes Window** *Property Sets Listbox* Property sets are groups of properties that can be applied to a sample(s), such as Vector, Host and Primer used. The software provides four templates: Cloning, PCR, FLP and pedigree. **Figure 15: Property Sets List Box**

Table 2:   Sample Setup Module, Main Window Descriptions

Item	Description
J (cont'd)	*Method Window* Provides a summary of the separation parameters defined for the selected method. Edit existing or create a new method by clicking on the **Edit** button. **Figure 16: Method Window** *Analysis Window* Used to: • Enable/disable the **Automatic Analysis** mode. When enabled, this mode causes the CEQ to automatically analyze each (previously selected) sample after it has run, using the Analysis Parameter Set selected in the drop-down menu. • Choose Sequence Analysis or Fragment Analysis **Parameter Sets** via the drop-down menu. • Enable/disable automatic printing of reports, view and/or edit the print report options. • Enable/disable automatic exporting of data, view and/or edit export options. **Figure 17: Analysis Window**
K	The *Status Bar* displays information concerning the current selection.
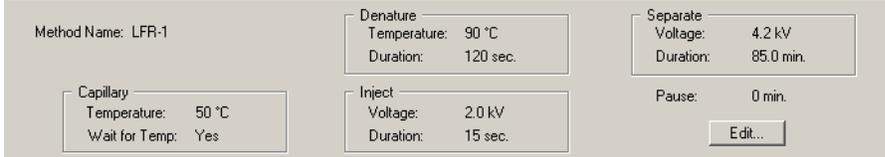
Menu Bar Options

The menu bar has the options shown in Figure 18. Tables 3 through 8 provide descriptions of the menu options.

Figure 18: Sample Setup Module, Menu Bar Items
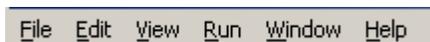
File Menu

Table 3:   Sample Setup Module, File Menu

Item	Description
New	Used to open an empty sample plate.
Open	Used to select an existing sample plate to open.
Close	Used to close the selected sample plate.
Save	Used to save the current sample plate.
Save As	Used to save the sample plate with a new name.
Import	Used to import a sample plate (Tab Delimited ASCII Text [*.txt] format).
Export	Used to export a sample plate (Tab Delimited ASCII Text [*.txt] format).
Properties	Provides an information window concerning the active sample plate.
Default Report Format	Used to define the report formats for new and imported sample plates.
Print Preview	Used to display a facsimile of a hardcopy printout of the active sample plate.
Print Setup	Used to define printer properties.
Print	Pull-right menu used 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	Pull-right menu with the option to send an image of the computer desktop or application window to the printer.
Lock	Used to prevent editing of the selected plate.
Exit	Used to close the Sample Setup module.
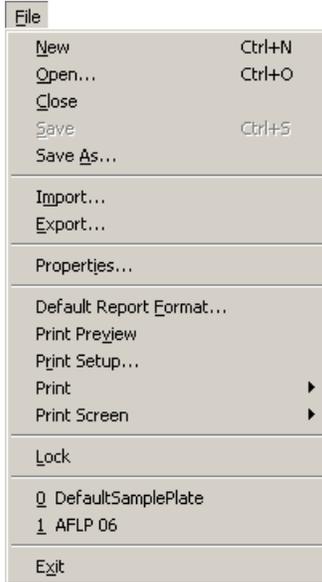
Edit Menu

Table 4:   Sample Setup Module, Edit Menu

Item	Description
Undo	Used to undo the last action performed.
Redo	Used to redo the last undo action.
Clear All	Used to empty all cell properties in the sample plate.
Select All	Used to select all cells in the sample plate.
Cut	Used to delete text or the applied properties in one or more cells of the sample plate.
Copy	Used to duplicate text or the applied properties in one or more cells of the sample plate.
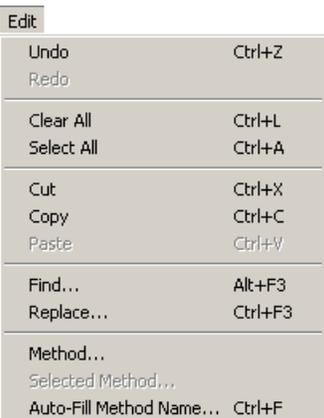
Item	Description
Paste	Used to insert copied or cut items at the cursor insertion point.
Find	Use this dialog box to search for text within the sample name entered in a sample plate.
Replace	Use this dialog to search and replace text within the sample names entered in a sample plate.
Method	Used to select a method to edit.
Selected Method	Used to view or edit the currently selected method, for a specific sample or sample set, from the dialog box shown below.
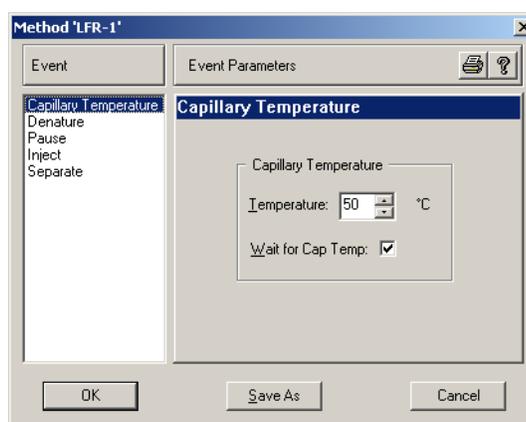
Item	Description
Auto-Fill Method Name	Used to select a method from the drop-down menu to be used with the selected sample set of the plate or all sample sets in the plate.
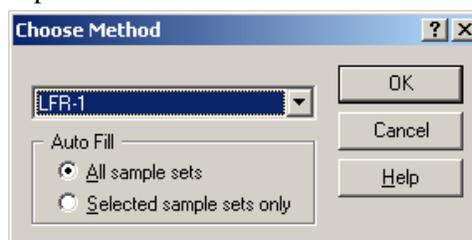
View Menu

Table 5:   Sample Setup Module, View Menu

Item	Description
Toolbar	Toggles between displaying/not displaying the toolbar.
Status Bar	Toggles between displaying/not displaying the Status Bar.
Cell Coordinates	Toggles between displaying/not displaying the coordinates of the plate cells.

Item	Description
Sample Property Sets	Used to create, delete or modify the property set.
Summary	Displays a summary of the currently selected plate.
View by Subject ID	This option toggles the sample plate cell labels between Sample Name and Subject ID.
Working Database	Displays a dialog box showing the database in use.

Run Menu

Table 6:   Sample Setup Module, Run Menu

Item	Description
Start	Invokes the Run module and loads the active sample plate.

Window Menu
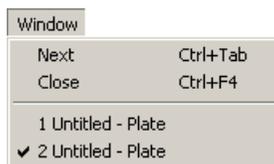
Table 7:   Sample Setup Module, Window Menu

Item	Description
Next	Toggles between open sample plates.
Close	Used to close the active sample plate or sample plate summary.

Help Menu
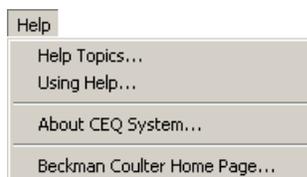
Table 8:   Sample Setup Module, Help Menu

Item	Description
Help Topics	Used to select and print specific topics in the Help file and/or search for information by: topic, index entry and/or keyword.
Using Help	Used to select and print specific topics in the *Windows* Help file and search for information by: topic, index entry and/or keyword.
About CEQ System	Used to access software, instrument and system information.
Beckman Coulter Home Page	Used to access the Beckman Coulter Home Page on the Internet.

Toolbar Icons

The toolbar (Figure 19) contains icons that correspond to common menu options. The information in Table 9 describes the function of each icon.

Figure 19: Sample Setup Module, Toolbar

Table 9:   Sample Setup Module, Toolbar

Icon	Description
	New - Used to open an empty sample plate.
	Open - Used to select an existing sample plate to open.
	Save - Used to save the current sample plate.
	Print Sample Plate - Used to print a detailed report of the active sample plate.
	Print Preview - Used to display a facsimile of a hardcopy printout of the active sample plate.
	Cut - Used to delete text in one or more cells of the sample plate.
	Copy - Used to duplicate text in one or more cells of the sample plate.
	Paste - Used to insert copied or cut items at the cursor insertion point.
	Unlock - Indicates that the selected sample plate is available for editing. Clicking on the icon locks the plate to prevent editing and changes the icon to .
	Run - Invokes the Run module and loads the active sample plate.
	Select All - This button is used to highlight (select) all cells in the sample plate.
	Clear All - This button is used to remove all cell properties in the sample plate.
	View Summary - View a summary of a specific sample plate by opening the desired sample plate and clicking on the View Summary icon. The Sample Plate Summary spreadsheet is displayed with a summary of each sample in the plate. This is a read-only spreadsheet and cannot be edited.

Figure 20: Sample Plate Summary

Icon	Description
	Help Topics - Used to select and print specific topics in the CEQ Help file and search for information by: topic, index entry and/or keyword.
	Context-Sensitive Help - Used to open the Help file related to a specific menu option.
	Run Sample Plate - Used to initiate the Run module and run the plate.

Run Module

The Run module provides the capability of executing pre-programmed sample plates and controlling individual functions of the instrument. Specifically, it is used for:

*   Running and viewing the progress of a sample plate
*   Viewing and changing display options
*   Specifying a sample set, buffer set or wetting tray position
*   Specifying the capillary holding temperature
*   Denaturing a sample
*   Aligning the capillaries
*   Injecting a sample
*   Performing a separation
*   Changing plates, wetting tray, capillary array or gel cartridge

Selecting the Run *icon* from the Main Menu (Figure 21) executes this module.

Figure 21: Main Menu, Run Icon
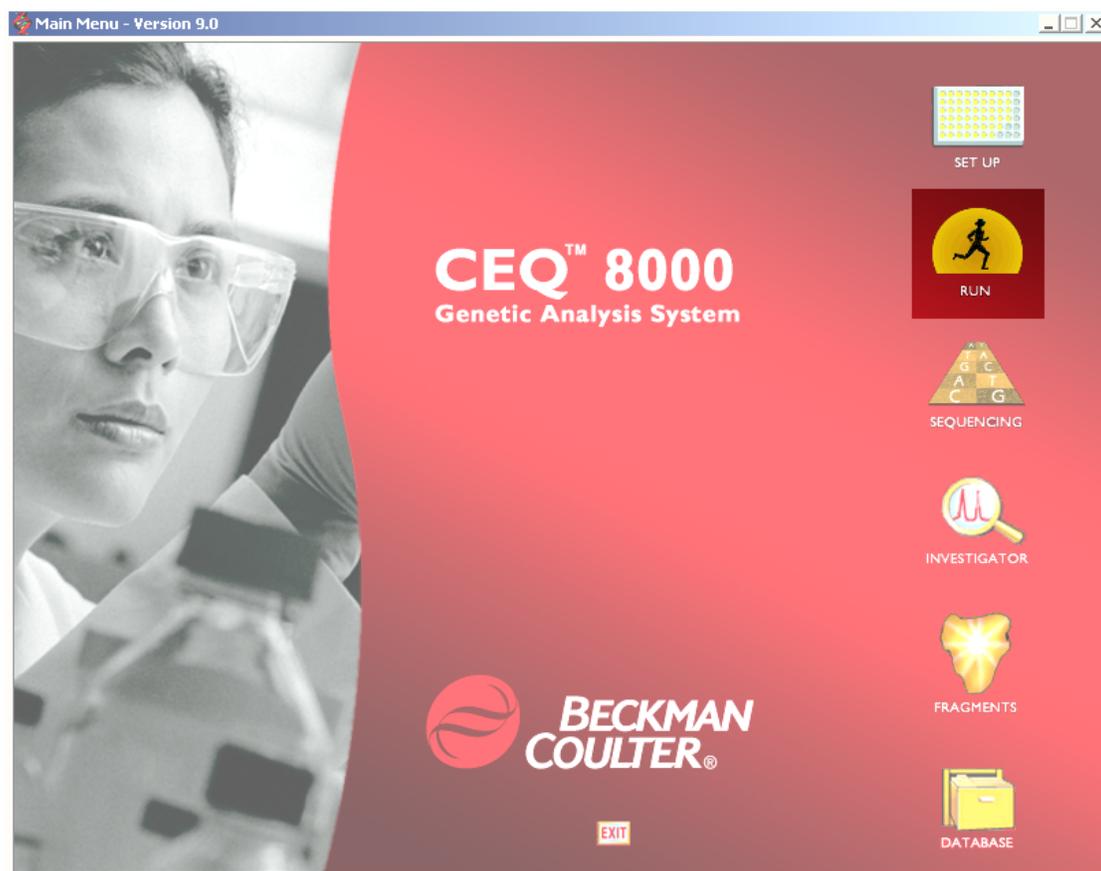
Main Window

The main window of the Run module is shown in Figure 22 and described in Table 10. Tables 11 through 18 provide descriptions of the menu options.

Figure 22: Run Module, Main Window
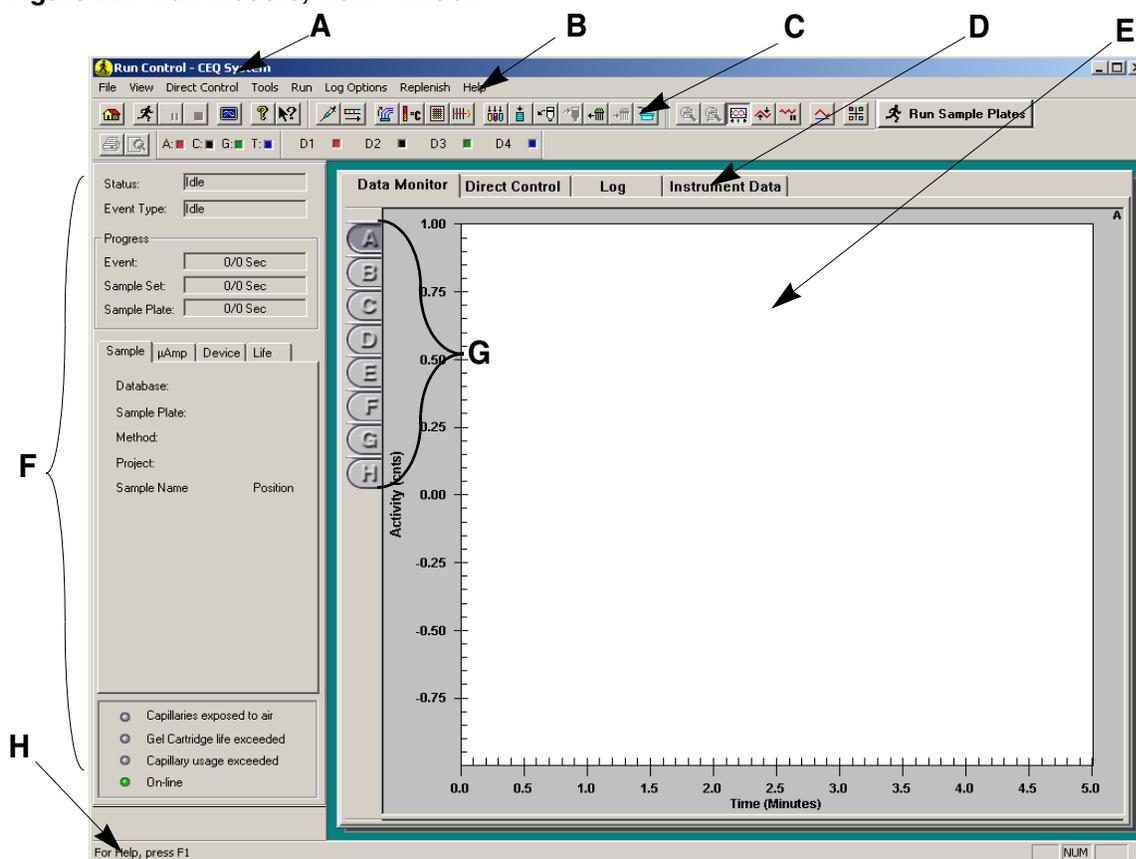
Table 10: Run Module, Main Window Descriptions

Item	Description
A	The *Title Bar* displays the name of the module (*CEQ Run*) and the user specified system name.
B	The *Menu Bar* is a list of the menu options (see *"Menu Bar Options"* on page 31).
C	The *Toolbars* contain the icons that execute pre-defined functions (see *"Toolbar Icons"* on page 45).
D	The *Window Selection Tabs* are used to toggle between windows (see *"Window Selection Tabs"* on page 53).
E	The *Display Area* graphically displays the raw data of the selected capillary or capillaries.
F	The *Status Monitor* provides information concerning the current state of the run, capillary and gel cartridge status and system on-line/off-line status.

Item	Description
G	*Capillary Buttons* - Letters **A** through **H** represent the eight capillaries in the array. Each button selected will have an associated pane shown in the Display Area.
H	The *Status Bar* displays messages such as 　• *Currently Active Database* - The active database name will be displayed. 　• *Pause to Load* - If Pause to load has been selected, the wait time will be displayed. The right areas of the status bar indicate which of the following keys are latched down: 　• *CAP* - The Caps Lock key is engaged. 　• *NUM* - The Num Lock key is engaged. 　• *SCRL* - The Scroll Lock key is engaged.
🏃 Run Sample Plates	*Run Sample Plate* - Used to run the currently active plate.

Menu Bar Options

The menu bar has the options shown in Figure 23. Tables 11 through 18 describe the options.

Figure 23: Run Module, Menu Bar Items

File View Direct Control Tools Run Log Options Replenish Help
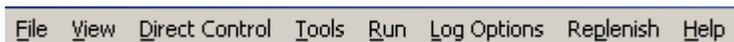
File Menu

Table 11: Run Module, File Menu
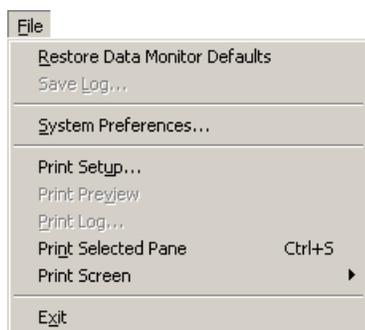
Item	Description
Restore Data Monitor Defaults	Restores the colors of the data monitor to the original default shipped with the instrument.
Save Log	Used to select the folder where the system log will be stored. The log is identified by the date and a unique identifier.
System Preferences	Used to view and/or change the: **System Name**, **Operator Name**, **Project Name** and **Dye Names** for Fragment Analysis and/or to activate or deactivate the alarms.
Print Setup	Used to define printer properties.
Print Preview	Used to display a facsimile of a hardcopy printout of the System Log prior to printing.
Print Log	Used to print the System Log.
Print Selected Pane	Used to print the selected pane.
Print Screen	Pull-right menu with the option to send an image of the computer desktop, application window or main window to the printer.
Exit	Closes the Run module.
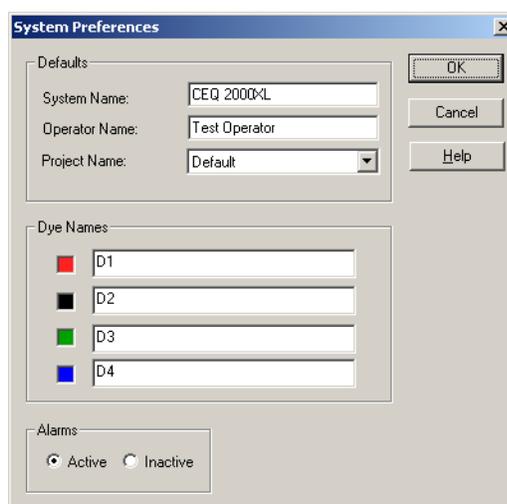
View Menu

Table 12: Run Module, View Menu
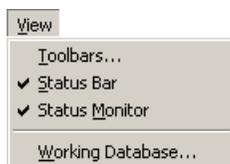
Item	Description
Toolbars	Used to select the toolbars to display in the Run module window.
Status Bar	Toggles between displaying/not displaying the Status Bar.
Status Monitor	Toggles between displaying/displaying the Status Monitor (see *"Status Monitor"* on page 51).
Working Database	Displays a dialog box showing the database in use.
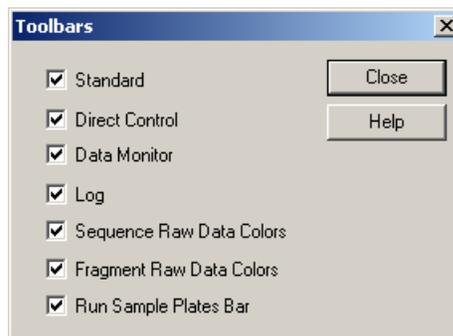
Direct Control Menu

Table 13: Run Module, Direct Control Menu
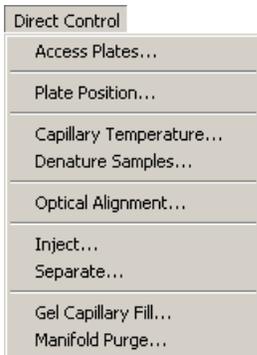
Item	Description
Unload Plates	Used to move the plate holder to the Load position at the front of the instrument to load or unload plates.
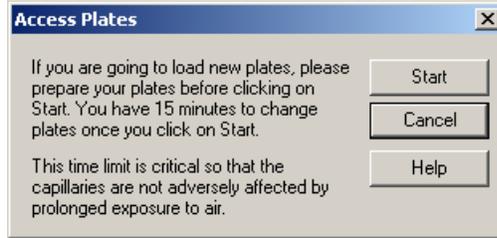
Item	Description
Plate Position	This dialog box is used to "graphically" select the well location where the capillaries will be immersed.
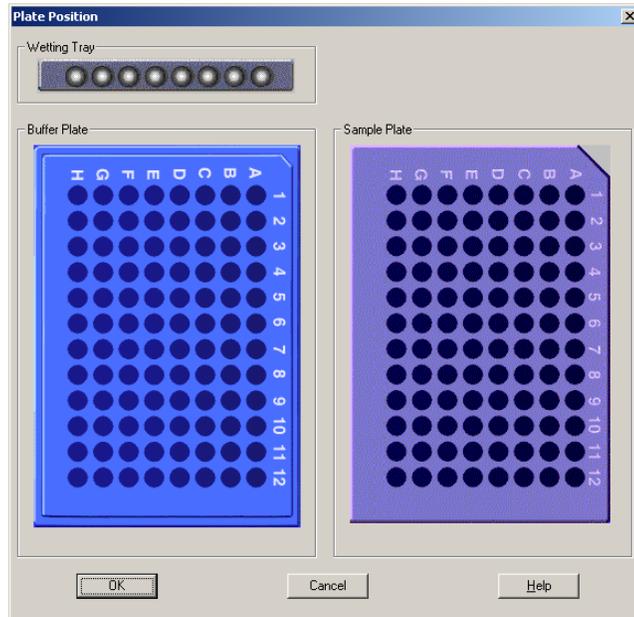
Capillary Temperature	Used to change the capillary holding temperature.
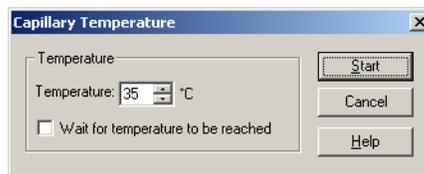
> *This temperature will be over-ridden at the end of each sample plate or when cancelled.*
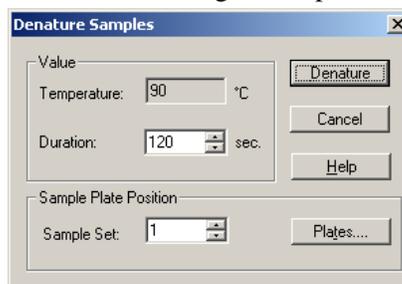
Denature Samples	Used to initiate the denaturing of samples.
Optical Alignment	Used to align the lasers with the detection windows of the eight capillaries. Data can be saved and viewed in the Sequence module.
Inject	Used to initiate the injection of the sample prior to separation.
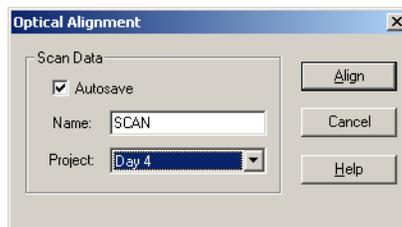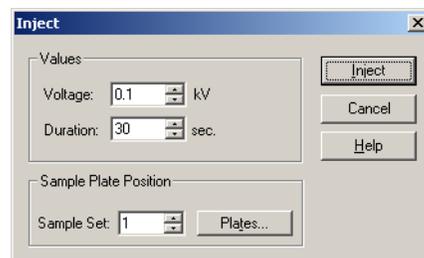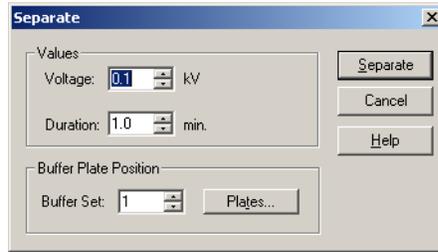
| Separate | Used to initiate the separation of injected samples. The data generated is not saved. |

| Gel Capillary Fill | Used to 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.) |

| Manifold Purge | Used to clear the manifold of air bubbles and/or remaining DNA fragments before the next separation process. |
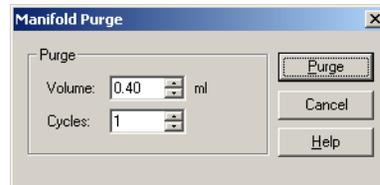
Tools Menu

Table 14: Run Module, Tools Menu

Item	Description
Autoscroll	Used to scale all data to the last 10 minutes of the run on the X axis and confine the display of data to the pane on the Y axis.
Autoscale	Used to scale data to the confines of the pane. If autoscaling is disabled, the data is scaled to its true values. Use this item to turn autoscaling on or off.
Pause Data Display	Used to pause the display of data. Pausing data does not stop the stream of data, just the display of data.
Unzoom	Used to undo one zoom level.

Table 14: Run Module, Tools Menu (Continued)

Item	Description
Unzoom All	Used to undo all zoom levels.
Display Options	Invokes the **Display Options** dialog box used to modify any or all of the following parameters: • Title • X Axis Options • Y Axis Options • Dye Traces • Current Traces • Colors See immediate changes in the selected pane by clicking on **Apply**.

Note	*Double-clicking the left mouse button in the selected pane, also brings up this dialog box.*

View Last Analysis	Used to open and display the most recently analyzed sample set. If the last sample set was analyzed with a sequence analysis parameter set, the Sequence Analysis module is launched. If the last sample set was analyzed with a fragment analysis parameter set, the Fragment Analysis module is launched
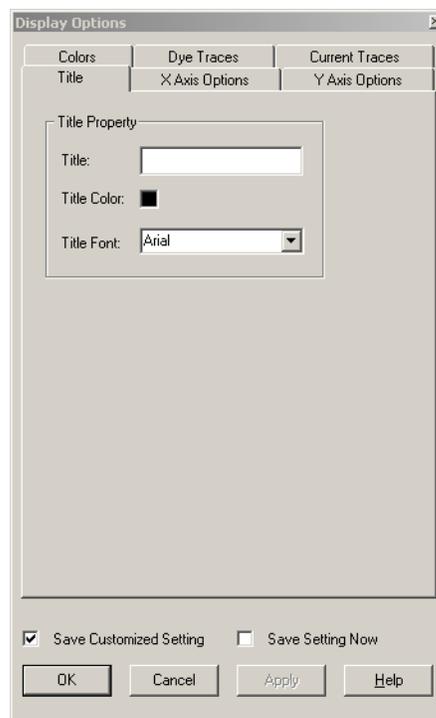
Run Menu

Table 15: Run Module, Run Menu

Run

- Start Sample Plate...
- Pause
- Pause to load...
- Stop System...
- Monitor Baseline...
- Diagnostics...
- Reset
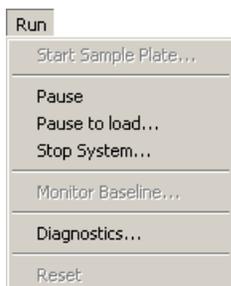
Item	Description
Start Sample Plate	Used to open and execute a specific sample plate.
Pause	Used to pause a running sample plate.
Stop System	Used to stop a running sample plate or direct control process.
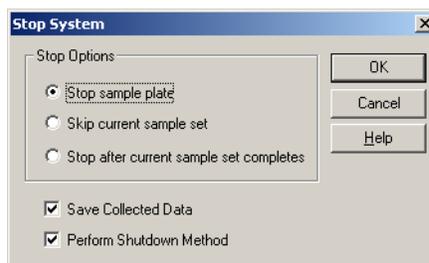
Stop Options - Specifies when to stop sample plate:

Stop sample plate will stop the currently running sample plate immediately,

Skip current sample set will skip the currently running sample set, *OR*

Stop after current sample set completes will stop the run after the currently running sample set has been completed.

Save Collected Data:

Save data already collected for the currently executing sample set.

Perform Shutdown Method:

Purge the system of any remaining DNA fragments and refill the capillaries with fresh gel after the sample plate has been cancelled. (The capillary temperature will be set to 35°C.)

Item	Description
	When stopping a direct control process: *(Stop System dialog box shown with Stop Options: Stop ALL selected, Stop High Voltage Only; buttons OK, Cancel, Help)* **Stop Options -** Specifies what process to stop: **Stop All** cancels all the currently running processes. **Stop High Voltage Only** stops the high voltage but continues to inject the sample.
Monitor Baseline	Baseline monitoring displays the baseline data trace. To ensure that the optics are working correctly and that the capillaries are clean, view the baseline when a new capillary array is installed, after the capillaries have been filled with gel for the first time and an optical alignment has been performed. The baseline trace should be low and relatively flat (less than 6000 counts). *(Monitor Baseline dialog box shown: Enable Monitor Baseline; Baseline Parameters: Autosave, View Injection Instrument Data checked; Name: BASELINE; Project: Default; buttons OK, Cancel, Help)* If **Autosave** is selected from the dialog box, baseline data will be stored under the Sample Data folder (for the selected project) in the Data Manager. If Monitor Baseline is not ended manually, baseline data will be collected until a sample plate is run or until six hours have elapsed from the start of the baseline monitoring process.
Diagnostics	Used to view the instrument version, laser power, PC Settings, monitor status, and to home the plates and/or the gel pump.
Reset	Used to re-synchronize system hardware with the firmware and to reset the system log.
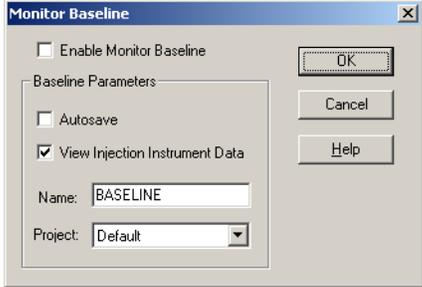
Log Options Menu

Table 16: Run Module, Log Options Menu
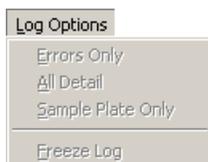
Item	Description
Errors Only	Causes the program to list *errors only* in the Log Window.
All Details	Causes the program to display *all details* in the Log Window including all messages and system activity.
Sample Plate Only	Causes the program to only list items related to a running *sample plate* in the Log Window.
Freeze Log	Used to freeze the display in the Log Window. (Freezing the log data does not stop the collection of data, just the display of data.)

Replenish Menu

Table 17: Run Module, Replenish Menu
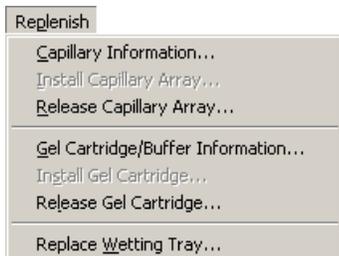
Item	Description
Capillary Information	Displays the **Capillary Information** dialog box to view the part number and serial number for the capillary array, the number of previous runs and the number of days that the capillary array has been on the instrument. The **Advanced** button shows additional capillary parameters.

Install Capillary Array	Used to install the capillary array. Verify or change the information requested, and then install the array.

Release Capillary Array	Used to remove the current capillary array. To install a new capillary array, select the **Replace capillary array** radio button. To install a manifold plug, select **Install manifold plug**. To clean the capillary windows, select **Clean capillaries**.

Gel/Buffer Information	Used to view the part number, lot number for the gel and/or buffer, the date and time of gel cartridge installation and the number of hours the cartridge has been on the instrument.

Install Gel Cartridge	Used to install a gel cartridge.

Release Gel Cartridge	Used to release the gel cartridge in preparation for installing a new cartridge or preparing the system for short or long-term storage. When selected, the following confirmation dialog will open.

Select **OK** to continue.

Enter the requested information and then:

- Select **Install Plug** and then install the Gel Plug or a clean, empty cartridge

  OR

- Select **Install Cartridge** to install a new cartridge.
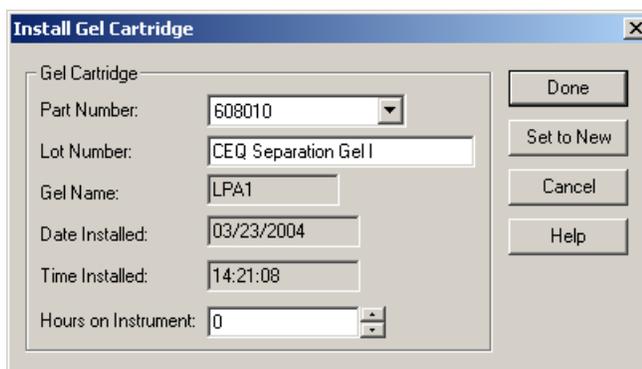
Replace Wetting Tray	Used to replace or re-fill the wetting tray. When selected, the following confirmation dialog will open.

Select the position where you wish the capillaries to be immersed after the wetting tray is replaced. If you wish to select the location graphically, click on the **Plates** button.
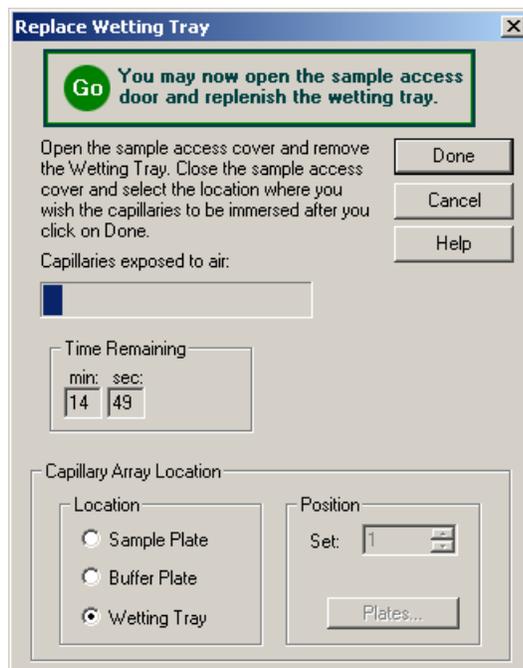
Help Menu
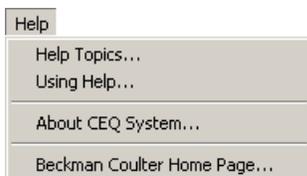
Table 18: Run Module, Help Menu

Item	Description
Help Topics	Used to select and print specific topics in the Help file and/or search for information by: topic, index entry and/or keyword.
Using Help	Used to select and print specific topics in the *Windows* Help file and search for information by: topic, index entry and/or keyword.
About CEQ System	Used to access software, instrument and system information.
Beckman Coulter Home Page	Used to access the Beckman Coulter Home Page on the Internet.

Toolbar Icons

There are six toolbars (Figure 24) used in the Run module: 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.

Figure 24: Run Module, Toolbars

Standard Toolbar

 (Table 19)

Direct Control Toolbar

 (Table 20)

Data Monitor Toolbar

 (Table 21)

Log Toolbar

 (Table 22)

Sequence Dye Colors Toolbar

A:■ C:■ G:■ T:■ (Table 23)

Fragment Dye Colors Toolbar

D1 ■ D2 ■ D3 ■ D4 ■ (Table 24)
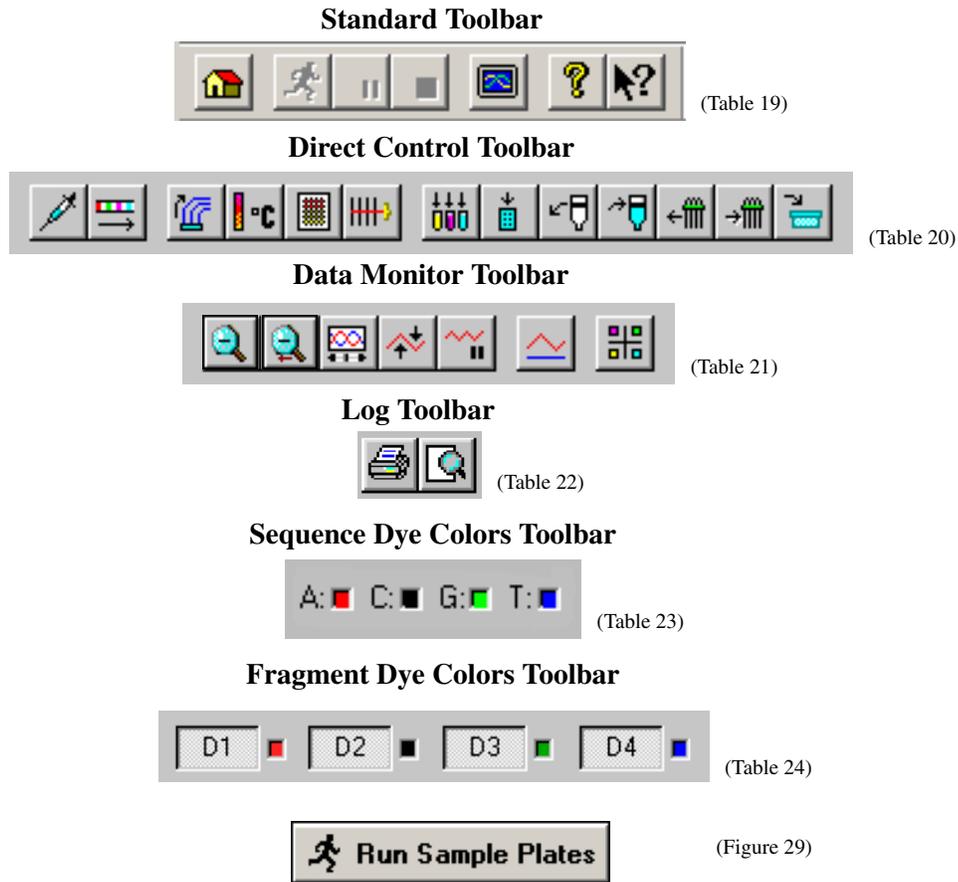
 (Figure 29)

Standard Toolbar

Figure 25: Run Module, Standard Toolbar

Table 19: Run Module, Standard Toolbar

Button	Description
	Restore Data Monitor Defaults - Used to restore the display configuration of the display monitor to the original default shipped with the instrument.
	Run Sample Plate - Used to open and execute a specific sample plate.
	Pause - Used to pause a running sample plate.
	Stop - Used to stop a running sample plate or direct control process.

Button	Description
🖼	*View Last Analysis* - Used to execute the Sequence Analysis module and display the last sample analyzed.
❓	*Help Topics* - Used to select and print specific topics in the CEQ Help file and search for information by: topic, index entry and/or keyword.
▶❓	*Context-Sensitive Help* - Used to open the Help file related to a specific menu option.

Direct Control Toolbar

Figure 26: Run Module, Direct Control Toolbar

Table 20: Run Module, Direct Control Toolbar

✎	*Inject* - Used to initiate the injection of the sample prior to separation.
⟶	*Separate* - Used to initiate the separation of injected samples. The data generated is not saved.
⟲	*Gel Capillary Fill*- Used to 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.)
°C	*Capillary Temperature* - Used to change the capillary holding temperature.
▦	*Denature Samples*- Used to initiate the denaturing of samples.
⫯	*Optical Alignment* - Used to align the lasers with the detection windows of the eight capillaries. Data can be saved and viewed in the Sequence Analysis module.
⫯⫯⫯	*Plate Position* - Used to select the plate or tray position where the capillaries will be immersed.
⊟	Displays the *Access Plates* dialog box which is used to move the plate holder to the Load position at the front of the instrument to load or unload plates.
◁⊟	*Release Gel Cartridge* - Used to release the gel cartridge in preparation for installing a new cartridge or preparing the system for short or long-term storage.
⊟▷	*Install Gel Cartridge* - Used to install a gel cartridge.
◁ⅲ	*Release Capillary Array* - Used to remove the current capillary array. To install a new capillary array, select the **Replace capillary array** radio button. To install a manifold plug, select **Install manifold plug**. To clean the capillary windows, select **Clean capillaries**.
ⅲ▷	*Install Capillary Array* - Used to install the capillary array. Verify or change the information requested, and then install the array.
⊟	*Replace Wetting Tray* - Used to replace or re-fill the wetting tray.

Data Monitor Toolbar

Figure 27: Run Module, Data Monitor Toolbar

Table 21: Run Module, Data Monitor Toolbar

🔍	*Unzoom* - Used to undo one zoom level.
🔍	*Unzoom All* - Used to undo all zoom levels.
🔲	*Autoscroll On/Off*- Used to scale all data to the last 10 minutes of the run on the X axis and confine the display of data to the pane on the Y axis.
📈	*Autoscale On/Off* - Used to scale data to the confines of the pane. If autoscaling is disabled, the data is scaled to its true values. Use this icon to turn autoscaling on or off.
〰	*Pause Data Update* - Used to pause the display of data. Pausing data does not stop the stream of data, just the display of data.
📉	*Monitor Baseline* - Baseline monitoring displays the baseline data trace. To ensure that the optics are working correctly and that the capillaries are clean, view the baseline when a new capillary array is installed, after the capillaries have been filled with gel for the first time and an optical alignment has been performed. The baseline trace should be low and relatively flat. If **Autosave** is selected from the dialog box, baseline data will be stored under the Sample Data node (for the selected project) in the Data Manager. If Monitor Baseline is not ended manually, baseline data will be collected until a sample plate is run or until six hours have elapsed from the start of the baseline monitoring process.
🔳	*Display Options* - Invokes the Display Options dialog box used to modify display parameters.
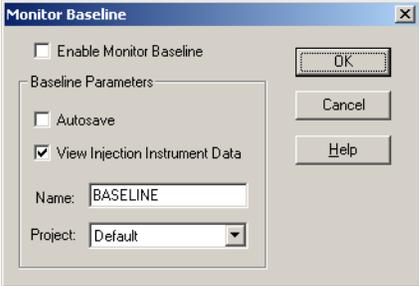
Log Toolbar

Figure 28: Run Module, Log Toolbar

Table 22: Run Module, Log Toolbar

🖨	*Print* - Used to print a single pane selected in the Display Area.
🔍	*Print Preview* - Used to display a facsimile of a hardcopy printout of the active sample plate.

Run Sample Toolbar

Figure 29: Run Sample Toolbar

When selected, this button invokes the *Sample Plate Run Confirmation* dialog box.

Sequence Dye Colors Toolbar

Figure 30: Run Module, Sequence Dye Colors Toolbar

Table 23: Run Module, Sequence Dye Colors Toolbar

Icon	Description
A: (red)	The color red is the default color assigned to the Adenine (**A:**) nucleotide bases in the Analyzed Data pane.
C: (black)	Black is the default indicator assigned to the Cytosine (**C:**) nucleotide bases in the Analyzed Data pane.
G: (green)	The color green is the default color assigned to the Guanine (**G:**) nucleotide bases in the Analyzed Data pane.
T: (blue)	The color blue is the default color assigned to the Thymine (**T:**) nucleotide bases in the Analyzed Data pane.

Fragment Dye Colors Toolbar

Figure 31: Run Module, Fragment Dye Colors Toolbar

Table 24: Run Module, Fragment Dye Colors Toolbar

Icon	Description
D1 (red)	The color red is the default color assigned to Dye #1 in the Fragment Data pane, and **D1** is the default name.
D2 (black)	The color black is the default color assigned to Dye #2 in the Fragment Data pane, and **D2** is the default name.
D3 (green)	The color green is the default color assigned to Dye #3 in the Fragment Data pane, and **D3** is the default name.
D4 (blue)	The color blue is the default color assigned to Dye #4 in the Fragment Data pane, and **D4** is the default name.

Status Monitor

The Status Monitor displays the state of the current run. Figure 32 shows the status monitor and Table 25 describes the areas.

Figure 32: Run Module, Status Monitor Displays
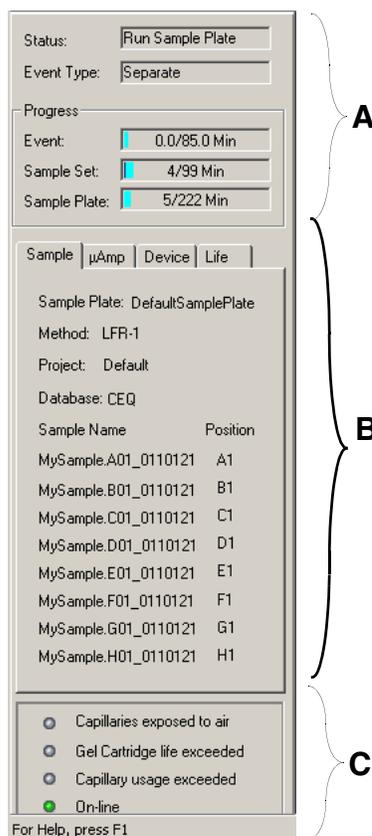
Table 25: Run Module, Status Monitor Displays

Item	Function
A	*Progress Indicator* - This area of the Status Monitor shows the Status, Event Type and Progress of a running sample plate or process. 📝 *Note* *Times displayed are approximations.* Status: Run Sample Plate Event Type: Denature Sample Progress Event: 2.0/2.0 Min Sample Set: 2/128 Min Sample Plate: 3/136 Min
B	*Information Tabs* - Toggles between the tabs shown below. • *Sample* Lists the sample plate, sample names and the method being used. Sample | µAmp | Device | Life Sample Plate: J1022 Method: LFR-1 Project: Default Database: U7_010401_TRAINING Sample Name — Position 102202_ja.A01_0110231 A1 102202_jb.B01_0110231 B1 102202_jc.C01_0110231 C1 102202_jd.D01_0110231 D1 102202_je.E01_0110231 E1 102202_jf.F01_01102312 F1 102202_jg.G01_0110231 G1 102202_jh.H01_0110231 H1 • *µAmp* Shows the approximate overall microamperes (µA) and voltage used during the separation. Sample | µAmp | Device | Life Voltage: 0.2 kV Total Current: 18 µA Capillaries A: 3.0 B: 3.0 C: 3.0 D: 3.0 E: 3.0 F: 3.0 G: 3.0 H: 3.0 • *Device* Displays the condition or value of devices or parameters. Sample | µAmp | Device | Life Plate Position: Sample: 1 Capillary Temperature: 35 °C Capillary Array: Present Manifold Plug: Not Present Cartridge: Gel Cartridge Barrel: Locked Sample Access Cover: Closed Capillary Access Cover: Closed Voltage: 2.0 kV Total Current: 20 µA • *Life* Displays the remaining gel, capillary usage and laser hours. Sample | µAmp | Device | Life Gel Cartridge Remaining: 10.7 ml Age: 0 hours Capillary Array Usage: 1 runs Age: 6.1 days Lasers Laser 1 Hours: 952 Laser 2 Hours: 952
C	*Indicators* - Provides indicators that pertain to 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. ○ Capillaries exposed to air ○ Gel Cartridge life exceeded ○ Capillary usage exceeded ● On-line
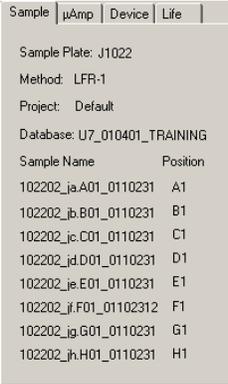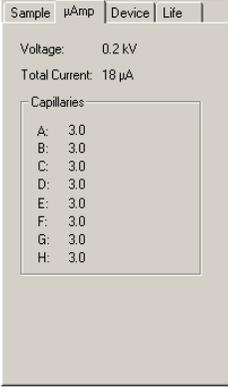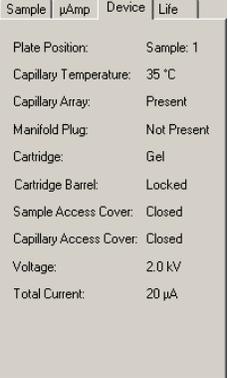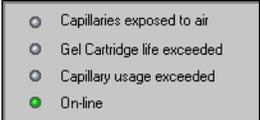
Window Selection Tabs

The Window Selection tabs provide access to the following windows: Data Monitor, Direct Control, Log and Instrument Data. These windows are described on the following pages.

Data Monitor Window

The Data Monitor window is shown in Figure 33. This window is accessed in the Display Area by selecting the Data Monitor *tab*. The window displays information associated with a sample's analysis.

Figure 33: Run Module, Data Monitor Window
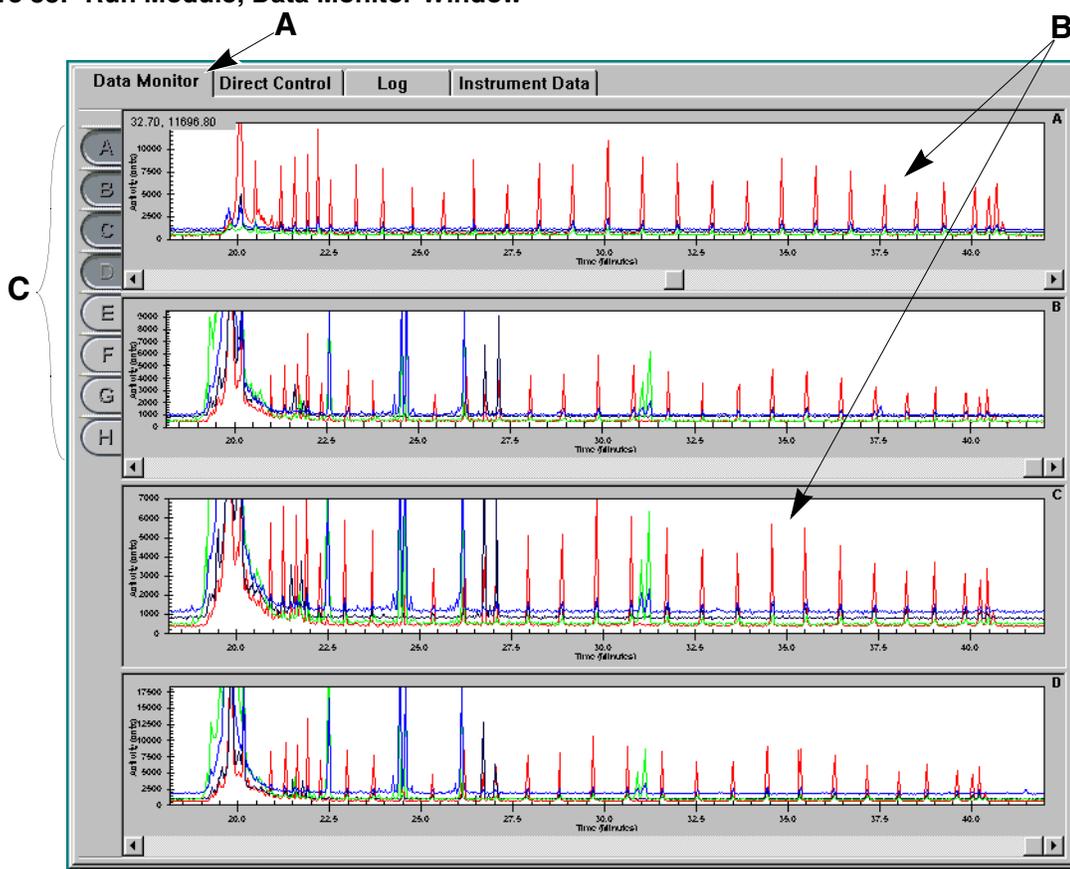
Table 26: Run Module, Data Monitor Window Descriptions

Item	Description
A	*Window Selection Tab* - Select this tab to access the Data Monitor window.
B	*Data Monitor Window* - This window displays the data for the running sample plate for the selected capillary buttons.
C	*Capillary Buttons* - These buttons (**A** through **H**) represent the eight capillaries of the array.

Direct Control Window

The Direct Control window is shown in Figure 34. This window is accessed in the Display Area by selecting the Direct Control *tab*.

This window contains hot areas and function buttons that perform some of the distinct tasks listed in the "Direct Control" and "Replenish" drop-down menus.

Figure 34: Run Module, Direct Control Window
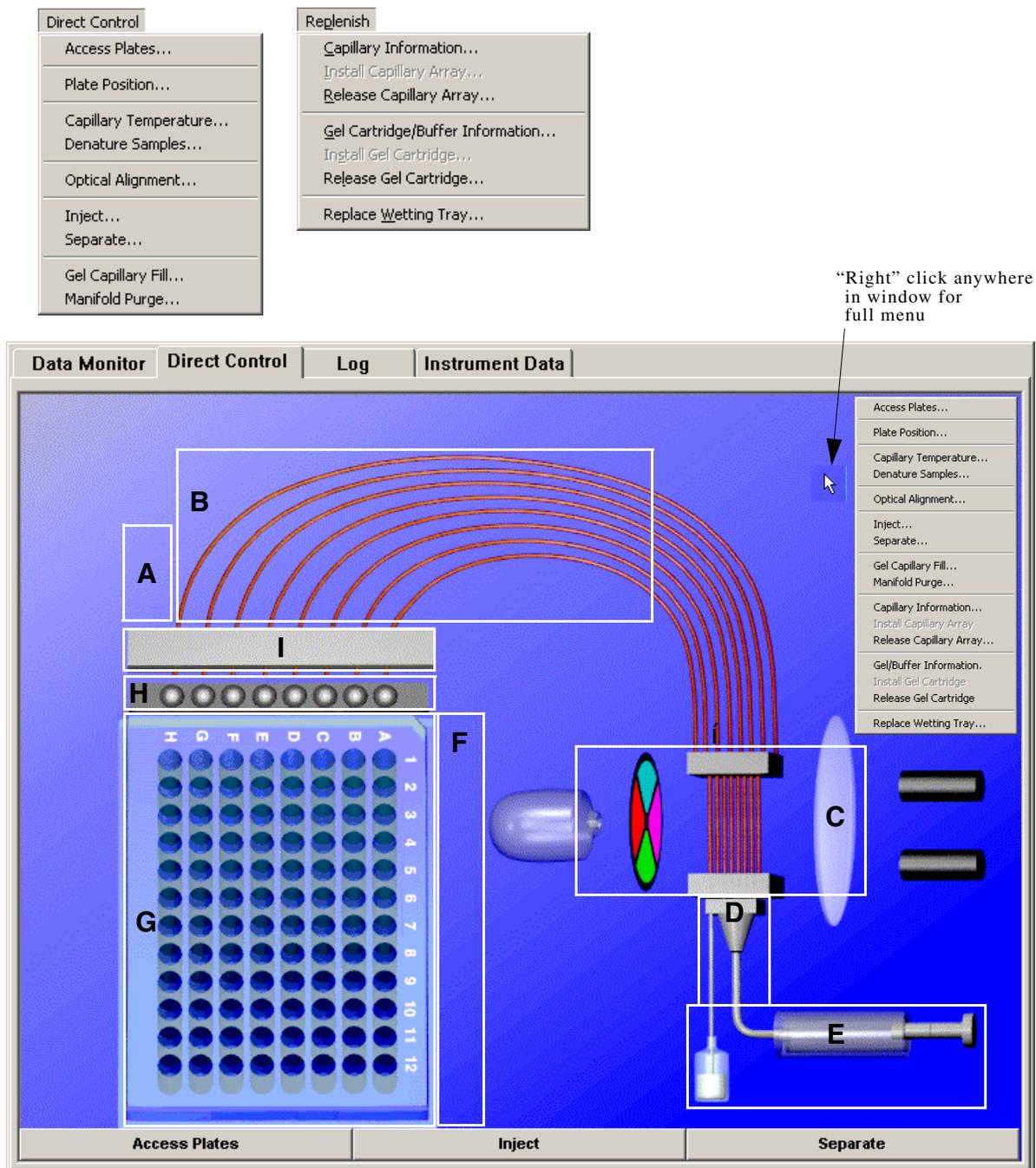
The circled numbers shown on the window in Figure 34 represent the locations of the hot areas in the Direct Control window. Each hot area, when selected, initiates a unique Direct Control task. Table 27 lists the tasks available in this window.

Table 27: Direct Control Window, Hot Areas

Item	Description
A	*Capillary Temperature* - Used to change the capillary temperature.
B	*Gel Capillary Fill* - Used to 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.)
C	*Optical Alignment* - Used to 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* - Used to clear the manifold of air bubbles and/or remaining DNA fragments before the next separation process.
E	*Install/Release Gel Cartridge* - Used to release and/or install a gel cartridge.
F	*Denature Samples* - Used to initiate the denaturing of samples.
G	*Plate Position* - Used to select the plate or tray position where the capillaries will be immersed.
H	*Wetting Tray* - Used to replace or re-fill the wetting tray.
I	*Install/Release Capillary* - Used to install the capillary array. Verify or change the information requested, and then install the array.
	Access Plates - Displays the **Access Plates** dialog box. This dialog box is used to: **Access Plates** • select the position for capillary immersion in the plate or wetting tray. • move the plates to the Load position at the front of the instrument to load or unload plates.
	Inject - Used to initiate the injection of the sample prior to separation. **Inject**
	Separate - Used to initiate the separation of injected samples. **Separate**

Log Window

The Log window (Figure 35) is accessed in the Display Area by selecting the Log *tab*. The log window provides all of the messages and activity for a sample plate run.

Figure 35: Run Module, Log Window
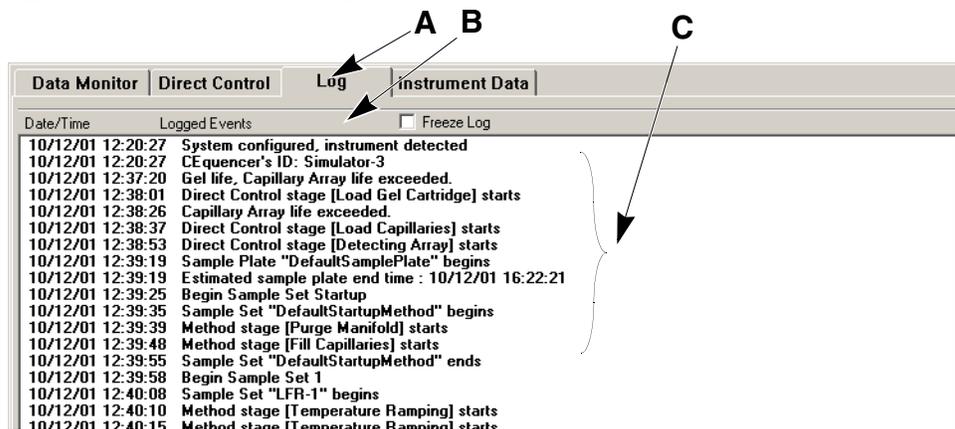
Table 28: Run Module, Log Window Descriptions

Item	Description
A	*Window Selection Tab* - Select this tab to access the Log window.
B	*Freeze Log* - Used to freeze the display in the Log window. (Freezing the log data does not stop the collection of data, just the display of data.)
C	The *Display Area* provides the list of logged events.

Instrument Data Window

The Instrument Data window (Figure 36) is accessed in the Display Area by selecting the Instrument Data *tab*. This window displays the current for the eight capillaries and the voltage level of the instrument for the current run.

Figure 36: Run Module, Instrument Data Window
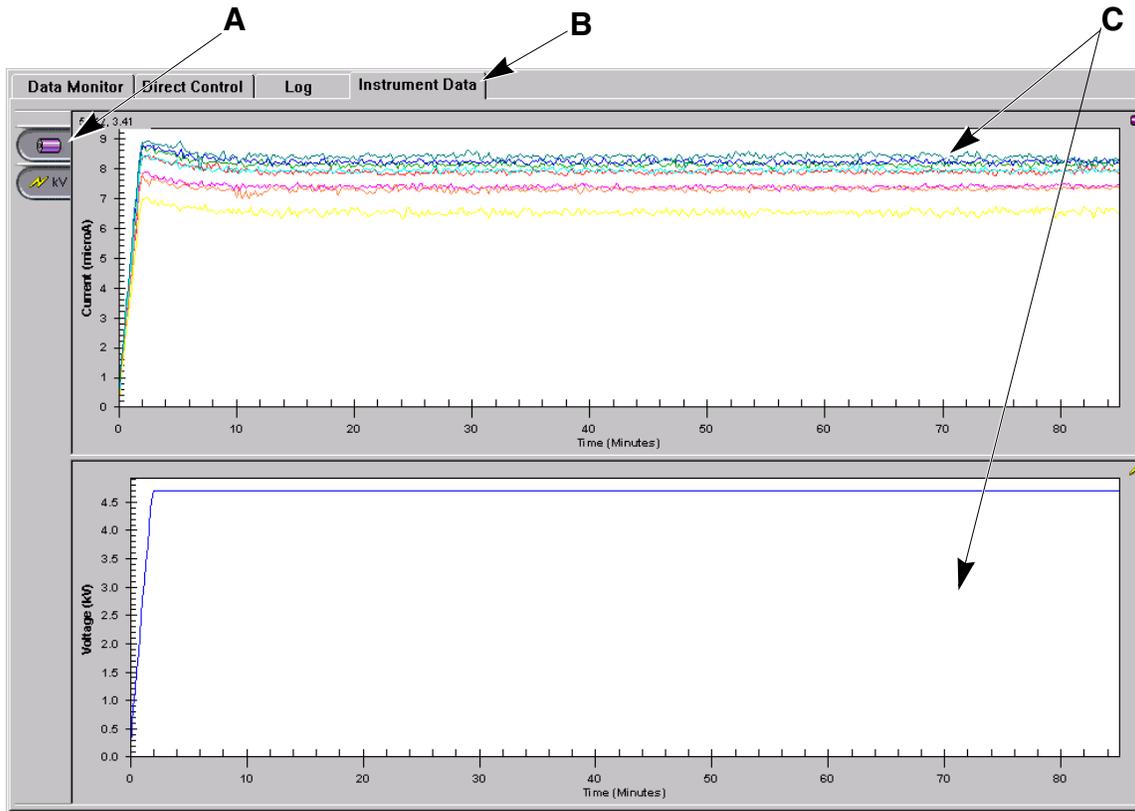
Table 29: Run Module, Log Window Descriptions

Item	Description
A	*Current and Voltage Buttons* - Used to select panes for display.
B	*Window Selection Tab* - Select this tab to access this window.
C	The *Display Area* displays the current and voltage levels of selected current run.

Sequence Analysis Module

The Sequence Analysis module is used to view, analyze, compare, manipulate and print data of the following types:

- Raw Data*
- Current Data*
- Voltage Data*
- Analyzed Data
- Base Sequences
- Optical Scan Data*
- Baseline Data*
- Quality Parameters*

  * These types of data cannot be manipulated.

This module accepts raw data and analyzed data. Editing and re-analysis functions provide the capability to verify the accuracy of the base calls. These data can also be exported to third party packages for further analysis.

Selecting the Sequence Analysis *icon* from the Main Menu (Figure 37) executes this module.

Figure 37: Main Menu, Sequence Analysis Icon
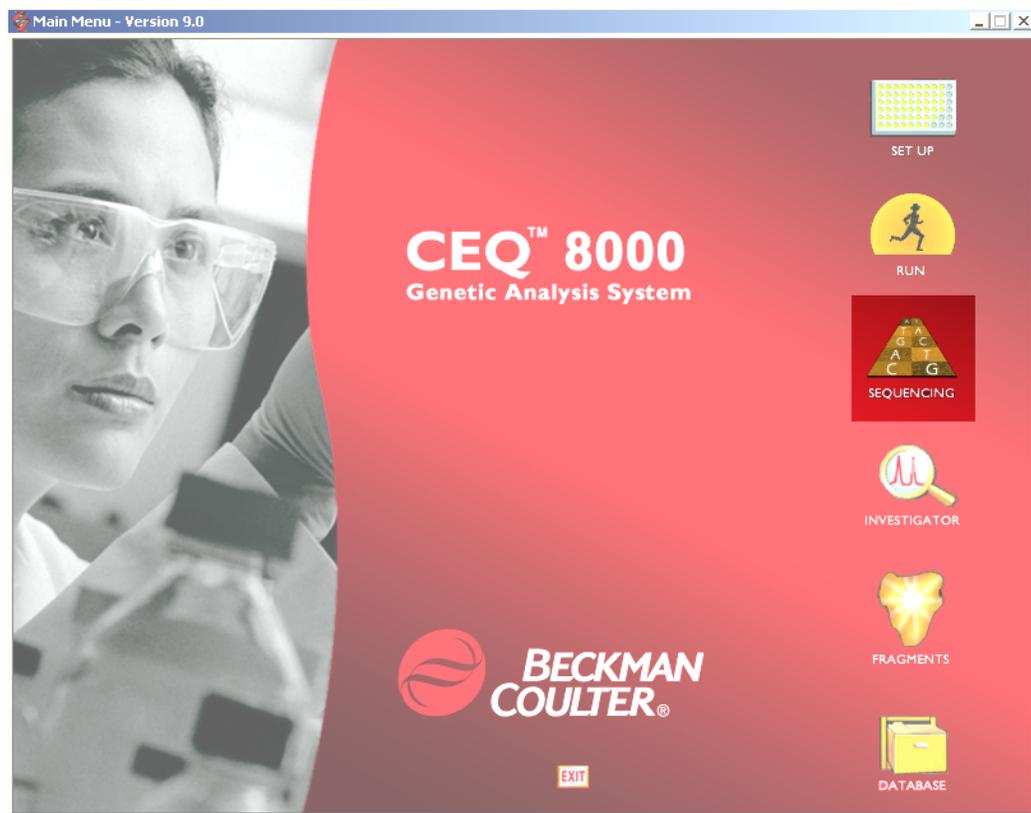
Main Window

The main window of the Sequence Analysis module is shown in Figure 38 and is described in Table 30.

Figure 38: Sequence Analysis Module, Main Window
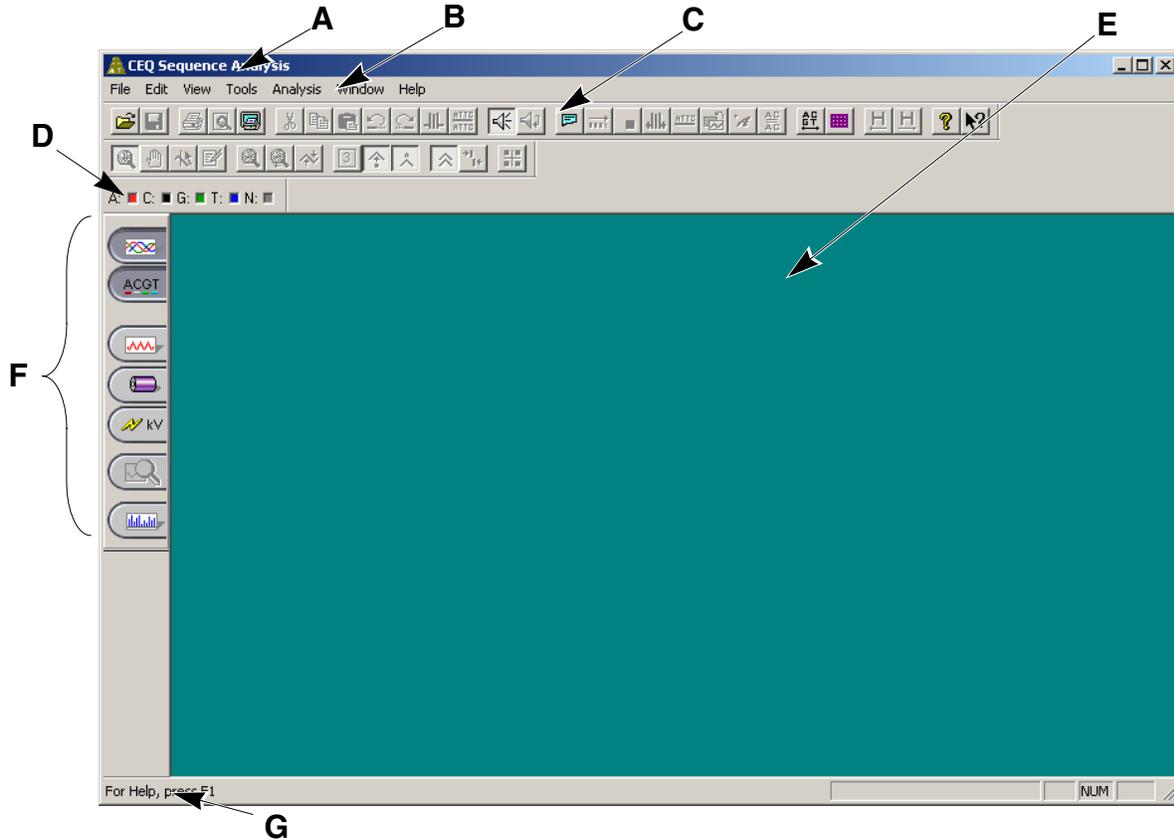
Table 30: Sequence Analysis Module, Main Window Descriptions

Item	Description
A	The *Title Bar* showing the module name (*CEQ Sequence Analysis*) and the currently active sample.
B	The *Menu Bar* is a list of the menu options (see *"Menu Bar Options"* on page 62).
C	The *Toolbar* contains the icons that execute pre-defined functions (see *"Toolbar Icons"* on page 73).
D	Double-clicking on a base sequence text letter (**A**, **C**, **G**, **T**) in the *Dye Colors* box brings up the **Color** dialog box. Change the dye color displayed *for that particular* dye.

Item	Description
E	This *Display Area* graphically displays the opened data.

F		*Analyzed Data*	Displays the data that has been analyzed for the active sample.
		Base Sequence	Displays a text view of the bases from the analyzed data for the active sample.
		Raw Data	Displays the raw data for the active sample.
		Current	Displays the current data for the active sample.
		Voltage	Displays voltage data for the active sample.
		Compare Data	Used to compare the active analyzed data set against another analyzed data set.
		Quality Parameters	Used to view a graphical representation of the Quality Values for the active sample.

G	The *Status Bar* displays information concerning the current selection.

Menu Bar Options

The menu bar has the options shown in Figure 39. Tables 31 through 37 provide descriptions of the menu options.

Figure 39: Sequence Analysis Module, Menu Bar Options

File   Edit   View   Tools   Analysis   Window   Help

File Menu

Table 31: Sequence Analysis Module, File Menu

Item	Description
Open	Used to open: Sample Data, Sequence Analysis Parameters, Optical Scan Data, Sequence Results and Sample Plate Results.
Close	Closes the open sample.
Close Tab	Closes the active tab.
Save	Saves existing data.
Save As	Used to save data to a new name.
Import	Used to locate data to be imported. **Standard Chromatogram Format** (***.scf**) and **Electropherogram Sample Data (*.esd)** are available format for importing into this module.
Export	Used to export data in one of the following formats: • Standard Chromatogram Format Ver 3.00 (*.scf) • Standard Chromatogram Format Ver 2.10 (*.scf) • Tab Delimited ASCII Text (*.txt) • FASTA and Quality (*.fasta) • PHRED and SCF (*.phd.1) • SEQ Sequence Text (*.seq) • ESD (*.esd) • CEQ (*.cq*)

Open...   Ctrl+O
Close
Close Tab

Save   Ctrl+S
Save As...

Import...
Export...
Export from Plate...

Preferences...

Properties...

Report Format...
Print Preview
Print Report   Ctrl+R
Print Selected Pane   Ctrl+P
Print Desktop

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

Exit

Item	Description
Export from Plate	Used to open or export samples from a specific sample plate. Multiple samples can be selected using the **Shift** key. (The **Sample Plate** toolbar must be open to use this menu option.)

Preferences	Used to: • view or change what panes will be displayed when a sample is first opened. • show the **Report Format** dialog box when **Print Report** is selected from the **File** menu. • open all associated results that belong to a sample when a sample(s) 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 displayed panes. • select audio playback speed.

Item	Description
Properties	Used to view the general properties of the currently selected item. 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, the instrument, and the operator name.
Report Format	Used to specify the format of the report that will be generated for the active sample.
Print Preview	Used to display a facsimile of a hardcopy printout of the selected sample plate.
Print Report	Used to print the report.
Print Selected Pane	Used to print the selected pane.
Print Desktop	Used to print the desktop, application or main window as defined in the **Preferences** dialog box.
Recent Objects	Lists the most recently opened objects. The first set of four is sample data and the second set of four is sequence results.
Exit	Closes the Sequence Analysis module.

Edit Menu

Table 32: Sequence Analysis Module, Edit Menu

Item	Description
Undo	Used to undo the last action performed.
Redo	Used to redo the last undo action.
Cut	Used to cut selected bases.
Copy	Used to duplicate selected bases.
Paste	Used to insert one or more copied or cut bases.
Audio Enable	Toggles between enabling or disabling audio. When enabled, as a base letter is typed, in **Edit** mode, the letter is announced audibly.

Item	Description
Apply Quality-based Trimming	Use this option to commit to the Quality-based trimming.
Apply Sequence-based Trimming	Use this option to commit to the Sequence-based trimming.
Audio Playback	Used to audibly announce a series of selected base sequence text.

View Menu

Table 33: Sequence Analysis Module, View Menu
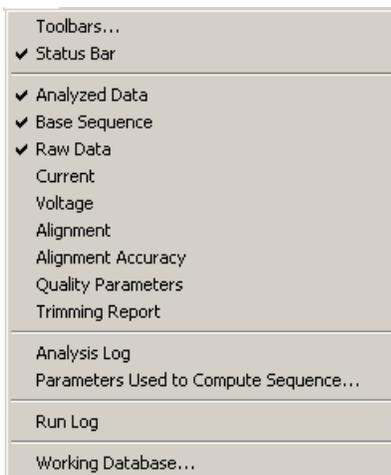
Item	Description
Toolbars	Used to select which toolbars to display.
Status Bar	Toggles between displaying or not displaying the Status Bar.
Analyzed Data	Displays the data that has been analyzed for the active sample.
Base Sequence	Displays a text view of the bases from the analyzed data for the active sample.
Raw Data	Displays the raw data for the active sample.
Current	Displays the current data for the active sample.
Voltage	Displays voltage data for the active sample.
Alignment	Displays the Alignment data for the active sample.
Alignment Accuracy	Displays the Alignment Accuracy data for the active sample.
Quality Parameters	Used to view a graphical representation of the Quality Values for the active sample.
Trimming Report	This menu item toggles (on/off) the Trimming Report pane.
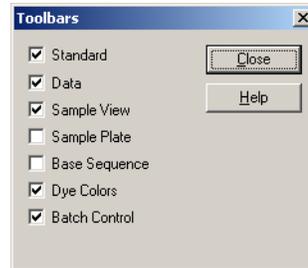
Item	Description
Analysis Log	Displays the Analysis Log for the active sample.
Parameters Used to Compute Sequence	Used to display the sequence analysis parameters used to produce the given analyzed data. The fields in this dialog box are read-only, and are not editable. This item is not available for data that has not yet been analyzed.
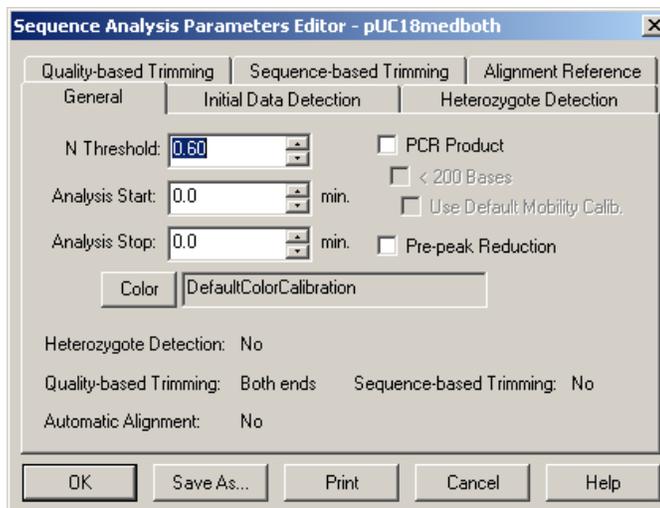
Item	Description
Run Log	Displays the messages received from the instrument during the sample run.
Working Database	Displays the name of the database in use.

Tools Menu

Table 34: Sequence Analysis Module, Tools Menu

Item	Description
Zoom	Used to magnify a specific area of data in the display.
Pan	Used to move analyzed data in the X and Y direction.
Align	Used to visually align bases and peaks in the analyzed data pane.
Edit	Used to insert, change and/or delete bases.
Unzoom	Used to undo one zoom level.
Unzoom All	Used to undo all zoom levels.
Autoscale	Used to scale data to the confines of the pane. If autoscaling is disabled, the data is scaled to its true values. Use this item to turn autoscaling on or off.
Base Spacing	Used to 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, synchronizes 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	Toggles the base sequence text between displaying on the top or bottom in the analyzed data pane.
Compare	Used to select an analyzed data set to compare to the current analyzed data set.
Compare Synch	Used to synchronize scaling, zooming and panning of two analyzed data sets while in the **Compare** mode.

Item	Description
Align Compare Views	Used to align selected points of each of the displayed analyzed data sets while in the **Compare** mode.
Display Options	Invokes the **Display Options** dialog box used to modify any or all of the following parameters: • Title • X Axis Options • Y Axis Options • Dye Traces • Current Traces • Colors • Quality Parameters See immediate changes in the selected pane by clicking on **Apply**.
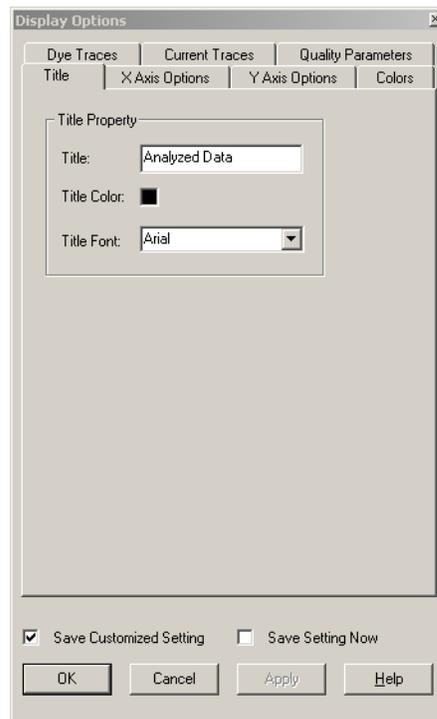
> **Note** *Double-clicking the left mouse button in the selected pane, also brings up this dialog box.*

Item	Description
Heterozygote Display Color	Opens the Heterozygote Display Colors dialog. This is used to change the color of the heterozygotes in the base sequence pane.

Analysis Menu

Table 35: Sequence Analysis Module, Analysis Menu Trim

Item	Description
Analyze	Used to analyze active sample data or reanalyze existing sequence results. When selected, the current Working Parameters dialog box is displayed. The analysis begins when **OK** is pressed.
Stop	Terminates the executing analysis.
Working Analysis Parameters	Used to view and/or change the analysis parameters to be used for subsequent analyses.
Restore Original Base Sequence	Used to return analyzed data display and base sequence text back to its original state (losing all edits).

Item	Description
Batch Analysis	Used to analyze multiple Sample Data or Sample Plate Results sequentially. Invokes the following dialog box.
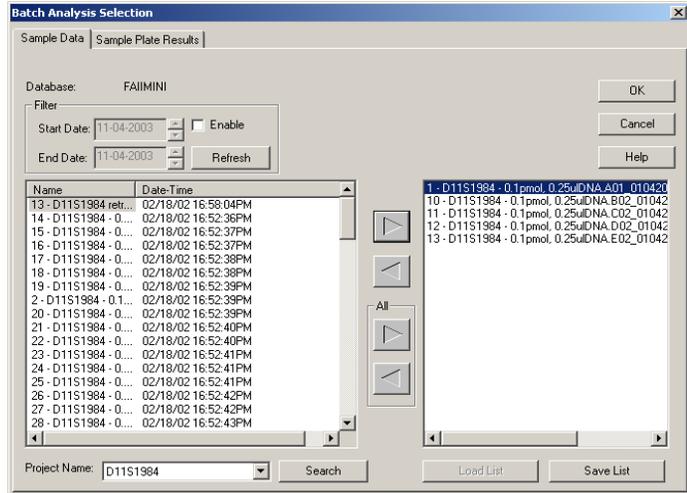
Once files are transferred to the right-side list box and **OK** is selected, the analysis parameters editor dialog box is displayed. Select the appropriate analysis parameters or edit an existing parameter set and click **OK**, batch processing begins and a window, similar to the one below, is displayed.
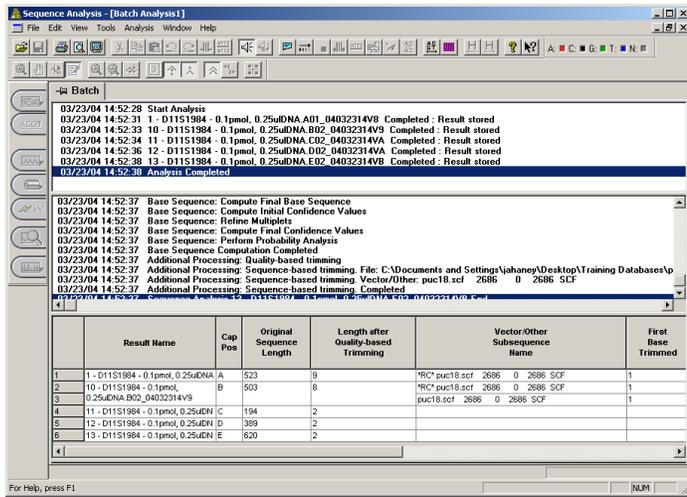
Item	Description
Reanalyze Batch	After performing a batch analysis, use this option to perform the analysis again.
View Selected Batch Sample Result	After performing a batch analysis, use this item to view the results of a selected sample in the upper pane of the **Batch Analysis** window.

Item	Description
Skip Current Sample	While performing a batch analysis, use this item to pass over the *sample* currently being analyzed. (This item is not available if **Sample Plate Results** was selected from the **Batch Analysis Selection** dialog box.)
Skip Current Sample Set	While performing a batch analysis, use this item to pass over the *sample set* currently being analyzed. (This item is not available if **Sample Data** was selected from the **Batch Analysis Selection** dialog box.)
Skip Current Sample Plate	While performing a batch analysis, use this item to pass over the sample *plate* currently being analyzed. (This item is not available if **Sample Data** was selected from the **Batch Analysis Selection** dialog box.)
Pause After Current Sample	Used to momentarily stop a batch analysis after the currently running sample has been analyzed. Use **Resume Batch Processing** to continue the analysis.
Resume Batch Processing	Used to resume a batch analysis after it has been paused.
Third Party Analysis	Used to launch the third party analysis package specified in the **Third Party Analysis Setup** dialog box.
Third Party Analysis Setup	Used to specify the path, third party analysis package (loaded on your PC) and the export format of the data.
Service Alignment	Used to: • align the sequence against puc18dG. • print the alignment report. • export the alignment data (as a text file).
Align with Template	Used to perform an alignment of the sequence against the current alignment settings.
Batch Alignment	Used to perform a batch alignment.
Trim Based on Quality	Opens the *Current Working Parameters* Dialog. Click **Edit** to open the *Quality-based Trimming* tab.
Trim Based on Sequence	Opens the *Current Working Parameters* Dialog. Click **Edit** to open the *Sequence-based Trimming* tab in the *Sequence Analysis Parameters* Editor.
Batch Trim	Opens the *Batch Trimming* Dialog box

Window Menu

Table 36: Sequence Analysis Module, Window Menu
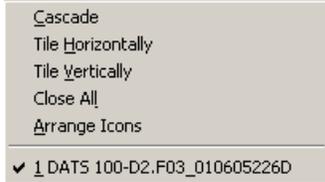
Item	Description
Cascade	Cascades the open windows.
Tile Horizontally	Tiles the windows in a horizontal orientation.
Tile Vertically	Tiles the windows in a vertical orientation.
Close All	Closes any currently active window(s).
Arrange Icons	Automatically arranges the icons.

Help Menu

Table 37: Sequence Analysis Module, Help Menu

Item	Description
Help Topics	Used to select and print specific topics in the Help file and/or search for information by: topic, index entry and/or keyword.
Using Help	Used to select and print specific topics in the *Windows* Help file and search for information by: topic, index entry and/or keyword.
About CEQ System	Used to access software, instrument and system information.
Beckman Coulter Homepage	Used to access the Beckman Coulter Homepage on the Internet.

Toolbar Icons

There are seven toolbars (Figure 40) used in the Sequence Analysis module: (1) Standard, (2) Data, (3) Sample View, (4) Sequence Dye Colors, (5) Batch Control, (6) Sample Plate and (7) Base Sequence. Each icon of these toolbars corresponds to a commonly used menu item. By default, all toolbars (except Sample Plate and Base Sequence) are displayed when the Sequence Analysis module is opened. The tables below describe the functions of each of the toolbar icons.

Figure 40: Sequence Analysis Module Toolbars

Standard Toolbar

(Table 38)

Data Toolbar

(Table 39)

Sample View Toolbar

(Table 40)

Sequence Dye Colors Toolbar

A: ■ C: ■ G: ■ T: ■ N: ☐

(Table 41)

Batch Control Toolbar

(Table 42)

Sample Plate Toolbar
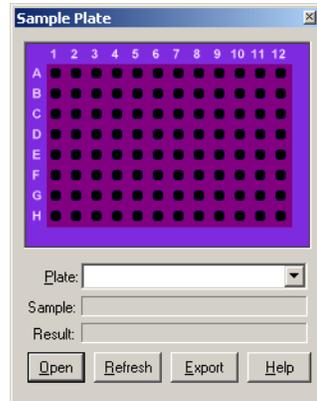
(Figure 46)

Base Sequence Toolbar
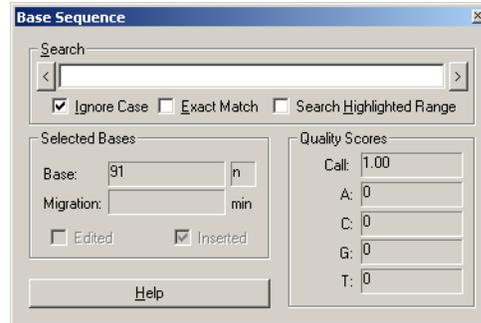
(Figure 47)

Standard Toolbar

Figure 41: Sequence Analysis Module, Standard Toolbar
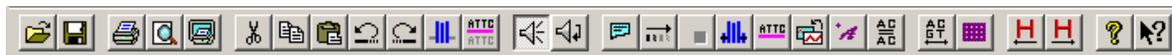
Table 38: Sequence Analysis Module, Standard Toolbar

Icon	Description
	Open - Used to open: Sample Data, Sequence Analysis Parameters, Optical Scan Data, Sequence Results and Sample Plate Results.
	Save - Saves existing data.
	Print - Used to print the report.
	Print Preview - Used to display a facsimile of a hardcopy printout of the selected sample plate.
	Print Desktop - Used to print the desktop, application or main window as defined in the **Preferences** dialog box.
	Cut - Used to cut selected bases.
	Copy -Used to duplicate selected bases.
	Paste - Used to insert one or more copied or cut bases.
	Undo - Used to undo the last action performed.
	Redo - Used to redo the last undo action.
	Apply Quality-based Trimming - Use this option to commit to the Quality-based trimming.
	Apply Sequence-based Trimming - Use this option to commit to the Sequence-based trimming.
	Audio Enable - Toggles between enabling or disabling audio. When enabled, as a base letter is typed, in **Edit** mode, the letter is announced audibly.
	Audio Playback - Used to audibly announce a series of selected base sequence text.
	Working Analysis Parameters - Used to view and/or change the analysis parameters to be used for subsequent analyses.
	Analyze - Used to analyze active sample data or reanalyze existing sequence results. When selected, the current Working Parameters dialog box is displayed. The analysis begins when **OK** is pressed.

Icon	Description
■	***Stop*** - Terminates the executing analysis.
	Trim Based on Quality - Use this option to perform a Trim based on Quality.
ATTC	***Trim Based on Sequence*** - Use this option to perform a Trim based on a Sequence.
	Restore Original Base Sequence - Used to return analyzed data display and base sequence text back to its original state (losing all edits).
	Third Party Analysis - Used to launch the third party analysis package specified in the **Third Party Analysis Setup** dialog box.
	Align - Used to perform an alignment of the sequence against the current alignment settings.
	Base Sequence Toolbar - Used to view the base sequence toolbar that allows you to search for text and ambiguity codes.
	View Sample Plate Toolbar - Used to view the sample plate toolbar that allows you to select which samples you wish to open or export.
H	***Heterozygote Backward Search*** - Used to search backwards for heterozygotes in the base sequence text pane.
H	***Heterozygote Forward Search*** - Used to search forward for heterozygotes in the base sequence text pane.
?	***Help Topics*** - Used to select and print specific topics in the Help file and/or search for information by: topic, index entry and/or keyword.
?	***Context-Sensitive Help*** - Used to open the Help file related to a specific menu option.

Data Toolbar

Figure 42: Sequence Analysis Module, Data Toolbar

Table 39: Sequence Analysis Module, Data Toolbar

Icon	Description
	Zoom Mode - Used to magnify a specific area of data in the display.
	Pan Mode - Used to move analyzed data in the X and Y direction.
	Align Mode - Used to visually align bases and peaks in the analyzed data pane.
	Edit Mode - Used to insert, change and/or delete bases.
	Unzoom - Used to undo one zoom level.
	Unzoom All - Used to undo all zoom levels.
	Autoscale - Used to scale data to the confines of the pane. If autoscaling is disabled, the data is scaled to its true values. Use this item to turn autoscaling on or off.
	Base Spacing - Used to 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, synchronizes 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 - Toggles the base sequence text between displaying on the top or bottom in the analyzed data pane.
	Compare Synch - Used to synchronize scaling, zooming and panning of two analyzed data sets while in the **Compare** mode.
	Align - Used to align selected points of each of the displayed analyzed data sets while in the **Compare** mode.
	Display Options -Invokes the **Display Options** dialog box.

Sample View Toolbar

Figure 43: Sequence Analysis Module, Sample View Toolbar

Table 40: Sequence Analysis Module, Sample View Toolbar

Icon	Description
	Analyzed Data - Displays the data that has been analyzed for the active sample.
	Base Sequence - Displays a text view of the bases from the analyzed data for the active sample.
	Raw Data - Displays the raw data for the active sample.
	Current - Displays the current data from the capillary arrays for the active sample.
	Voltage - Displays the voltage data from the instrument for the active sample.
	Compare Data - Used to compare the active analyzed data set against another analyzed data set.
	Quality Parameters - Used to view a graphical representation of the Quality Values for the active sample.

Dye Colors Toolbar

Figure 44: Sequence Analysis Module, Dye Colors Toolbar

Table 41: Sequence Analysis Module, Dye Colors Toolbar

Icon	Description
A: (red)	The color red is the default color assigned to the Adenine (**A:**) nucleotide bases in the Analyzed Data pane.
C: (black)	Black is the default indicator assigned to the Cytosine (**C:**) nucleotide bases in the Analyzed Data pane.
G: (green)	The color green is the default color assigned to the Guanine (**G:**) nucleotide bases in the Analyzed Data pane.
T: (blue)	The color blue is the default color assigned to the Thymine (**T:**) nucleotide bases in the Analyzed Data pane.
N: (gray)	The color gray is the default color assigned to ambiguous nucleotide bases in the Analyzed Data pane.

Batch Control Toolbar

Figure 45: Sequence Analysis Module, Batch Control Toolbar

Table 42: Sequence Analysis Module, Batch Control Toolbar

Icon	Description
	Batch Analysis - Used to analyze multiple Sample Data or Sample Plate Results sequentially.
	Batch Alignment - Used to select sequence results or sample plate results to align.
	Reanalyze Batch - After performing a batch analysis, use this option to perform a new analysis on the same data set.
	Batch Trimming - Used to Trim multiple Sample Data or Sample Plate Results sequentially.
	Stop Analysis - Terminates the executing analysis.
	Pause - Used to momentarily stop a batch analysis after the currently running sample has been analyzed. Use **Resume Batch Processing** to continue the analysis.
	Resume - Used to resume a batch analysis after it has been paused.
	Skip Sample - While performing a batch analysis, use this item to pass over the sample currently being analyzed. (This item is not available if **Sample Plate Results** was selected from the **Batch Analysis Selection** dialog box.)
	Skip Set - While performing a batch analysis, use this item to pass over the sample set currently being analyzed. (This item is not available if **Sample Data** was selected from the **Batch Analysis Selection** dialog box.)
	Skip Plate - While performing a batch analysis, use this item to pass over the sample plate currently being analyzed. (This item is not available if **Sample Data** was selected from the **Batch Analysis Selection** dialog box.)
	View Results - After performing a batch analysis, use this item to view the results of a selected sample in the upper pane of the **Batch Analysis** window.

Sample Plate Toolbar

Figure 46: Sequence Analysis Module, Sample Plate Toolbar
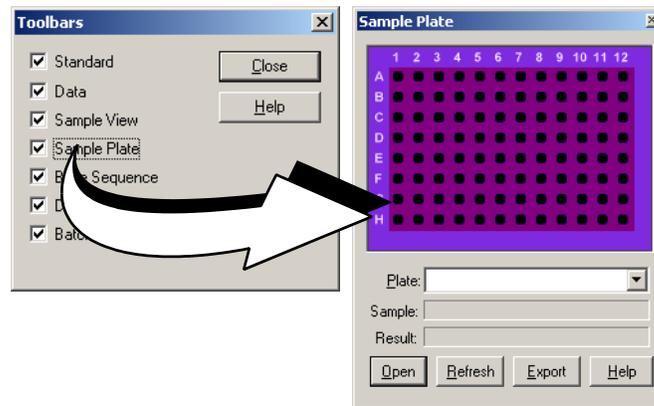
Accessed from the **View | Toolbars** menu option or the ▦ icon on the Standard toolbar.

Used to specify the samples to open or export. Select the desired plate from the drop-down menu. Valid samples appear as filled wells. Wells not specified as samples appear dark, or empty. Click on 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 are displayed in the **Sample** and **Result** text boxes.

> **Note** *To select contiguous samples, click on the first sample in the series, hold down the* **Shift** *key and then click on the last sample in the series. The selected samples are highlighted.*

Base Sequence Toolbar

Figure 47: Sequence Analysis Module, Base Sequence Toolbar

Accessed from the **View | Toolbars** menu option or the [icon] icon on the Standard toolbar.

Use this dialog box to search for specific base sequences and to view quality parameters. Enter the desired string of bases and mouse 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 below:

Character	Description
|	The alternation character (|) 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 + sign in the expression.
?	The question mark (?) matches the character to its left 0 or 1 times.
[ ]	Brackets ([and]) 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, check the **Ignore Case** checkbox. To search for the exact match for text (i.e., not IUB codes), check the **Exact Match** checkbox. To search for text within a range, highlight the range of interest in the base sequence pane and check the **Search Highlighted Range** checkbox.

The IUB codes to enter when the **Exact Match** check box is unchecked are listed below.

N = G, A, T, or C
V = G, A, or C
B = G, T, or C
H = A, T, or C
D = G, A, or T
K = G or T
S = G or C
W = A or T
M = A or C
Y = C or T
R = A or G

CEQuence Investigator Module

CEQuence Investigator is a module used for comparing sequences to known reference sequences. For each comparison, one or two new sequence results, in either orientation, are used 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.

A number of tools are provided for inspecting and editing the data before the final report is generated. 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 sequence(s) and indicates the locations of the discrepancies. Together, these alignment, inspection, editing and reporting features make CEQuence Investigator an ideal tool for identifying mutations or polymorphisms in DNA.

Figure 48: Main Menu, Sequence Investigator Icon

Main Window

Figure 49 shows the CEQuence Investigator screen layout. This consists of a title bar, menu bar, several toolbars, the display window and the status bar. A description of each screen element is described in Table 43.

Figure 49: CEQuence Investigator Module, Main Window
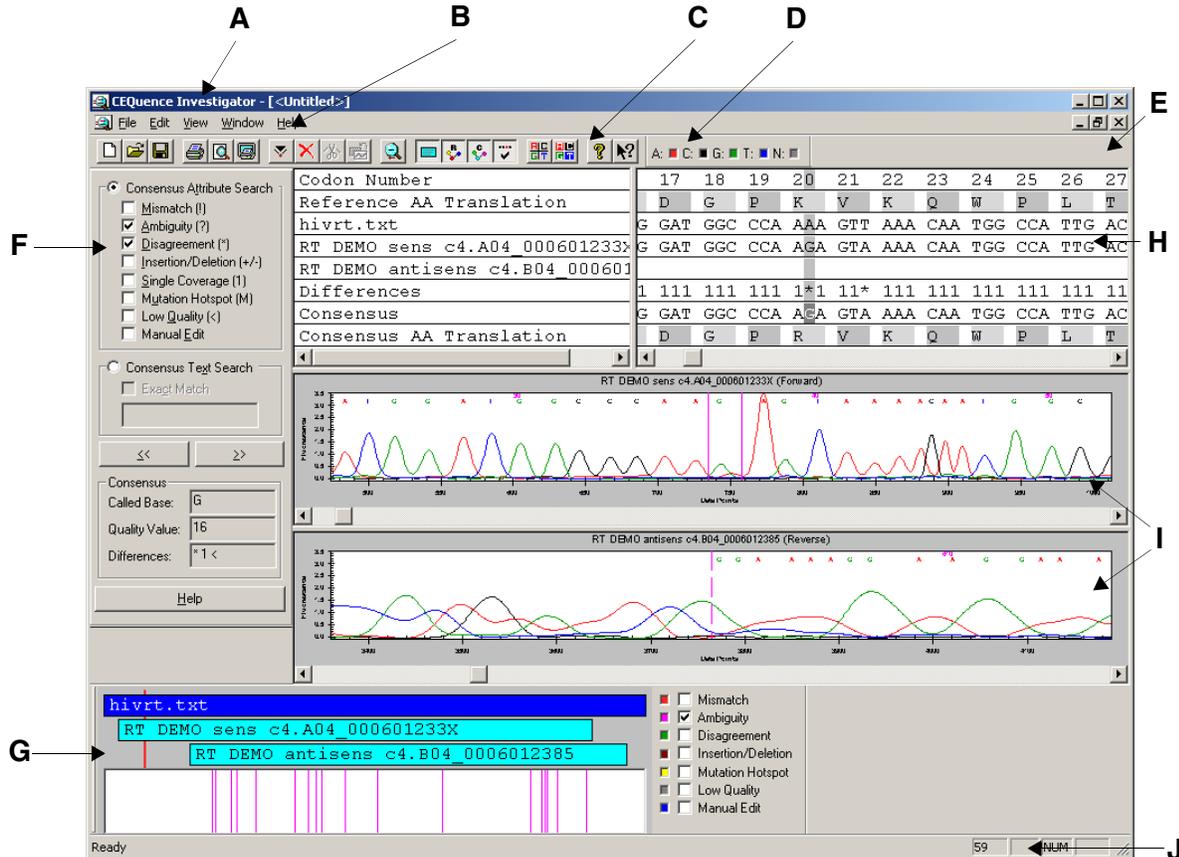
Table 43: Screen Layout and Descriptions

Item	Description
A	*Title Bar* - The Title Bar shows the module name, the active document name and has the standard minimize , maximize and close icons at the top-right of the window.
B	*Menu Bar* - The Menu Bar contains a list of drop-down menu items that display various executable options.
C	*Standard Toolbar* - Each button on the toolbar, when activated, executes a pre-defined function.
D	*Dye Colors Toolbar* - These user-selectable colors are used in the trace data view and the bases in the text view when the text coloring is activated.
E	*Display Window* - This area is used to graphically display the specific window or windows associated with the current task.

Item	Description
F	*Navigator Toolbar* - Offers search and navigation tools within an alignment.
G	*Discrepancy Map Toolbar* - Displays color-coded locations of any type of attributes selected. You may view the coverage of the reference and sample sequence(s).
H	*Alignment Panes* - Displays the text alignment results, including the following elements: Base and codon numbering, reference and consensus amino acid translations, reference sequence, sample sequence(s), consensus sequence and the difference line.
I	*Trace Data Panes* - Displays trace data and base sequence results for the selected sample sequence(s).
J	*Status Bar* - The Status Bar displays information concerning the current selection, including the position of the currently highlighted text.

Menu Bar Options

Table 44: CEQuence Investigator Module, File Menu

Item	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	Opens an existing compared sequence result.
Close	Closes the document.
Save	Saves the active document to a Windows folder.
Save As	Save the current document to a new name and/or folder.
Export	Exports the current document as a text (*.txt) or a protein file (*.pro).
Preferences	Sets the preferences for the CEQuence Investigator.
Properties	Used to view the properties of the current document.
Report Format	Sets the elements that will be printed and adjusts the printer settings.
Print Preview	Displays a preview of the active document as it will be printed as specified in the Report Format dialog box.
Print	Prints the active document as specified in the Report Format dialog box.
Print Desktop	Prints the active desktop area as specified in the Preferences dialog box.
Recent Objects	Used as a short cut to open previously opened documents.
Exit	Exits the CEQuence Investigator module.

Table 45: CEQuence Investigator Module, Edit Menu

Item	Description
Insert	Inserts a space before the currently selected position.
Delete	Deletes the selected position.
Replace	Replaces the highlighted position with a selected IUB code.
Trim	Removes contiguous selected bases that contain an end base in the sample sequence base-call text.
Search	Used to search Forward and Backward for the attributes or text specified in the Navigator toolbar.
Restore To Original	Restores the compared sequence result to its original state (all changes will be removed).
Quality Threshold	Changes the quality threshold below which bases in the consensus will be identified as having low quality.
Flip Traces	Moves the sequence trace shown in the top trace pane and exchanges its position with the bottom trace.

Table 46: CEQuence Investigator Module, View Menu
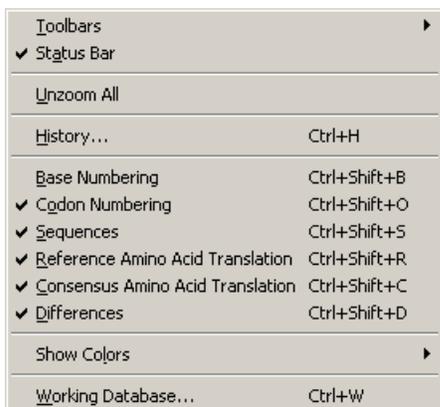
Item	Description
Toolbars	Toggles view/hide each toolbar in a submenu.
Status Bar	Toggles view/hide the status bar.
Unzoom All	Shows each sequence in its entirety.
History	Opens the history log.
Base Numbering	Toggles on/off base numbering.
Codon Numbering	Toggles on/off codon numbering.
Sequences	Toggles view/hide the Sequences.
Reference Amino Acid Translation	Toggles view/hide the Reference Amino Acid Translation.
Consensus Amino Acid Translation	Toggles view/hide the Consensus Amino Acid Translation.
Differences	Toggles on/off the sequence differences.
Show Colors	Toggles on/off text and background color.
Working Database	Displays the active database.

Table 47: CEQuence Investigator Module, Window Menu

Item	Description
Cascade	Cascades the open windows.
Tile Horizontally	Tiles the windows in a horizontal orientation.
Tile Vertically	Tiles the windows in a vertical orientation.
Close All	Closes all open windows.
Arrange Icons	Arranges all icons on the desktop.
Open file list	Displays a list of all open documents.

Table 48: CEQuence Investigator Module, Help Menu

Item	Description
Help Topics	Used to select and print specific topics in the CEQ 2000 Help file and/or search for information by: topic, index entry and/or keyword.
Using Help	Used to select and print specific topics in the *Windows* Help file and/or search for information by: topic, index entry and/or keyword.
About CEQ System	Used to access software, instrument and system information.
Beckman Coulter Home Page	Used to access the Beckman Coulter, Inc. Home Page on the internet.
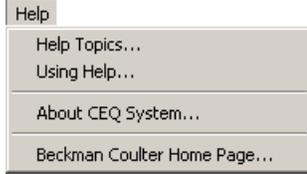
Toolbar Icons

The toolbars provide quick and easy access to commonly used menu items. Each toolbar (Figure 50) contains buttons that correspond to commonly used menu items. Click the icon to execute the function. By default, all the toolbars are displayed when the module is opened. The information in Table 49 and Table 50 describe the function of each of these buttons.

Figure 50: Toolbars

Standard Toolbar

Dye Color Toolbar

Navigator Toolbar

Δισχρεπανχψ Μαπ Τοολβαρ
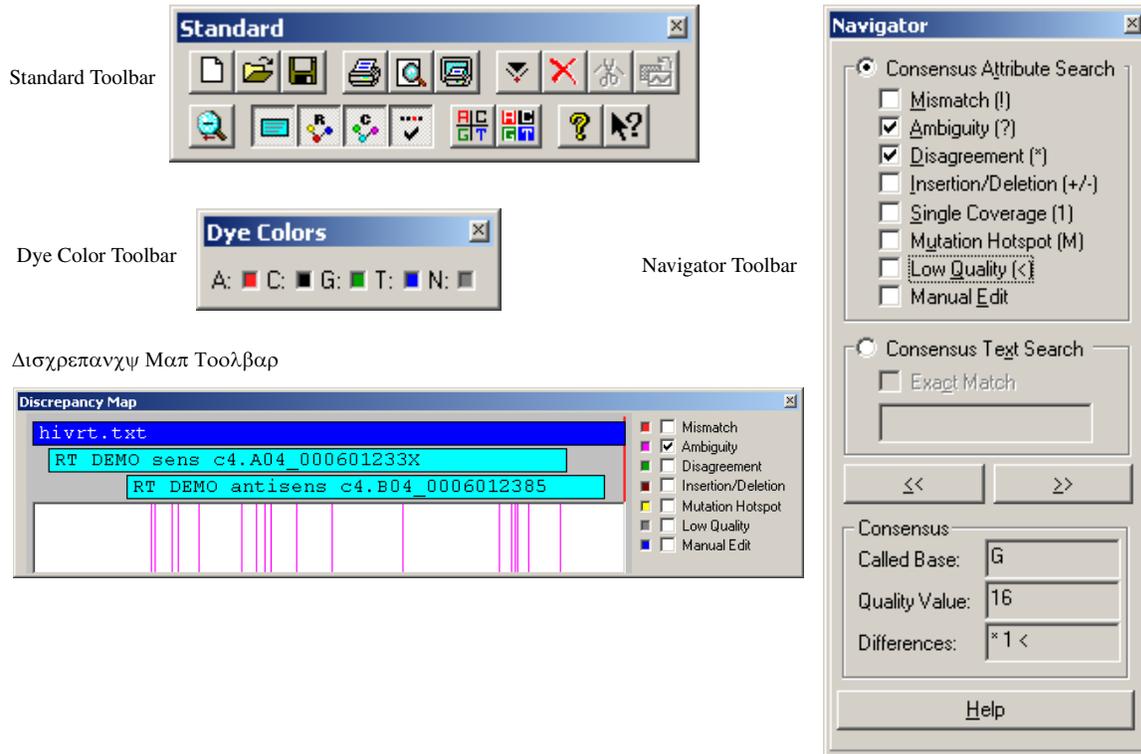
Figure 51: Standard Toolbar Buttons

Table 49: Standard Toolbar Button Description

Button	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 - Opens an existing compared sequence result.
	Save - Saves the active document to a Windows folder.
	Print - Prints the active document as specified in the Report Format dialog box.

Button	Description
	Print Preview - Displays a preview of the active document as it will be printed as specified in the Report Format dialog box.
	Print Desktop - Prints the active desktop area as specified in the Preferences dialog box.
	Insert - Inserts a space before the currently selected position.
	Delete - Deletes the selected position.
	Trim - Removes contiguous selected bases that contain an end base in the sample sequence base-call text.
	Restore To Original - Restores the compared sequence result to its original state (all changes will be removed).
	Unzoom all - Displays both sequence traces in their entirety.
	Toggle Sequences - Displays or hides the sequence(s) in the text view.
	Toggle Reference AA Translation - Displays or hides the reference amino acid translation line in the text view.
	Toggle Consensus AA Translation - Displays or hides the consensus amino acid translation line in the text view.
	Toggle Differences - Displays or hides the differences line in the text view.
	Dye Color Coded Text- Displays base text in the specified dye colors when selected. Displays the base text in black and white when unselected.

Button	Description
	Dye Color Coded Text block - Displays the base text background in the specified dye colors when selected.
	List Help Topics - Opens CEQ System Help contents.
	Display Context Sensitive Help - Click on this button first and then on a menu item, toolbar button, or window area in question for context specific help.

Dye Colors Toolbar

Figure 52: Dye Colors Toolbar

Table 50: Dye Colors

Icon	Description
A (red)	The color red is the default color assigned to the Adenine (**A**) nucleotide in the Data view.
C (black)	The color black is the default color assigned to the Cytosine (**C**) nucleotide in the Data view.
G (green)	The color green is the default color assigned to the Guanine (**G**) nucleotide in the Data view.
T (blue)	The color blue is the default color assigned to the Thymine (**T**) nucleotide in the Data view.
N (gray)	The color gray is the default color assigned to ambiguous nucleotides other than A, C, G or T.

> **Note**
> *Colors can be changed by the user and are persistent. These colors are used in the trace data view and for the bases in the text view, when text coloring is activated.*

Fragment Analysis Module

The Fragment Analysis module processes fragment data from the CEQ platform and provides size and allele information for detected peaks. The results may be viewed, exported and printed. Parameters used to identify alleles are organized as locus tags that can be customized by the user.

Analysis parameter sets provide parameters specific to an experiment, allowing the user to define size calibration and locus tags to be used for processing data. The analysis of data can be performed manually or automatically. The editing and re-analysis functions provide the capability to verify the accuracy of the peaks.

The Fragment Analysis module is used to graphically view, analyze, manipulate, compare and print data of the following types:

• Raw Data*
• Fragment Data
• Fragment Lists
• Information about locus tags and assigned alleles

Also available are Current Data* and Voltage Data*.

* These types of data cannot be manipulated.

Selecting the Fragment Analysis *icon* from the Main Menu (Figure 53) executes this module.

Figure 53: Main Menu, Fragment Analysis Icon
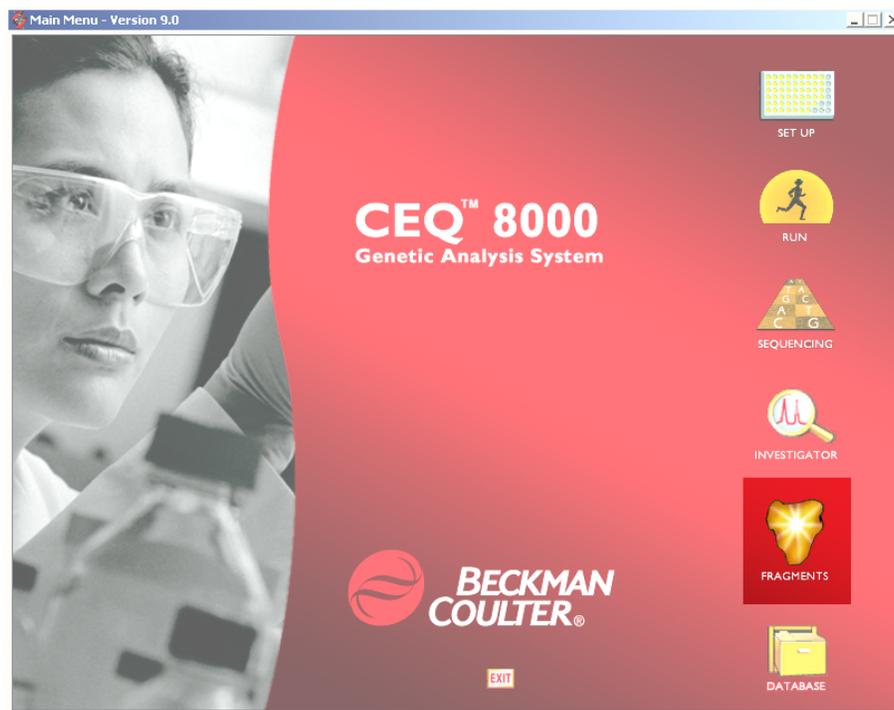
Main Window

The main window of the Fragment Analysis module is shown in Figure 54 and described in Table 51.

Figure 54: Fragment Analysis Module, Main Window
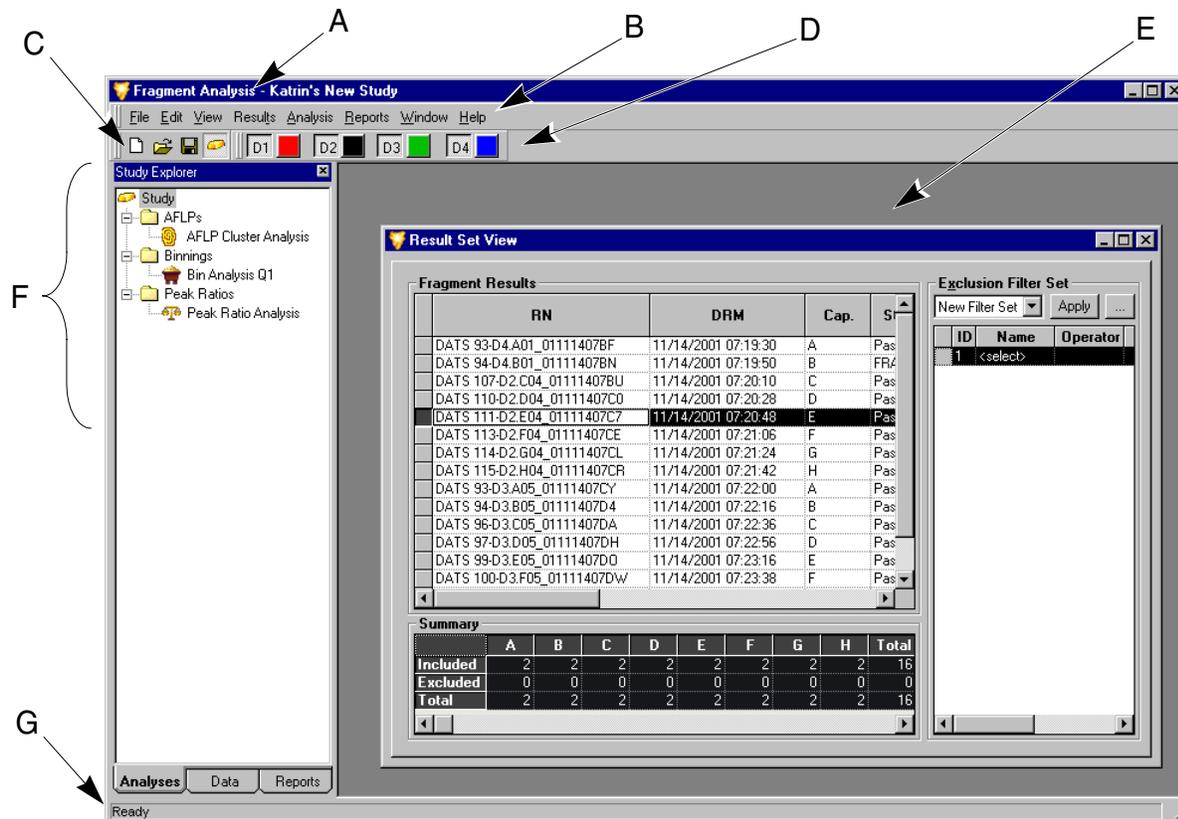
Table 51: Fragment Analysis Module, Main Window Descriptions

Item	Description
A	The *Title Bar* showing the module name (*Fragment Analysis*) and the name of the currently open Study.
B	The *Menu Bar* is a list of the menu options (see *"Menu Bar Options"* on page 99).
C	The *Toolbar* contains the icons that execute pre-defined functions (see *"Toolbar Icons"* on page 104).
D	Clicking on a dye color in the *Dye Colors* box brings up the **Color** dialog box. Change the dye color displayed for that particular dye. Clicking on the dye name (**D1, D2, D3, D4**) will remove that dye trace from all traces.
E	This *Display Area* graphically displays the opened data.
F	The *Study Explorer* frame displays and provides access to all of the parameters available in the selected Study. These are divided into the tabbed sections; *Analyses*, *Data* & *Reports* located at the bottom of the Study Explorer frame.
G	The *Status Bar* displays information concerning the current selection.

Menu Bar Options

The menu bar has the options shown in Figure 55. Tables 52 through 59 provide descriptions of the menu options.

Figure 55: Fragment Analysis Module, Menu Bar Options

File Menu

Table 52: Fragment Analysis Module, File Menu
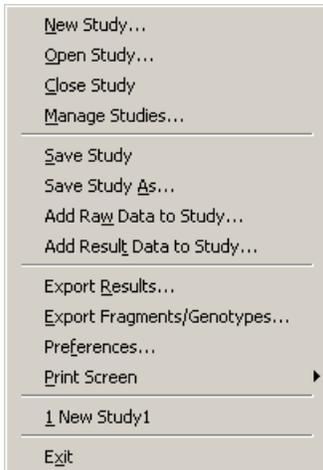
Item	Description
New Study	Used to start a new Study from either raw data or analyzed results.
Open Study	Used to open an existing Study.
Close Study	Closes the active Study
Manage Studies	Opens the Study Manager to view and select all Studies saved to the database.
Save Study	Used to save the new or edited Study.
Save Study As	Used to save the current Study with a new name.
Add Raw Data to Study	Used to select additional data (raw data) to be added to the Study.
Add Result Data to Study	Used to select additional data (previously analyzed result data) to be added to the Study.
Export Results	Used to export data in one of the following formats: • Text (Tab Delimited) (*.txt) • CEQ format (*.cq) • CRV (*.CRV)
Export Fragments / Genotypes	Used to export the fragment / genotype data. • CSV (Comma Delimited) (*.csv) • Linkage (Pedin)(*.pre) • Discovery Manager (DMPopulation)(*.txt) • Genotype Summary Report (Tab Delimited)(*.gsr)

Item	Description
Preferences	Used to: • select for automatic display of the Study window when the Fragment Analysis module is launched. • select to graph all electropherograms by a function of either size or time. • select the number of egrams to be cached. • select the initial view when the Single Result View of a sample is selected. • select to have the system display a warning message before resetting all manually included or excluded bins during an AFLP analaysis. • select to have the system display a warning dialog before reanalysis if old results in the study will be replaced by new results.
Print Screen	Pull-right menu with the option to send an image of the computer desktop, application window or main window to the printer.
Recent Studies	Lists the most recently opened Studies.
Exit	Closes the Fragment Analysis module.

View Menu

Table 53: Fragment Analysis Module, View Menu
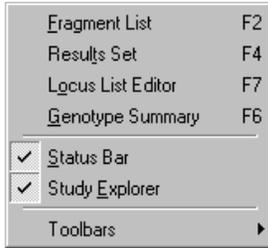
Item	Description
Fragment List	Displays the Fragment List. The Fragment List contains information for all analyzed fragments included in the Study, such as size, height, area, locus and allele ID.
Results Set	Displays the Results Set View. The Result Set contains information for all of the analyzed samples included in the Study.
Locus List Editor	Opens a dialog that helps create the LocusList.txt file used for the Genotype Summary View (GSV).
Genotype Summary	The Genotype Summary View grid displays a study's genotype information (loci and alleles).
Status Bar	Toggles between displaying or not displaying the Status Bar.
Study Explorer	Displays the Study Explorer. The Study Explorer displays and provides access to all of the parameters available in the selected Study.
Toolbars	Used to select which toolbars to display.
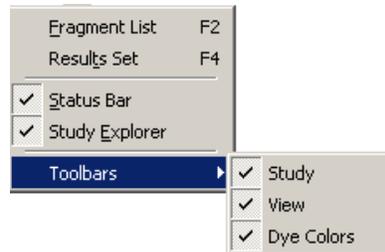
Results Menu

Available when the Result Set View is displayed

Table 54: Fragment Analysis Module, Results Menu

Item	Description
Show Excluded	Used to show fragment results that were excluded from the Fragment Results Grid.
Show Capillary Summary	Displays a table that identifies the number of samples that are included and excluded in the Result Set within each capillary.

Fragments Menu

Available when the Fragment List is displayed

Table 55: Fragment Analysis Module, Fragments Menu

Item	Description
Show Excluded	Used to show fragments that were excluded from the Fragment List.
Manually Add Peaks	Provides an option to manually select peaks to be included in the fragment list.
Save Fragment Grid As CSV	Used to save the Fragment Grid as a Comma Delimited File (*.csv)

Analysis Menu

Table 56: Fragment Analysis Module, Analysis Menus

Item	Description
Reanalyze Results	Presents the options to reanalyze the results: • from sample data (opens the Analysis Parameters dialog) • using new parameters (opens the Analysis Parameters dialog) • using additional/edited locus tags (opens the Reanalyze Results dialog)
Analysis Parameters	Used to: • view the current working analysis parameters. • edit the working analysis parameters (maintained in memory for the duration of the application session). • select a previously saved parameter set to be used in the analysis.
New AFLP Analysis	Used to begin a new AFLP Analysis.
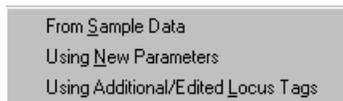
Item	Description
New Binning Analysis	Used to begin a new Binning Analysis.
New Peak Ratio Analysis	Used to begin a new Peak Ratio Analysis.
Run LOH Analysis	Used to run a Loss of Heterozygosity Analysis

Reports Menu

Table 57: Fragment Analysis Module, Reports Menu

Item	Description
New Report	Opens the New Report dialog box. Used to select a template and context type for the report.
Refresh	Refreshes the display of the selected report.

Window Menu

Table 58: Fragment Analysis Module, Window Menu
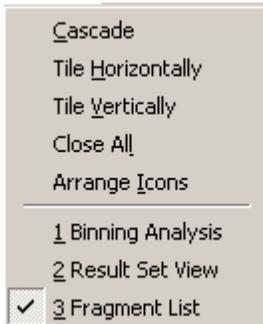
Item	Description
Cascade	Cascades the open windows.
Tile Horizontally	Tiles the windows in a horizontal orientation.
Tile Vertically	Tiles the windows in a vertical orientation.
Close All	Closes all currently active windows.
Arrange Icons	Automatically arranges the icons.

Help Menu

Table 59: Fragment Analysis Module, Help Menu

Item	Description
Help Topics	Used to select and print specific topics in the Help file and/or search for information by: topic, index entry and/or keyword.
Using Help	Used to select and print specific topics in the *Windows* Help file and search for information by: topic, index entry and/or keyword.
About CEQ System	Used to access software, instrument and system information.
Beckman Coulter Homepage	Used to access the Beckman Coulter Homepage on the Internet.

Toolbar Icons

While most of the features of the Fragment Analysis module are readily available through the menu selections, there are three toolbars available with buttons for some of the most commonly used commands.

By default, both toolbars are displayed when the Fragment Analysis module is opened. The tables below describe the functions of each of the toolbar icons.

Figure 56: Fragment Analysis Module, Toolbars

Study Toolbar

Dye Colors Toolbar

View Toolbar
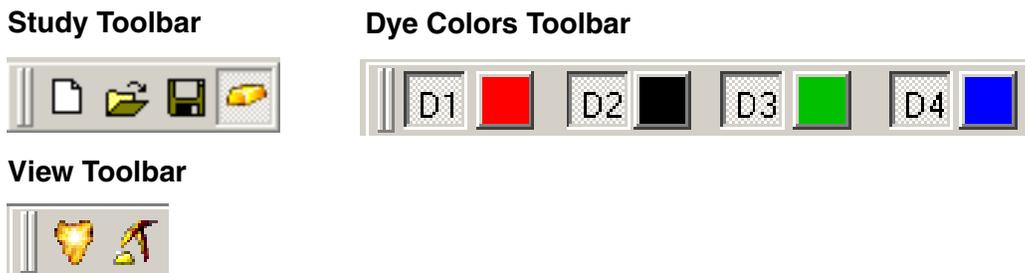
Table 60: Fragment Analysis Module, Toolbars

Icon	Description
	Used to create a new Study from either raw data or result data.
	Open - Used to open an existing or recent Study.
	Save - Saves the new or edited Study.
	Study Explorer - Used to display the Study Explorer.
	Fragment List - Used to open the Fragment List.
	Result Set - Used to open the Result Set View.
D1 (red)	The color red is the default color assigned to Dye #1 in the Fragment Data pane, and **D1** is the default name.
D2 (black)	The color black is the default color assigned to Dye #2 in the Fragment Data pane, and **D2** is the default name.
D3 (green)	The color green is the default color assigned to Dye #3 in the Fragment Data pane, and **D3** is the default name.
D4 (blue)	The color blue is the default color assigned to Dye #4 in the Fragment Data pane, and **D4** is the default name.

Data Manager Module

The Data Manager module is used to create, save and modify databases containing:

- Filter Sets
- Fragment Analysis Parameters
- Fragment Results
- STR Locus Tags
- Methods
- Optical Scan Data
- Sample Data
- Sample Plates
- Sample Plate Results
- Sequence Analysis Parameters
- Sequence Results
- SNP Locus Tags
- Standards

Selecting the Data Manager *icon* from the Main Menu (Figure 57) executes this module.

Figure 57: Main Menu, Data Manager Icon

Main Window

The main window of the Data Manager module is shown in Figure 58 and described in Table 61.

Figure 58: Data Manager Module, Main Window
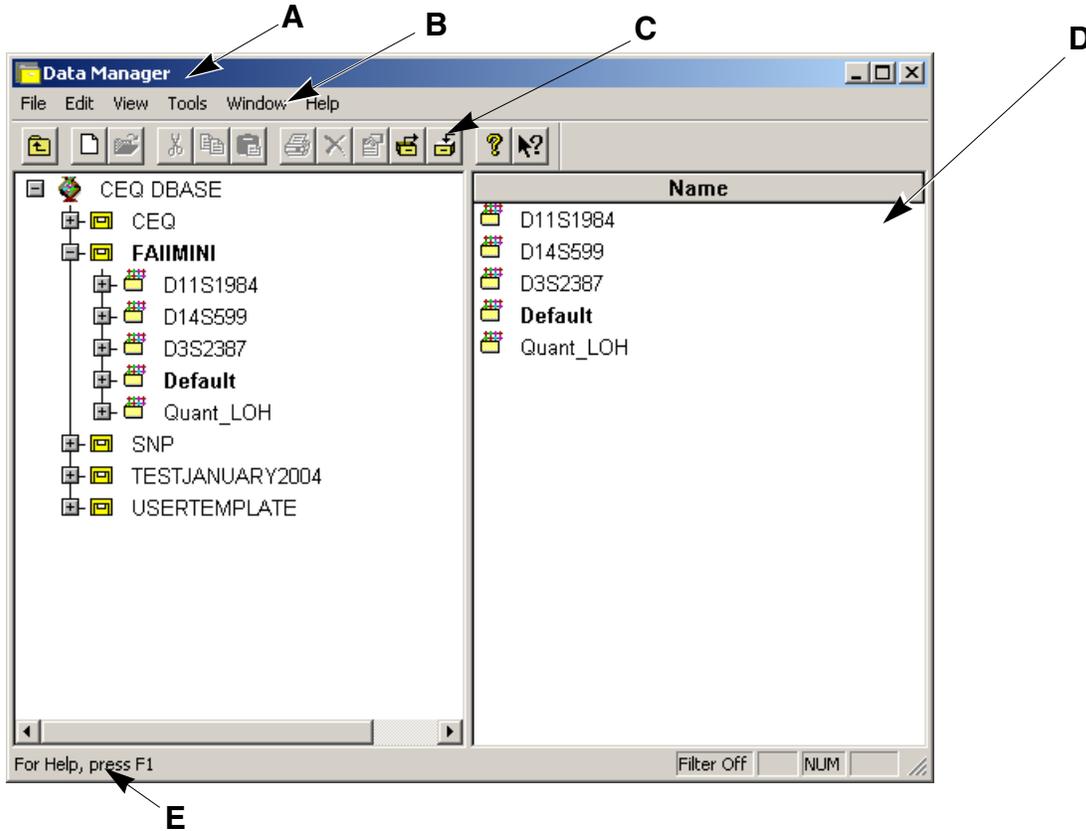
Table 61: Data Manager Module, Main Window Descriptions

Item	Description
A	The *Title Bar* showing the module name (*CEQ Data Manager*).
B	The *Menu Bar* is a list of the menu options (see *"Menu Bar Options"* on page 109).
C	The *Toolbar* contains the icons that execute pre-defined functions (see *"Toolbar Icons"* on page 113).
D	The *Display Area* lists the files contained in the selected node.
E	The *Status Bar* displays information concerning the current selection.

Menu Bar Options

The menu bar has the options shown in Figure 59. Tables 62 through 67 provide descriptions of these menu options.

Figure 59: Data Manager Module, Menu Bar Items

File Edit View Tools Window Help

File Menu

Table 62: Data Manager Module, File Menu

Item	Description
New	Used to create a new Project or Standard in a database.
New Database	Used to create a new database.
Open	Used to open and/or edit Fragment Results, Optical Scan Data, Sample Data, Sequence Results, Sample Plate Results or Standards.
Print	Used to print a report of the selected item.
Print Setup	Used to define printer properties.
Print Screen	Pull-right menu with the option to send an image of the computer desktop or application window to the printer.
Delete	Used to delete items, projects and databases. **Note** *The working database cannot be deleted!*
Rename	This item is used to rename items, projects and databases.
Properties	Displays the current database and modification date as well as providing other information concerning the selected item.
Import	Used to import a file in one of the following formats: • Standard Chromatogram Format v2.10 and v3.00 (*.scf) • ESD (*.ESD) • CEQ (*.cq*) A project must be selected to import items.

File
New   Ctrl+N
New Database...   Ctrl+B
Open...   Ctrl+O
Print...   Ctrl+P
Print Setup...
Print Screen   ▶
Delete
Rename
Properties...
Import...
Export...
Set As Working Database
Exit

Item	Description
Export	Used to export a file in one of the following formats: • Standard Chromatogram Format v2.10 and v3.00 (*.scf) • CEQ (*.cq*) • ESD (*.esd) • Tab Delimited ASCII Text (*.txt) • SEQ (*.seq) • FASTA (*.fasta) • PHRED (*.phd.1)
Set as Working Database	Defines the currently selected database as the default database.
Exit	Closes the Data Manager module.

Edit Menu

Table 63: Data Manager Module, Edit Menu

Edit
Cut	Ctrl+X
Copy	Ctrl+C
Paste	Ctrl+V
Find...	Ctrl+F
Find Next	F3
Select All	Ctrl+A

Item	Description
Cut	Used to delete an item.
Copy	Used to duplicate an item, project or database.
Paste	Used to insert one or more copied or cut items, projects or databases.
Find	Used to locate a specific item, project or database.
Find Next	Searches for the next occurrence of the item or folder defined in the **Find** dialog box.
Select All	Selects all items under the highlighted node.

View Menu

Table 64: Data Manager Module, View Menu

Item	Description
Toolbar	Toggles between displaying or not displaying the toolbar.
Status Bar	Toggles between displaying or not displaying the Status Bar.
Refresh	Rebuilds the window display to reflect the most recent changes.
Filter By	Used to filter the list (in the right-hand side window) by the dates modified.
Database	Displays all items in the selected database.
Sample Run History	This dialog box is used to display a list of items that were run in a project during specified dates.

Tools Menu
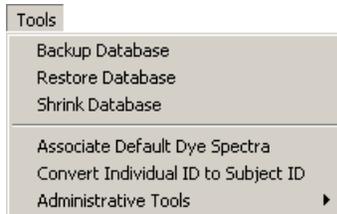
Table 65: Data Manager Module, Tools Menu

Item	Description
Backup	Used to make a backup copy of the selected database.
Restore	Used to restore a previously backed up copy of a database to the Data Manager.
Shrink Database	Use this menu option to reduce the size of the currently selected database.
Associate Default Dye Spectra	Associates previously saved data with a new default dye spectra.
Convert Individual ID to Subject ID	This menu option (Figure 20) is used to convert all sample data records that use Individual IDs to records that use Subject IDs. This applies only to the working database only and is active only when the working database is selected. All other modules must be closed prior to the start of conversion.
Administrative Tools	**Add CeqUsers Group to SQL Login** Select this menu item to add the "CEQUSERS" to the SQL login. *Only members of the "CEQUSERS" group are given access the CEQ databases.*

Window Menu

Table 66: Data Manager Module, Window Menu

Item	Description
New Window	Opens a new window.
Cascade	Cascades the open windows.
Tile	Tiles the windows in a horizontal orientation.
Arrange Icons	Automatically arranges the icons.
Close	Closes the currently active window.

Help Menu
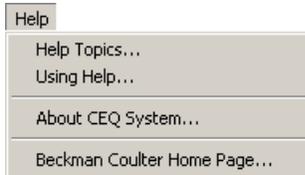
Table 67: Data Manager Module, Help Menu

Item	Description
Help Topics	Used to select and print specific topics in the Help file and/or search for information by: topic, index entry and/or keyword.
Using Help	Used to select and print specific topics in the *Windows* Help file and search for information by: topic, index entry and/or keyword.
About CEQ System	Used to access software, instrument and system information.
Beckman Coulter Home Page	Used to access the Beckman Coulter Home Page on the Internet.

Toolbar Icons

There is one toolbar (Figure 60) used in the Data Manager module. Each icon of this toolbar corresponds to a common menu item. Table 68 describes the function of each of the toolbar icons.

Figure 60: Data Manager Module, Toolbars

Table 68: Data Manager Module, Toolbar

Icon	Description
	Up One Level **-** Moves the directory structure tree up one level.
	New **-** Used to create a new project or database.
	Open **-** Used to open and/or edit Standards.
	Cut **-** Used to delete items, projects and databases.
	Copy **-** Used to duplicate an item, project or database.
	Paste **-** Used to insert one or more copied or cut items, projects or databases.
	Print **-** Used to print a report of the selected item.
	Delete **-** Used to delete items, projects and databases.
	Properties **-** Displays the current database and modification date and other information concerning the selected data file.
	Backup Database **-** Creates a backup of the database.
	Restore **-** Used to restore the database with the backup.
	Help Topics - Used to select and print specific topics in the Help file and search for information by: topic, index entry and/or keyword.
	Context-Sensitive Help - Used to open the Help file related to a specific menu option.

Operating the System

This section contains step-by-step procedures for common tasks necessary to prepare and use the system on a daily basis. It also provides procedures to perform the tasks involved in analyzing/managing samples and sample data.

Set-Up & Procedures

Although the following procedures are independent of the set-up and quick-start of the system, perform the procedures below in the sequence provided.

Preparing Sample

1. Prepare sample for sequence or fragment analysis in accordance with the instructions contained in either the CEQ DTCS or the CEQ Quick Start Chemistry Kits or the PCR Reagent manufacturers kit for Fragment Analysis.

Starting-Up the System

2. Set the PC power switch to the ON position and wait for Windows to start.
3. Set the CEQ instrument power switch to the ON position.
4. On the Desktop, select **Start | Programs | CEQ System | Control Center** (or **double-click** the CEQ System desktop icon). The instrument will initialize and after several seconds the CEQ Main Menu will be displayed.

Creating a Database and Project Folder

Opening the Data Manager Module

5. From the CEQ Main Menu, click on the **DATABASE** icon and verify that the Data Manager window is displayed.

| Note |

The system can be configured so that the CEQ Main Menu: (1) remains in the background at all times (default); (2) collapses to the CEQ System Toolbar when a CEQ System module is launched; (3) collapses to the Windows Taskbar when a CEQ System module is launched. This behavior is controlled by right clicking in the title bar of the main menu or taskbar button and "checking" the desired option. Unchecking all options will prevent the main menu from collapsing.

Creating a Database

User's Database Template

A database template called USERTEMPLATE is added upon installation of the CEQ software. This database is used to store newly created methods and parameters. 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.

The USERTEMPLATE database cannot be set as the Working Database, but it can be deleted. If the USERTEMPLATE database is deleted, no template will be used for subsequent databases. If a new template is required, create a new one named USERTEMPLATE.

6. In the Data Manager window, select **File | New Database**.
7. In the **New Database** dialog box, enter the name for this new database, select **Set as Working Database** (if desired) and select **OK**.

Creating and Naming a Project Folder

8. In the Data Manager window, highlight the desired database where the project will reside and select **File | New**.
9. Highlight the new project and select **File | Rename**.
10. Enter a descriptive name for this project and press **Enter**.
11. Close the Data Manager module (**File | Exit**).

Setting up a Sample

Starting the Sample Setup Module

12. From the CEQ Main Menu, click the **SETUP** icon and verify that the **Sample Plate Selection** dialog is displayed.
13. Select the **Create a new sample plate** radio button and click **OK**.

Setting up the Sample Plate

14. Set up the sample plates by naming the desired cells.
15. Add any associated notes or property information.
16. Select a method from the drop-down menu at the bottom of the sample set.
17. Select the appropriate analysis parameters (as well as report and export parameters, if needed).
18. Enter the barcode in the Barcode field.
19. Select **File | Save As**. Enter a name for the sample plate.

20. Select the project you just created from the **Project Name** drop-down menu and click **OK**.

Running a Sample

Starting the Run Module

21. From the CEQ Main Menu, click on the **Run** icon and verify that the CEQ Run window is displayed.

Installing the Capillary Array

22. Check the capillary life and usage on the life tab and if necessary, perform the procedure "***Removing and Replacing the Capillary Array***" on page 336, and then return here.

Installing a Gel Cartridge

23. Check the gel life and usage on the life tab and if necessary, perform the procedure "***Removing and Replacing a Gel Cartridge/Gel Pump Plug***" on page 343, and then return here.

Installing the Gel Waste Bottle

24. If necessary, perform the procedure "***Replacing the Gel Waste Bottle***" on page 356, and then return here.

Preparing Plates for a Run

25. Load samples into the sample plate and cover with mineral oil.
26. Load buffer (250-300 µL) into the buffer plate.
27. Fill the wetting tray with D.I. water to the level indicator marker.

⚠ **CAUTION** **No more than one 96-well plate should be processed without replenishing the Wetting Tray.**

Periodically check the liquid level in the wetting tray. Liquid level should NEVER be allowed 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.

Setting-Up and Starting the Run

27. Select **Run | Start Sample Plate** from the Run menu.

28. In the **Sample Plate Run Confimation** dialog box (Figure 61), select the desired project and the side (left/right) for each prepared sample plate and click **OK**.

Figure 61: Sample Plate Run Confirmation Dialog
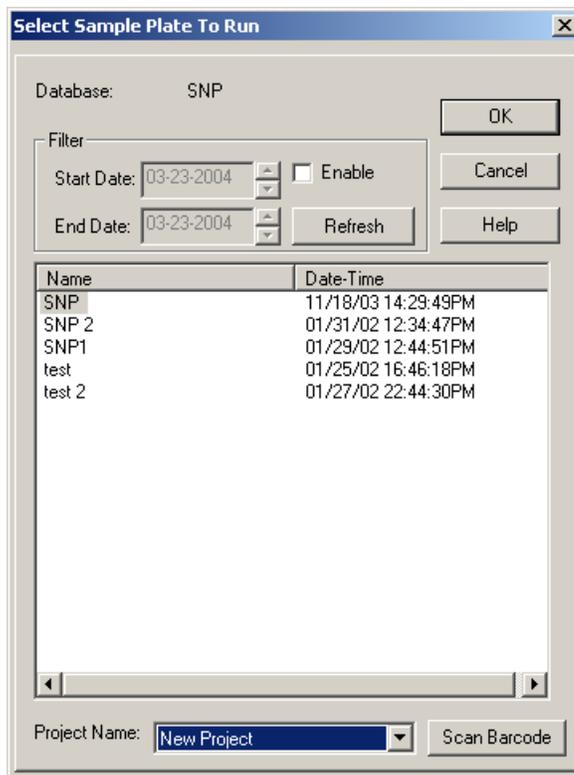
29. In the **Confirm Configuration** dialog box (Figure 62), verify the sample and buffer set locations.

Figure 62: Confirm Configuration Dialog
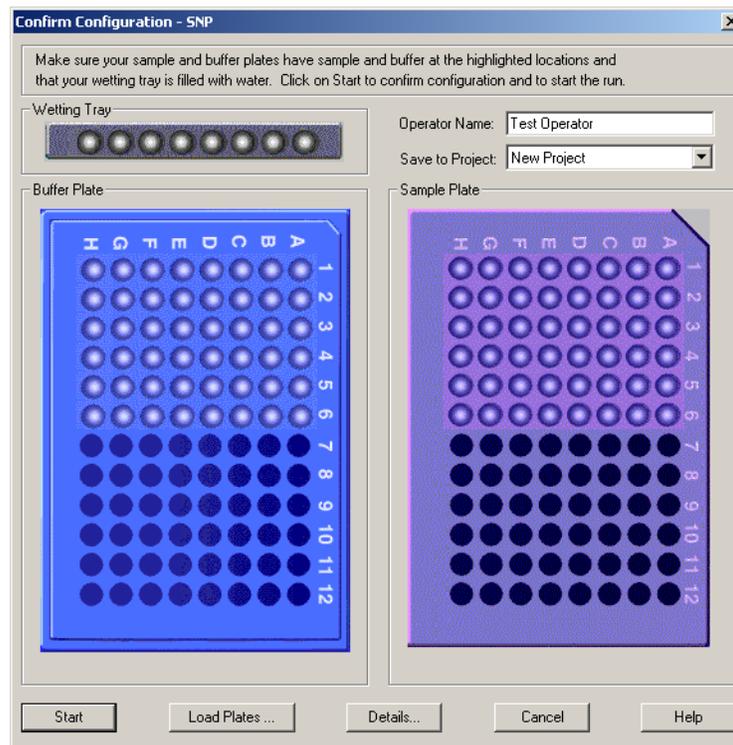
30. Click **Load Plates**. The Access Plates dialog in Figure 63 will appear.

Figure 63: Access Plates Dialog
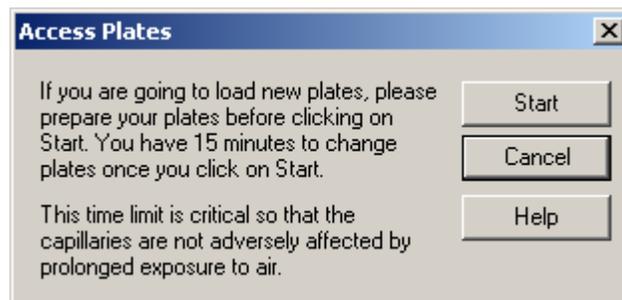
31. Click **Start** to continue.

32. Install the plate(s) and click the **Plate Loaded** check box (Figure 64) for each side, if 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 minutes to load the plates.*

Figure 64: Capillaries Exposed Dialog
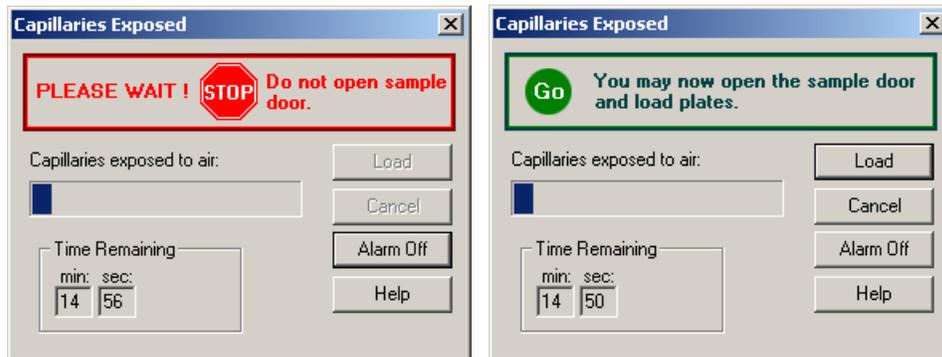
33. Click **Load** to continue.

> **Note** *Refer to "Installing the Wetting Tray' on page 328, "Loading the Sample Plate' on page 330 and "Loading the Buffer Plate and Evaporation Cover' on page 331.*

34. Click **Start** to begin the Run.

Sample Setup Procedures

Open an Existing Sample Plate

From the CEQ Main Menu, click on the **SETUP** icon and verify that the *Sample Plate Selection* dialog is displayed.

To open an existing sample plate:

1. Click the **Open an existing sample plate** radio button
2. Select the sample plate name (described below) and click **OK**.

When the **Open an existing sample plate** radio button is selected, the Sample Plate selection dialog is activated. Available sample plates, for the selected Project, are listed in the window.

> *Note*
>
> *The "DefaultSamplePlate" has been removed from the CEQ Database. If working with an existing Database, "DefaultSamplePlate" may still exist in the Sample Plates list.*
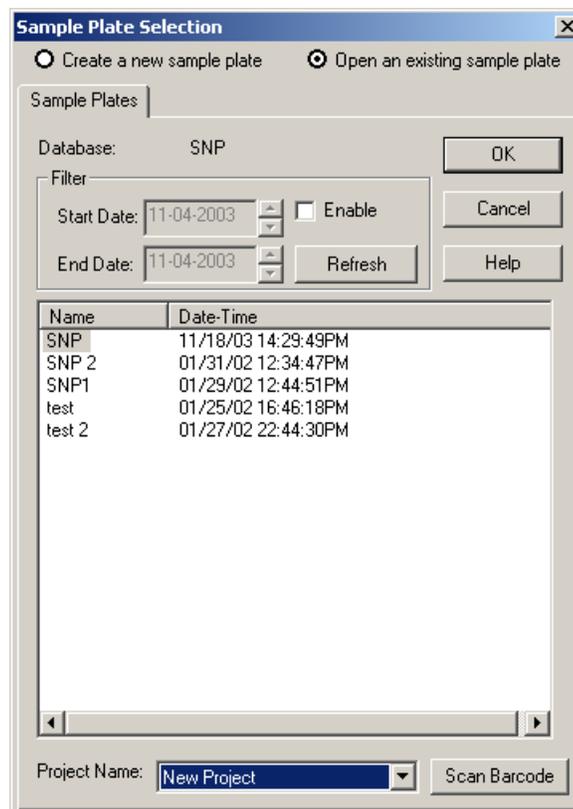
Figure 65: Sample Plate Selection – Open an Existing Sample Plate

Scan Barcode

Use the Scan Barcode option to search for a sample plate using its barcode. From the drop-down list, select the **Project Name** that contains the sample plate barcode. Click **Scan 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 wild cards "*" and "?" in your search. If more than one match is found, the Barcode Selection dialog will be opened to allow selection of the proper plate.

Figure 66: Scan Barcode Dialog

> **Note** *To search all projects for the sample name, select "-(none)-" from the Project Name drop-down before clicking Scan 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, click on the project down-arrow, then on the desired project. The list box will be updated accordingly.*

> **Note** *To change the working database, first close all CEQ System modules, and access the Data Manager module. Set the desired working database by clicking on the database you wish to set as the working database, then clicking on Set as Working Database under the File menu.*

> **Note** *You can also use the right click option to set the working database. Left click on the desired database to highlight it, then right click. Select Set as Working Database from the menu.*

To filter the list, check the **Enable** check box and enter the beginning and ending dates for the items you wish to view. Click on Refresh.

Creating a New Sample Plate

From the CEQ Main Menu, click on the **SETUP** icon and verify that the *Sample Plate Selection* dialog is displayed.

To create a new sample plate:

1. Click on the **Create a new sample plate** radio button.
2. Click **OK**. A blank sample plate will open.
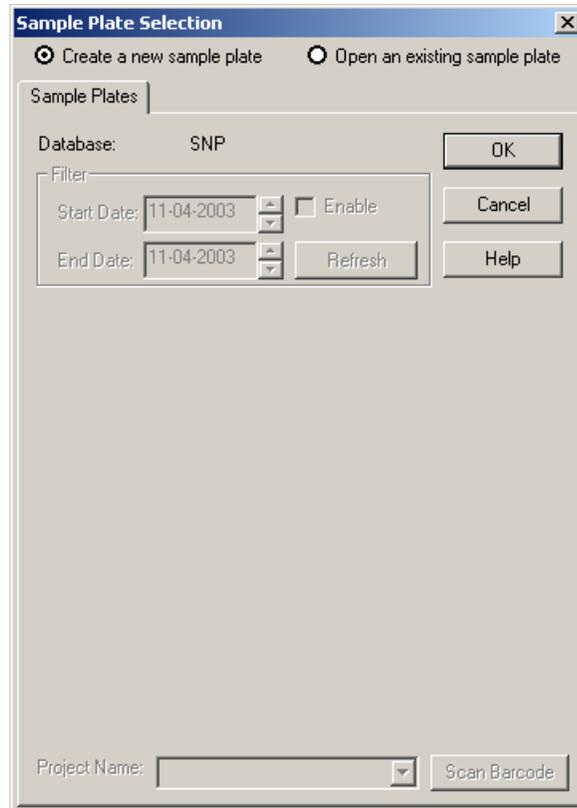
Figure 67: Sample Plate Selection – Create a New Sample Plate

3. Select (highlight) the cell or cells where the sample(s) will reside.
4. Enter the name of the sample(s) in the **Sample Name** text box and then press the **Enter** key.
5. Enter the barcode for the plate in the **Barcode** text box.
6. Apply sample specific information in the **Note** window of the **Note** tab and apply property set and values.

    a. Assign a method to each sample set. To edit the method or create a new method, see "*Creating or Editing a Method*" on page 134, and then return here.

b. To automatically analyze data after a sample set run, click on the **Analysis** tab and then on the **Automatic Analysis** check box in the **Analysis** window. The sample data will be analyzed with the parameter set selected from the drop-down menu. See "Assigning Analysis Parameter Sets" on page 135. and then return here.

c. To automatically print a report after a sample set run, see *"Specifying Sample Plate Print Options"* on page 136, and then return here.

d. To automatically export data after a sample set run, see *"Specifying Sample Plate Export Options"* on page 136, and then return here.

5. Select **File | Save As** from the menu.

6. In the **Save As** dialog box:

a. Select a **Project Name** from the drop-down menu**.**

b. Enter a name for this plate in the name field.

c. Select **OK** to save the plate.

Sample Selection

Individual samples can be selected using the mouse. In addition, the entire plate, row or column can be selected with one mouse click. Using the **Shift** or **Ctrl** keyboard buttons, contiguous and non-contiguous cells can be selected.

Selecting the Entire Sample Plate

Select the entire sample plate by mouse-clicking the grid box on the upper-left corner of the plate grid (see Figure 68). To remove the selection, mouse-click any sample in the grid.
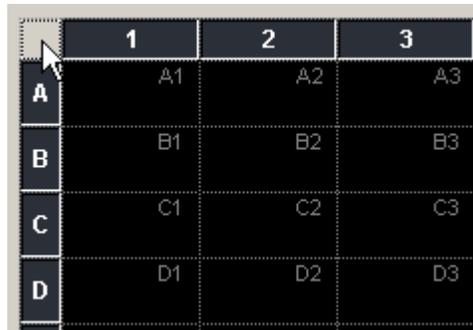
Figure 68: Select the entire plate

Selecting the Entire Row

Place the mouse pointer over the row label on the left-hand side of the row to be selected. A small black arrow will replace the mouse pointer (see Figure 69). Click once to select the row. To remove the selection, mouse-click any sample in the grid.
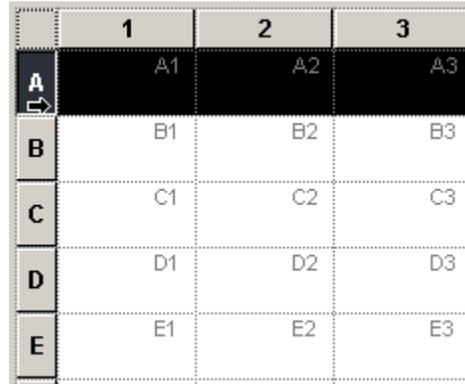
Figure 69: Select the entire row

Selecting the Entire Column

Place the mouse pointer over the column label at the top of the column to be selected. A small black arrow will replace the mouse pointer (see Figure 70). Click once to select the column. To remove the selection, mouse-click any sample in the grid.
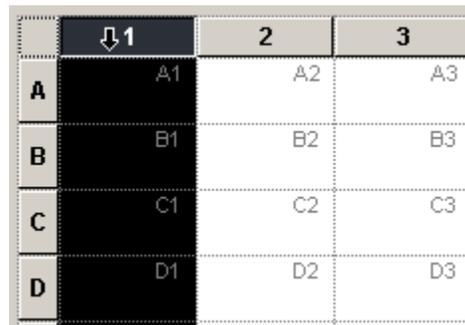
Figure 70: Select the entire column

Selecting Contiguous Cells

Contiguous cells can be selected by following the steps below.

1. Mouse-click on the first cell in the column or row to be selected.
2. Press and hold down the **Shift** key.
3. Mouse-click the last cell (in the column or row) to be selected.

> **Note** *If (using the above steps) cell A1 is selected and then cell D3 is selected, all the cells between A1 and D3 will be selected. (See Figure 71)*

4. Release the **Shift** key.

Figure 71: Select contiguous cells

Select Non-Contiguous Cells

Non-contiguous cells can be selected using the steps below.

1. Mouse-click the first cell to be selected.
2. Press and hold down the **Ctrl** key.
3. Mouse-click all the remaining cells for the selection.
4. Release the **Crtl** key. (See Figure 72)

Figure 72: Select non-contiguous cells

View by Sample Name/Subject ID

This Menu option toggles the sample plate view between Sample Name and Subject ID. It operates in conjunction with the radio buttons found next to the Sample Name and Subject ID fields.
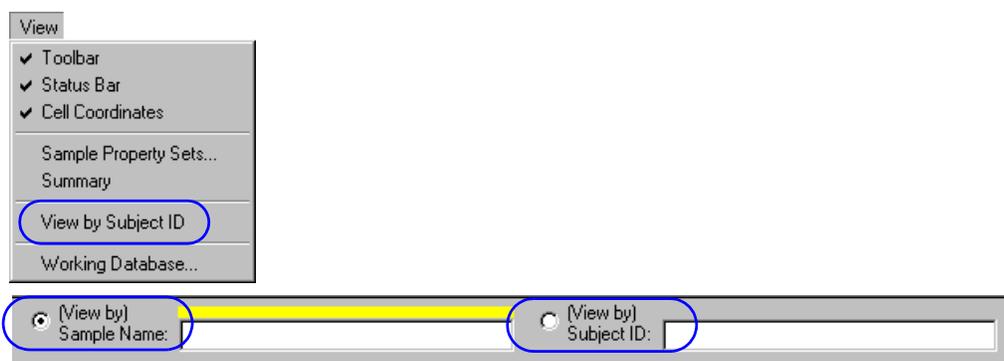
Figure 73: View by toggle

Naming Samples and Assigning Subject IDs

Each Subject ID entered will be appended with its location in the plate. For instance, a sample named John at position A3 in the plate, will be named John.A3.

The sample name field is automatically populated if a subject ID is entered before a sample name. It can be changed at any time.

To name a single sample:

1. Select the cell where the sample resides in the plate.
2. Enter the name of the sample in the **Sample Name** text box and press the **Enter** key.
3. Enter the Subject ID in the **Subject ID** text box and press **Enter**.

To name contiguous samples:

1. Select the cells where the samples reside in the plate by clicking and dragging the mouse cursor.
2. Enter the name of the samples in the **Sample Name** text box and press the **Enter** key.
3. Enter the Subject ID in the **Subject ID** text box and press **Enter**.

To name all wells in the plate:

1. Click the **Select All** icon.
2. Enter the name of the samples in the **Sample Name** text box and press the **Enter** key.
3. Enter the Subject ID in the **Subject ID** text box and press **Enter**.

To name multiple non-contiguous samples that are not within the same sample set:

1. Select the first cell.
2. Hold down the Control key while selecting the additional samples.
3. Enter the name of the samples in the **Sample Name** text box and press the **Enter** key.
4. Enter the Subject ID in the **Subject ID** text box and press **Enter**.

An edited cell, within the plate editor, will turn green. The Plate Status indicator will also display "Plate Edited." This indicates that the plate must be saved. Save the plate to retain the changes. A *Save Changes* dialog will remind you of this if you attempt to close the plate before saving it.

By placing the mouse pointer over a named cell, a "tool tip" will appear showing both the sample name and subject ID.

Turning Cell Coordinates On or Off

To view or hide cell coordinates, select **View | Cell Coordinates** from the menu. (When checked, coordinates are displayed. When unchecked, coordinates are not displayed.)

Using Find

To search for text in the sample plate, select **Edit | Find** and enter the desired text in the **Search what** text field. Click **Find Next** to find the next occurrence of the entered text.

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.

Check **Match whole word only** to locate text that is flanked by spaces. If you do not select "Match whole word only," the system will find the text, even if it is part of another word.

Check **Match case** to find the entered text exactly the way you type it into the field. If you do not select "Match case," the system will find all occurrences of the text, regardless of case.

Check **Use wildcards** to include the following wildcard characters in your search:

Wildcard	Description
"*"	The asterisk is used to denote any letter(s) before or after the entered text.
"?"	The question mark is used to represent any single letter.
"[!]"	The exclamation point within brackets is used to make the query negative. Enter the letter following the "!" within the brackets, and the system will find anything that is not the entered text. For example, [!A] will find any string that does not start with A.

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

Using Replace

To search and replace text in the sample plate, select **Edit | Replace** and enter the desired text in the **Find what** text field. Enter the text you wish to replace with in the **Replace with** text field.
Click **Find Next** to find the next occurrence of the entered 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 replace text.

Check **Match whole word only** to locate and replace text that is flanked by spaces. If you do not select "Match whole word only," the system will find the text, even if it is part of another word.

Check **Match case** to find and replace the entered text exactly the way you type it into the field. If you do not select "Match case," the system will find and replace all occurrences of the text, regardless of case.

Check **Use wildcards** to include the following wildcard characters in your search:

Wildcard	Description
"*"	The asterisk is used to denote any letter(s) before or after the entered text.
"?"	The question mark is used to represent any single letter.
"[!]"	The exclamation point within brackets is used to make the query negative. Enter the letter following the "!" within the brackets, and the system find will find anything that is not the entered text. For example, [!A] will find any string that does not start with A.

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

Using Property Sets

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

In the Property Set Table, use the Tab or arrow keys to move from cell to cell. The Tab key moves the focus from property to value and then to the next row and the arrow keys move in the direction selected. Pressing the Enter key moves the focus down the table one cell at a time, within the same column. Refer to Figure 74 and Figure 75.

Figure 74: Property Set Editor
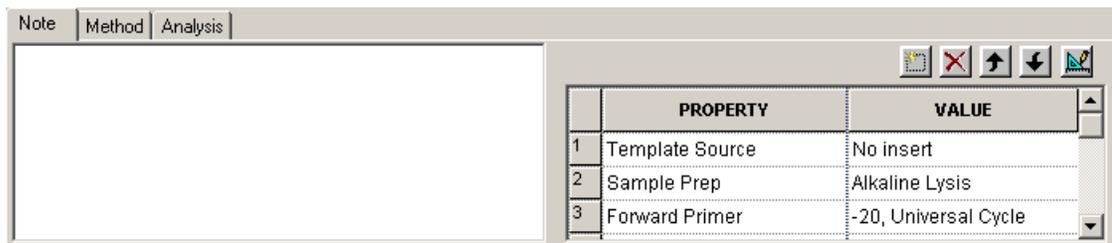
Figure 75: Sample Property Sets Dialog
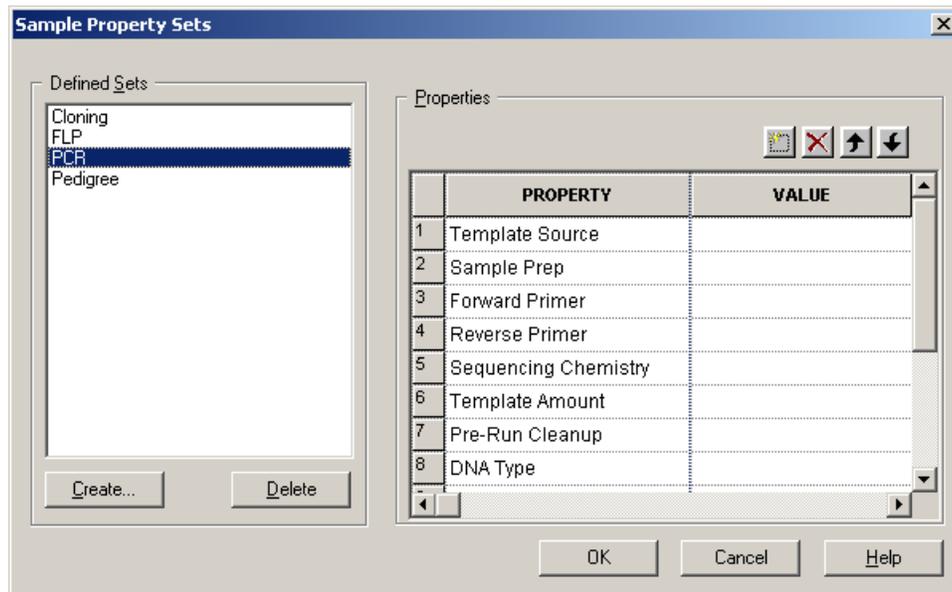
To select an entire column for sorting, click on its header. The column will be highlighted indicating the column is in selection mode.

To select a row, click on its number. As with the column selection, the appearance of the row will be highlighted indicating the row is in selection mode.

To select consecutive rows, drag the mouse through the blocks while holding the left mouse button. The row can be moved up or down one cell at a time by clicking the toolbar arrow ⬆ ⬇ buttons.

To delete the contents of the row(s), select the desired row(s) and click the **Delete** button ☒ . The selected row will be removed from the property set.

In order to prevent accidental deletions, a warning message will appear before allowing the user to delete one or more rows. Press **Yes** to delete the contents or **No** to return to the editor. See Figure 76.

To enter a new property, click 🔲 . A new row will be inserted into the property table. Enter the new property name and value into the cells of the new row.

Figure 76: CEQ Sample Setup Dialog
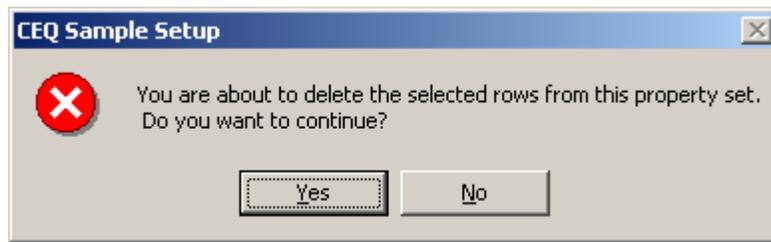
To assign a pre-defined property set, click on the Sample Property Sets button 📈 and select from the defined property sets (Figure 75).

To create and store a custom property set, click the **Create** button 🔲 from the Defined Sets portion of the Sample Property Sets dialog and enter a name in the Create Property Set dialog (Figure 77). Settings from an existing property set can be copied into the new one by using the **Copy settings from** drop-down menu or by manually adding properties using the 📈 button.

Figure 77: Create Property Set Dialog
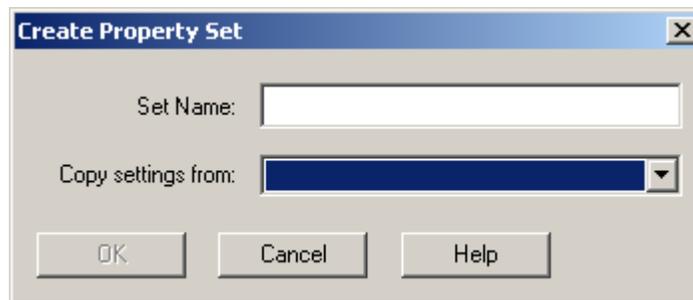
Assigning Methods

The method is the program of events the system uses to collect the data. The method controls the hardware, i.e. the temperatures, voltages and times, which work together to gather data. The system comes with several methods that are optimized for the different software applications. The *Frag* methods are used to collect data for fragment analysis. The *LFR* methods are used to collect sequence data.

To assign a method to one sample set:

After creating a new sample plate, the Method selection for each column is left blank. To assign a method, select a cell in the column with the mouse, then click the down-arrow on the method selection list (see Figure 78) to select the method. Once a method is chosen, the Method tab is activated and the method can be edited.

> **Note** *A method must be assigned to each named row before the sample plate can be saved. If any named row is not assigned a method and a save is attempted, a warning dialog will list each column that must be assigned a method.*
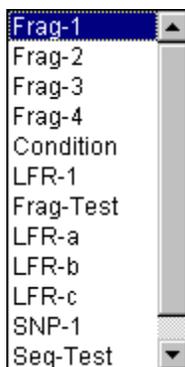
Figure 78: Select Method

To apply a method to multiple sample sets:

1. Highlight the desired sample sets as explained in *"Sample Selection'* on page 124.
2. Select **Edit | Auto-Fill Method Name** from the menu.
3. In the **Choose Method** dialog box, select a method from the drop-down menu.
4. In the **Auto Fill** area of the dialog box, select **Selected sample sets only** and then click **OK**.

To apply a method to all sample sets:

1. Select **Edit | Auto-Fill Method Name** from the menu.
2. In the **Choose Method** dialog box, select a method from the drop-down menu.
3. In the **Auto Fill** area of the dialog box, select **All sample sets** and then click **OK**.

To view the selected method, click the **Method** tab.

Condition Method

The *Condition* method (see Figure 78) should be used before a run if the system has not been used for a long period of time. Performing this method will fill the capillaries with gel 6 times. Then, after separation, thoroughly purge the manifold with fresh gel followed by a capillary fill.

> *Note* *Thoroughly clean the wetting tray with D.I. water before every run to remove old or dried gel.*

Creating or Editing a Method

To create and/or edit a method, perform the following steps.

1. Select **Edit | Method** from the Sample Setup Main menu.
2. Highlight the desired method in the **Choose Method to Edit** dialog box and click **OK**.
3. In the **Method - Capillary Temperature** dialog box:

   a. Enter a temperature between 35 and 60° C.
   b. Select the **Wait for Cap Temp** option (if desired).
   c. Click **OK** to exit the **Method** dialog box or continue with step 4 to make additional changes to the method.

4. Select **Denature** from the **Event** list and then:

   a. Enter a duration between 0 and 180 seconds (if 0 is selected, there will be no denaturation).
   b. Click **OK** to exit the **Method** dialog box or continue with step 5 to make additional changes to the method.

3. Select **Pause** from the **Event** list and then:

   a. Enter a time duration between 0 and 10 minutes.
   b. Click **OK** to exit the **Method** dialog box or continue with step 6 to make additional changes to the method.

3. Select **Inject** from the **Event** list and then:

   a. Enter an injection voltage between 0.1 and 12.0 kV.
   b. Enter a time duration in seconds.
   c. Click **OK** to exit the **Method** dialog box or continue with step 7 to make additional changes to the method.

4. Select **Separate** from the **Event** list, and then:

   a. Enter the separation voltage between 0.1 and 12.0 kV.
   b. Enter a time duration in minutes.
   c. Click **Advanced** to specify the two separation phases for the method. Click **OK** to return to the **Method** dialog box.

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

    d. Click **OK** to exit the **Method** dialog box or continue with step 8 to make additional changes to the method.

5. In the **Save As** dialog box:

    a. Select a **Project Name** from the drop-down menu.
    b. Enter a name in the name field.
    c. Click **OK**.

Assigning Analysis Parameter Sets

Analysis parameter sets define the conditions specific to an experiment used in data processing. *Sequence Analysis Parameter Sets* define the start and end times for the analysis of raw data, the threshold below which bases will be called as 'N's, and the information necessary to detect the start of data to be analyzed: 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 will be considered peaks. *Fragment Analysis Parameter Sets* are used to process the fragment data to estimated fragment sizes and to identify alleles. Parameter sets are created or edited in 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 to be used, assign quantitation values, and define several other factors that will affect how your data will be analyzed.

For sequence analysis, select a Sequence Analysis Parameter Set. For fragment analysis, select a Fragment Analysis Parameter Set.

Assigning a Parameter Set

To perform an analysis on a sample, select the cell/cells as described in *"Sample Selection'* on page 124 and select the **Perform Analysis** check-box, then click the down-arrow on the Parameter Set (see Figure 79) to assign a Parameter Set to each selected sample. Save the sample plate.

Figure 79: Analysis tab

Specifying Sample Plate Print Options

To define the print options and report format of the currently open sample plate, perform the following steps.

1. With a cell highlighted, select the **Analysis** tab at the bottom of the window.
2. Select the **Print Report** check box to print a report immediately after completion of the run.
3. Select the **Edit Print Format For Plate** button.
4. In the **Report Format** dialog box, verify or change the **Printer**, **Page Layout** and **Copies** options.
5. In the **Sample Elements** area of the **Report Format** dialog box, select each element to be printed.
6. If desired, select the **Options** button and verify or change the **Raw Data**, **Sequence Analysis Result Data**, **Fragment Analysis Result Data, Base Sequence Grouping** and **Current Trace Options** selections in the **Print Options** dialog box and then click **OK**.
7. If desired, select the **Colors** button and verify or change the trace colors of the **Raw Data** and/or **Analyzed Data** and then click **OK**.
8. Select **OK** from the **Report Format** dialog box to save the changes and close the dialog box.

Specifying Sample Plate Export Options

Import/Export Sample Plate

Import Sample Plate

Sample plates can be imported from files stored in the tab-delimited text (*.txt) file format. Import a sample plate using the following procedure.

1. Open the *Sample Setup Module*.
2. When presented with the *Sample Plate Selection* dialog, click **Cancel**.
3. From the menu bar, select **File | Import.**
4. In the Import dialog, navigate to the folder containing the file.
5. Select the file.
6. Click **OK**.

Export Sample Plate

The sample plate can be exported for use in compatible equipment or to manually edit the plate information. To export the sample plate follow the steps below.

1. Open the sample plate that will be exported. If it is already open, make sure it has been saved before proceeding.
2. From the menu bar, 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, a suffix to the new file will be added.

> **Note** *If* **Resolve Filename Conflicts** *is not selected and a file with the same name will be overwritten, a warning will appear 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 save location for the file.
5. Enter a filename or leave the name as it is.
6. Click **OK**.

Apply Trimming

Selecting the **Apply Trimming** option will remove the trims from the sequence before it is exported. All internal trims will be replaced by the letter "x".

Export Only If Sequence > X nt

The resulting sequence, after trimming, may be to small to be useful. Use the **Export Only If Sequence > X nt** option to specify a minimum size that the sequence must be when exporting. Only those sequences greater than the set number of nucleotides will be exported

Reports

A report can be printed for individual samples. To do so, select the cell/cells, as described in *"Sample Selection'* on page 124, and select the Print Report check-box. To modify the report format, click the **Edit Print Format For Plate** button and select the appropriate options (see Figure 80). The selections made in the *Report Format* dialog will apply to all selected samples.

Trimming Log

A Trimming Log option has been added to the Report Format dialog (see Figure 80). This log (see Figure 81) details the parameters used in Quality-based and Sequence-based trimming. It includes the original sequence length, the length after quality-based trimming (see *"Quality-based Trimming Tab'* on page 162), the length after sequence-based trimming (see *"Sequence-based Trimming Tab'* on page 163), the vectors trimmed and the base sequence in its original and trimmed forms.

Figure 80: Report Format dialog

Figure 81: Trimming Log

Lock

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

View Summary

A read-only window showing the attributes of the currently selected plate in tabular format is displayed by selecting **View | View Summary**.

Run Procedures

Opening the Run Module

From the CEQ Toolbar, click on the Run icon and verify that the *Run* window is displayed.

Defining System Preferences

To define the system preferences, perform the following steps.

1. Select **File | System Preferences** from the menu.
2. In the **System Preferences** dialog box:

   a. Enter the **System Name** and **Operator Name**.
   b. Select the **Project Name**.
   c. Define **Dye Names** for the Fragment Analysis module, if desired.
   d. Enable or disable the alarms and then select **OK**.

Running a Sample Plate

To run two sample plates that have been created and saved, perform the following steps.

1. Select **Run | Start Sample Plate** from the menu.
2. In the **Sample Plate Run Configuration** dialog, select a **Project Name** from the drop-down for the Left and Right Plates and highlight the desired sample plate.
3. In the **Confirm configuration** dialog box, click **Load Plates**. Load the wetting tray, sample plate and buffer plate by following the instructions in the **Unload** and **Capillaries Exposed** dialog boxes. Click **Load** to finish loading plates.
4. In the **Confirm Configuration** dialog box, verify the sample position in the plate and then select **Start** to initiate the run.

Database Size

Prior to running a sample plate, define the working database in the Data Manager. If a database that is greater than 500MB in size is selected, when a sample plate is created and run, the following warning will appear.

Figure 82: 500 MB Warning Dialog
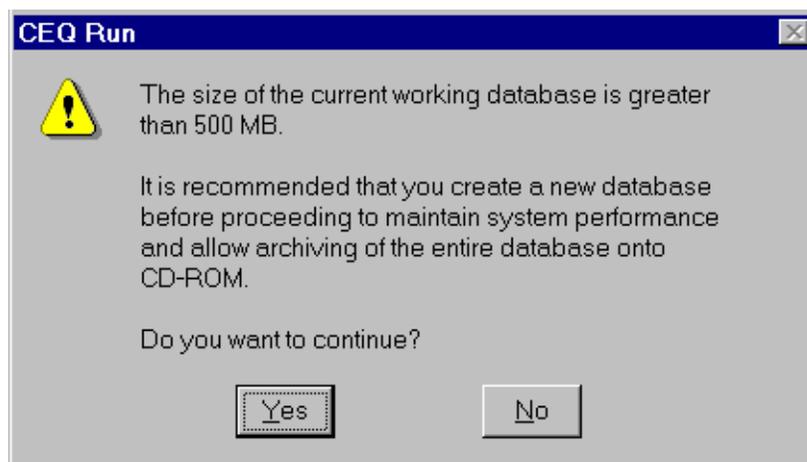
Note*If you do not create and select a new database, the system will use the default database (CEQ.mdb) to store your data.*

If you have created a sample plate in a database that is too large and are presented with the warning, perform the following set of procedures to transfer your sample plate to a new database and process the plate for data storage in a new database.

1. Click **No** to dismiss the warning dialog box.
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 by highlighting CEQDBAS and clicking **New** [ ] button.
6. Enter a name for the database and click **OK**.
7. Select the new database and set it as the Working Database.
8. Copy the sample plate from the previous working database to the new database.
9. Click **OK All** to copy any objects referenced by the sample plate to the new database.
10. Exit the Data Manager module and launch the Run module.
11. Select **Run | Start Sample Plate**.
12. Select the new sample plate and click **OK**.
13. The plate will be processed and the data will be stored in the new database.

Note *It is recommended that you archive databases that are larger than about 500MB.*

Gel Re-estimation Feature

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

You will be notified and the system will perform a gel volume re-estimation if you attempt to run a plate when the total cartridge gel volume is less than 1.5 mL *more* than the minimum amount needed to process the plate.

If the system determines that there might not be enough gel to run the plate, it will perform a 2-step re-estimation process automatically.

In the first step, the system will "home" the gel pump and the message in Figure 83 will appear.

Figure 83: Gel Volume Re-estimation - Step 1 Dialog
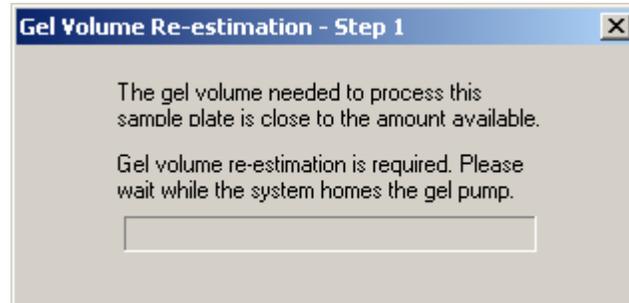
In the second step, the Gel Pump plunger will be engaged and the system will accurately determine the gel volume.

The dialog box in Figure 84 will be displayed.

Figure 84: Gel Volume Re-estimation - Step 2 Dialog
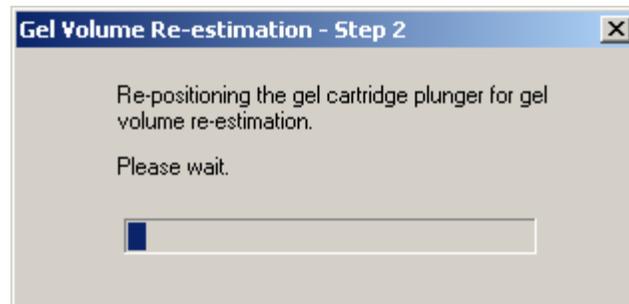
If the gel volume is *greater* that 1.5 mL more than the minimum amount needed to process the plate, the plate will run and the system will not re-estimate the gel volume.

Gel Re-Estimation Procedure

1. Launch the Run module to run a sample plate.
2. Select **Run | Start Sample Plate** to process the sample plate.

> **Note** *You can view the gel cartridge volume by clicking the* **Life** *tab on the Status Monitor.*

3. If the system determines that the gel cartridge volume is less than the volume needed to safely process the plate, you will be presented with the two re-estimation dialog boxes before the system starts running the sample plate.

The system will measure the gel volume accurately and will determine whether or not to proceed with the run.

4. If the actual volume (re-estimated by the system) is *greater* than 1.5 mL more than the total volume needed to run the plate, the system will automatically start the plate.

5. If the actual volume (re-estimated by the system) is *less* than 1.0 mL more than the total volume to run the plate, you will be presented with the dialog box in Figure 85.

Figure 85: Gel Usage Confirmation Dialog
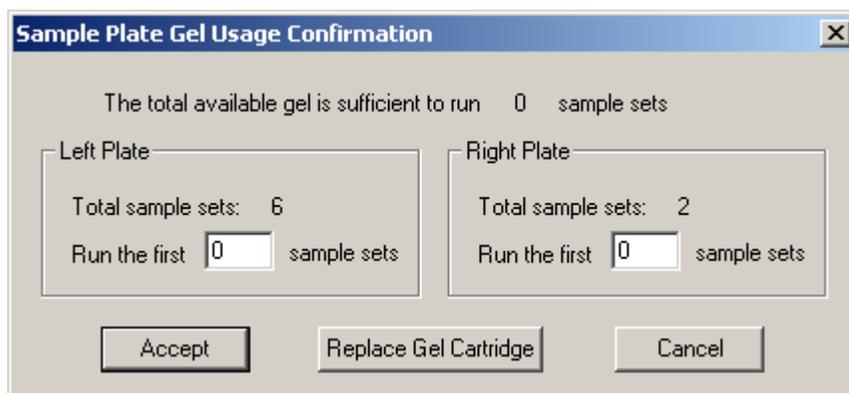
6. Click **Accept** to proceed with the run. If the run is selected to continue, specify the sample sets to run. Select **Replace Gel Cartridge** to replace the gel cartridge before the run.

Gel Volume Notification

A dialog box will warn you if you attempt any operation requiring gel when there is not enough gel in the cartridge to perform the operation.

You will be presented with the warning message in Figure 86 if you attempt to perform a capillary gel fill when the cartridge does not have enough gel.

Figure 86: Gel Warning Dialog
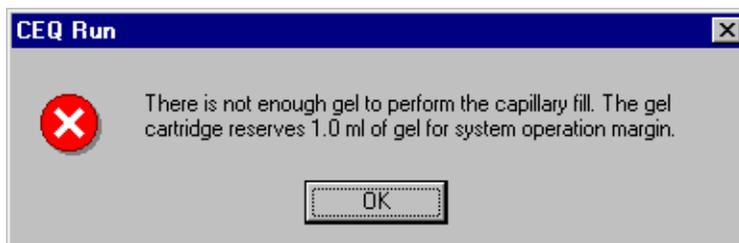
You will also be presented with the dialog box in Figure 87 if you attempt to run a sample plate and the gel cartridge does not have enough gel to process any sample sets in that plate.

Figure 87: Gel Usage Confirmation Dialog
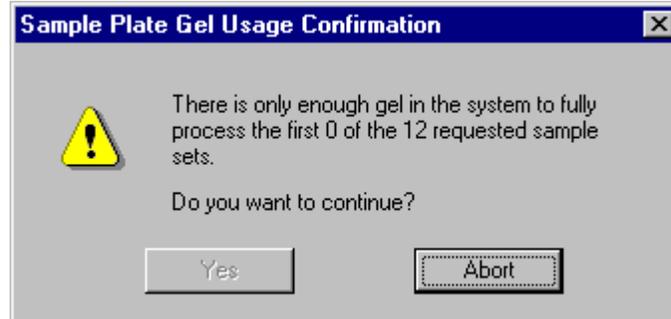
You must click **Abort** to end the run and return to the Run main window.

Gel Plug Warning

When the *Run Module* initializes, it will check the gel cartridge condition. If a gel plug is installed, the dialog in Figure 88 is displayed.
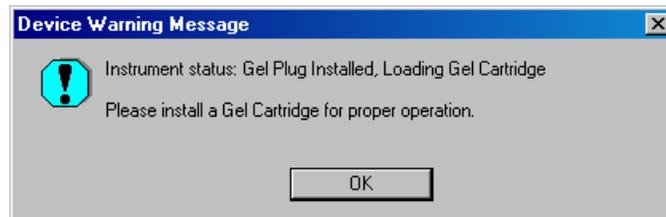
 Figure 88: Gel plug installed warning

The *Device* tab indicates the state of the Cartridge, as well. When a gel plug is present, the *Device* tab indicates *plug* in the *Cartridge* field, as shown in Figure 89. A gel cartridge must be installed before the next run. When a cartridge is present, the *Device* tab indicates *Gel* in the *Cartridge* field, as shown in Figure 90.
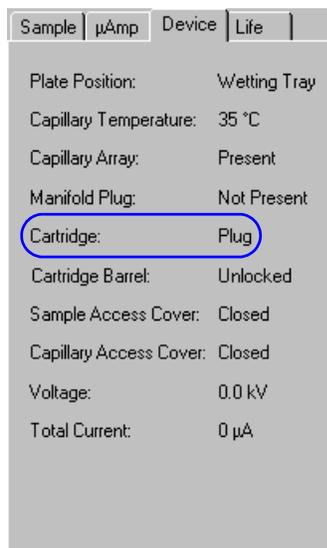
Figure 89: Device Tab – Gel Plug installed
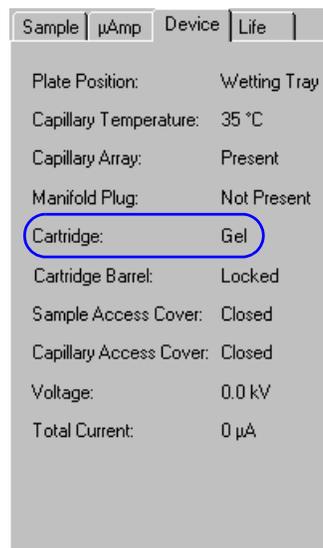
Figure 90: Device Tab – Gel Cartridge installed

Pausing a Sample Plate Run

To pause the currently executing sample plate, click the **Pause** ❚❚ button. The system will pause at the next executable step of the sample plate. To resume the run, click the **Pause** ❚❚ button again.

Stopping a Sample Plate Run

To stop the currently executing sample plate, perform the following steps.

1. Click the **Stop System** ▪ button from the menu.

Figure 91: Stop System Dialog
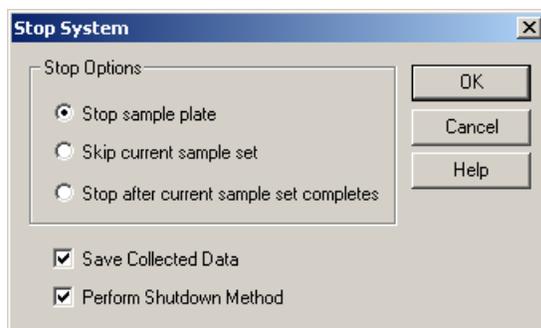
2. In the **Stop System** dialog box:

   a. Select the desired **Stop Options** radio button.

b. Select the **Save Collected Data** option (if desired).

c. Select the **Perform Shutdown Method** (to purge the manifold and capillaries and set capillary chamber temperature to 35°C), then select **OK**.

Viewing the Last Analysis Performed

To view the last analysis performed on the CEQ System, perform the following steps.

1. With a sample plate running (and with **Automatic Analysis** selected), select **Tools | View Last Analysis** from the menu.

2. In the **Sample Plate** dialog box, select a sample or multiple samples and then click **Open**. If the last analysis was a sequence analysis, the Sequence Analysis module will be launched and the sequence analyzed data displayed.

Sequence Analysis Procedures

Viewing Sample Data

To view sample data, perform the following steps.

1. Select **File | Open** from the menu.

Figure 92: Open Dialog
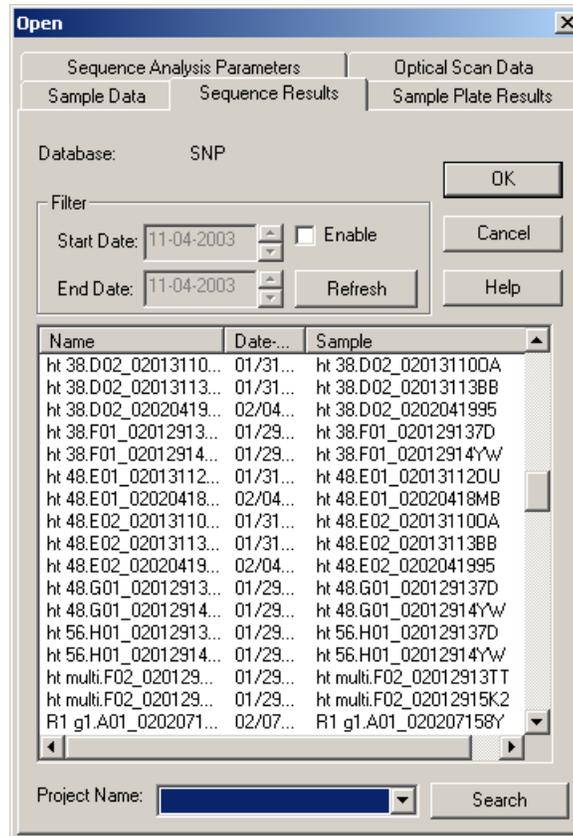
2. In the **Open** dialog box, select the appropriate tab (**Sample Data**, **Sample Plate Results**, **Sequence Results**, **Sequence Analysis Parameters** or **Optical Scan Data**), highlight the desired item and then select **OK**.

3. Click on the **Sample View** (See "Sample View Toolbar" on page 151.) toolbar icons to display the desired data.

Below is a description of each tab in the **Open** dialog box.

Sample Data: This tab displays the list of raw data and baseline data that resides in the selected project. On the right-hand side of the list box you may view the date and time the sample was run. You may want to open a raw data set to analyze it.

Sequence Results: This tab displays the list of analyzed data that resides in the selected project. On the right-hand side of the list box you may view the date and time the sample was run as well as the raw data from which the sequence result was derived. 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 analysis only.

To filter the list, check 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: This tab displays the list of sample plates run in the selected project. Selecting a sample plate result displays the Sample Plate toolbar. Click on the desired sample(s), then click on Open. The desired sample(s) are opened.

Sequence Analysis Parameters: Displays 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, the threshold above which data will be considered peaks, the data start criteria, the heterozygote detection parameters and the alignment template and parameters. You may also edit sequence analysis parameters under the Analysis tab in the Sample Setup module.

Optical Scan Data: This tab lists the alignment optical scan data available in the selected project. Optical scan data is the result of the two lasers scanning the capillaries in order to determine the position of the capillary windows where the lasers will focus when collecting data. If the scan is successful eight peaks are displayed for each laser, denoting that all eight capillary windows were established.

Sample View Toolbar

Icon	Description
	Analyzed Data - Displays the data that has been analyzed for the active sample.
	Base Sequence - Displays a text view of the bases from the analyzed data for the active sample.
	Raw Data - Displays the raw data for the active sample.
	Current - Displays the current data from the capillary arrays for the active sample.
	Voltage - Displays the voltage data from the instrument for the active sample.
	Compare Data - Used to compare the active analyzed data set against another analyzed data set.
	Quality Parameters - Used to view a graphical representation of the Quality Values for the active sample.

Analyzing Sequence Data

Editing Sequence Analysis Parameters

The Sequence Analysis Parameters Editor dialog box consists of five tabs: General, Start of Data, Heterozygote Detection, Alignment Template and Alignment Parameters. You will define the sequence analysis parameters in the following dialog box.

To define the Sequence Analysis Parameters, perform the following procedure.

1. Select **Analysis | Working Analysis Parameters** to open the Working Parameters dialog box.
2. Click **Edit** in the Working Parameters dialog box.
3. The Sequence Analysis Parameters Editor dialog box will open, as shown in Figure 93.

Figure 93: Sequence Analysis Parameters Editor Dialog
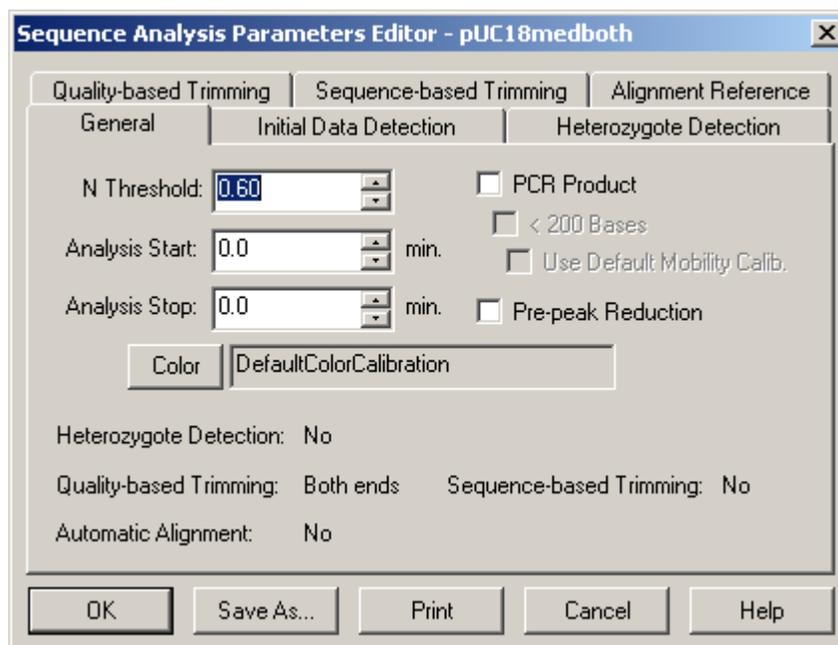
4. Make appropriate changes and click **Save As** to create a new Sequence Analysis Parameter name or click **OK** to save and exit the editor.

General Tab

The general tab is displayed by default.

N Threshold

This value is the threshold for the probability of correctness, below which a called base will be changed to an "N." The range is 0.00 to 1.00 and the default is 0.60.

> **Note** *If Detect Heterozygotes is checked in the Heterozygote Detection tab, this field is disabled.*

In the CEQ System Software, 'N's are assigned to regions of data where it is likely that an insertion or deletion exists, or where the identity of a called base(s) is ambiguous. The N Threshold is used to specify the Call Score below which an 'N' will be assigned to the called base.

> **Note** *It is important to set the N Threshold properly if you are exporting data to any program that can not use CEQ Quality Values, such as BLAST or FASTA, or to most primer design programs.*

To specify the threshold, enter the desired value, between 0.00 and 1.00, in the N Threshold text box. A value of 0.99 will call all peaks as indeterminate (N's) except the high quality peaks. A value of 0.50 will call only the lowest quality peaks as 'N's. A value of 0.00 will not call any peaks as 'N's. The value of 0.60 is the recommended starting threshold. Subsequently, the threshold and/or the stringency of your alignment tool should be adjusted to suit your needs.

> | Note | *After setting the N Threshold, the data must be reanalyzed.*

> | Note | *If you had set a N Threshold of 0.99, out of 100 bases called as 'N's, 99 of those would have been correct. The higher the N Threshold, the higher the number of correct bases will be called as 'N's. Again, it is important to set the N Threshold properly, as good quality data is lost when the N Threshold is set too high.*

Click **OK**. Any called bases or regions of data that have a Call Score below the specified threshold will be called as 'N's.

Analysis Start

Enter the time in minutes from the start of raw data to the time when data analysis is to begin. The range is 0.0 to 180.0 and the default is 0.0.

> | Note | *An entered Analysis Start or Stop time will override any automatically determined start or stop time. In addition, if you enter a stop time that exceeds data collection time, analysis will terminate at the end of data collection.*

Analysis Stop

Enter the time in minutes from the start of the raw data to where data analysis will end.

> | Note | *If an Analysis Stop time is entered and PCR Product (used to determine the end of analyzable data) is checked, the system will use the earlier of the two stop times to conclude the analysis.*

PCR Product

This check box indicates that the sample was generated from a PCR product and signals the system to end analysis when a sudden drop of signal lasts for more than two minutes. This prevents the analysis of baseline data.

<200 Bases

This field is enabled when the **PCR Product** option is checked. This feature has been included to allow for the sequencing of shorter fragments.

Use Default Mobility Calibration

The analysis algorithm determines the mobility corrections (Mobility Calibration) to be applied to each of the dye channels for every sample analyzed; stored mobility calibrations are not used. When processing short PCR samples, the software has too little data to determine an accurate mobility calibration from the data itself. Because of this, we recommend using the Default Mobility Calibration now supplied with the software. Reprocessing in such a manner may produce results with more consistent peak spacing (especially in the first 50 or so bases).

In some cases, the Default Mobility Calibration may prove to be inappropriate. If the bases near the beginning of the analyzed data appear to be inconsistently spaced, try processing the data with this option unchecked.

Pre-peak Reduction

Data collected from the CEQ System may have small peaks ("pre-peaks") approximately one base prior to the peaks representing full-length DNA sequencing fragments. These pre-peaks appear in the same channel and have two confirmed sources:

- The presence of primers shorter than the full-length primer (N-1, N-2, etc. primers) are present during the sequencing reaction, and
- The presence of a run of a single base (e.g. polyA) in the template leads to a "copying" error.

To enable the system to attempt to identify and reduce the inclusion of pre-peaks in the analyzed data, check the **Pre-peak reduction** check box in the **General** portion of the **Sequence Analysis Parameters Editor** dialog box.

> *If the primers used for the sequencing reaction are known to contain or suspected of containing N-1 primers as well as full-length primers, the Pre-peak reduction check box should be checked.*

Color Calibration

The values in the color matrix show the cross talk between the filters and the emissions for A, C, G, and T. The base calling algorithm is designed to remove signal due to cross talk. Click **Final Values** to view the computed color calibration after the run. Click **Initial Values** to view the color calibration values prior to the run. If the color calibration successfully removed peaks due to cross talk, it should be saved so it will apply to data with residual cross-talk signal. If the data is going to be reanalyzed using a known good color calibration, do not specify that the color calibration is to be recomputed.

Check the **Compute color matrix** check box to re-compute the color calibration the next time the data is analyzed. (This option is not available if accessed from the Parameters Used to Compute Sequence menu option under the View menu.)

Check the **Use Final Values as Initial Values** check box to use the final values to re-compute the color calibration the next time the data is analyzed. Otherwise the system will use the initial values to re-compute the color calibration. (This option is not available if accessed from the Parameters Used to Compute Sequence menu option under the View menu.)

Initial Data Detection

Select the Initial Data Detection tab to define the parameters that the system will use to define the start of usable data when an Analysis Start time, on the *General* tab, is not entered. See Figure 94.

The default settings are different when the **<200 Bases** option is selected. The default settings are:

> Threshold: 40
> Delay: 0.20
> Signal to Noise: 7.00
> Minimum Duration: 2.00

Figure 94: Initial Data Detection tab
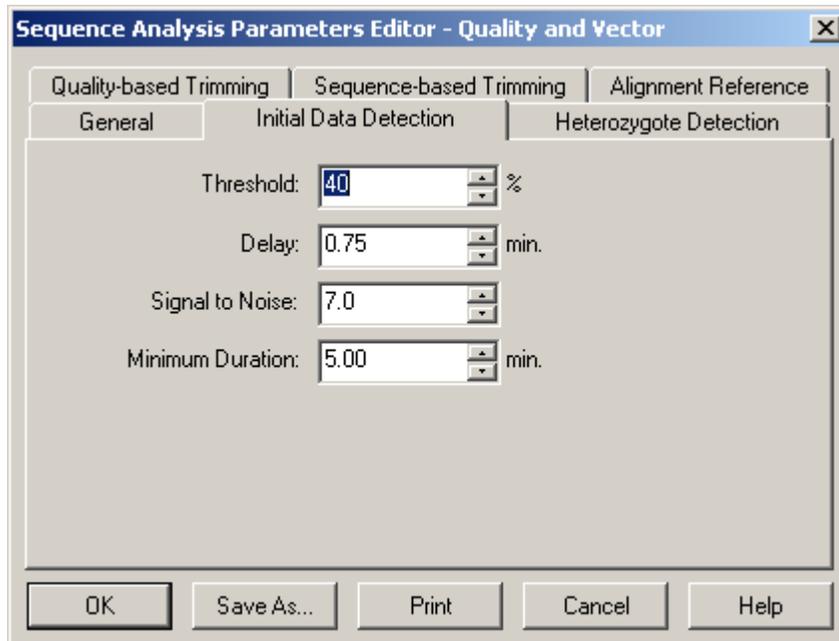
Threshold

The percent threshold is the percent of the maximum of the energy profile of all the data above which the signal must rise before start of data is detected. The data must stay above the threshold for the amount of the minimum duration without gaps lasting greater than 21 seconds. The range is 0 to 100 and the default is 40.

Delay

The start of data Delay is the delay, in minutes, between the detected start of data and the data that will be analyzed. The range is 0.00 to 180.00 and the default is 0.75 minutes.

> **Note** *The default value will be 0.20 minutes if <200 Bases is selected on the General tab.*

Signal to Noise

This is the signal to noise ratio for significant data. The range is 3.00 to 1000.00 and the default is 7.00.

Minimum Duration

The minimum duration is the duration, in minutes that the signal to noise threshold specified above has to be exceeded with no gaps greater than 21 seconds. The range is 0 to 50.00 minutes and the default is 5.00.

> **Note** *The default value will be 2.00 minutes if <200 Bases is selected on the General tab.*

> **Note** *If a value outside of an expected range is entered, a message box will be displayed requesting that a valid value be entered.*

Heterozygote Detection Tab

Select the **Heterozygote Detection** tab as shown in Figure 95 to define the parameters the system will use to detect heterozygotes. These parameters will only be used if the **Detect Heterozygote** option is selected in the *General* tab.

Figure 95: Heterozygote Detection Tab

Detect Heterozygotes

When this item is checked, the analysis will include heterozygote detection based on the remaining parameters specified in the *Heterozygote Detection* tab.

When the **Detect Heterozygotes** check box is checked, the **Call Threshold** is disabled (grayed) but the current Call Threshold value remains in the text box. The system will use a value of zero for the Call Threshold and will not call "N's" when attempting to detect heterozygotes.

% of Average Peak Spacing

Use this parameter to determine the range around each peak that the system will search for a possible heterozygous peak. The system will determine the local peak to peak spacing using an average of the spacing for a 10 base range around the base of interest. The system will then find the specified percent (e.g., 70%) of the local peak to peak spacing and look in that range from the center of each peak for a possible heterozygous peak. For instance, if a value of 70% is entered, the system will look 35% of the average peak spacing to the right and 35% to the left for additional peaks. The range is 0 to 100% and the default is 70%.

Height Ratio

Use this parameter to define the minimum percent of the primary peak's height that a second peak falling within the defined range must be to be considered a heterozygous peak. The range is 0 to 100% and the default is 30%.

Sensitivity

Use this parameter to define the sensitivity of the system when looking at the sharpness of the peak. A high value leads to a high degree of sensitivity and will detect "peaks" that are not very well formed, i.e., "blips" in the baseline. A lower value will lead to a low degree of sensitivity and will not detect "peaks" unless they are well-define and are shaped more like a DNA fragment peak. The range is 0 to 1.0 and the default is 0.25.

Range

This value defines the nucleotide range in the called sequence in which the system will look for heterozygous peaks. The system will not look for heterozygous peaks outside this nucleotide range. The range max is dependent upon the length of the analyzed sequence for the current sample.

Range From (Minimum)

If **Range From** is zero, detection will start at the first called base. The range is 0 to 2000 and the default is 50.

Range to (Maximum)

If **Range to** is zero, the algorithm will use the **Range End** value.

If both **Range From** and **Range to** are zero, detection will continue to the end of the called base sequence.

If **Range End** is zero, the system will use the **Range to** value.

If both **Range From** and **Range End** have a non-zero value, the algorithm will choose the *earlier* of the two values.

Range End ("or to")

This item specifies how many bases, before the last called base, to stop looking for heterozygotes.

If the value in **Range End** is zero, the algorithm will use the **Range to** value.

If both **Range End** and **Range to** are zero, detection will continue to the end of the called base sequence.

If **Range to** is zero, the system will use the **Range End** value.

If both **Range to** and **Range End** have a non-zero value, the algorithm will choose the earlier of the two values.

> **Note** *If a value is outside of an expected range, a message box will be displayed requesting that a valid value be entered.*

The system will use the IUB ambiguity codes in Table 69 to represent heterozygous bases.

Table 69: IUB Ambiguity Codes

Code	Definition	Mnemonic
A	Adenine	A
C	Cytosine	C
G	Guanine	G
T	Thymine	T
R	AG	puRine
Y	CT	pYrimidine
K	GT	Keto
M	AC	aMino
S	GC	Strong 3 H bonds
W	AT	Weak 2 H bonds
B	CGT	Not A
D	AGT	Not C
H	ACT	Not G
V	ACG	Not T
N	AGCT	aNything

The system will report the heterozygous bases in the Analyzed Data and in the Base Sequence Data.

Alignment Template Tab

When the *Alignment Template* tab is clicked, the dialog box in Figure 96 is shown.

If **Automatic Alignment** is checked on the *General* tab, the options on this tab, along with the information on the *Alignment Parameters* tab, will be used for the alignment operation.

Figure 96: Alignment Reference Tab
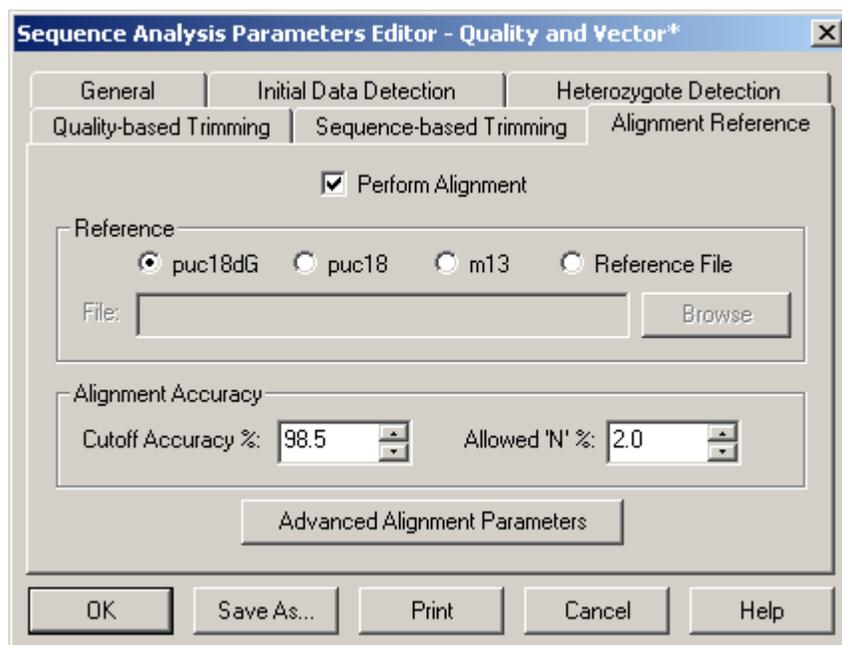
Reference

The user can choose from three templates that are stored as part of the software or choose a reference file from a disk.

If the **Reference File** radio button is not selected, then the text box and the **Browse** button below it are disabled.

If the **Reference File** radio button is selected, then the text box below it and the **Browse** button are enabled, allowing the user to enter the path of the desired reference file or browse for a file.

Browse

The Browse button will allow to search for Text files (*.txt) and Sequence files (*.seq).

Cutoff Accuracy.

Enter the desired **Cutoff Accuracy**. The alignment will detect the base number in the called sequence at which the accuracy falls below this value. The range is 0.0 to 100.0% and the default is 98.5%.

Allowed N's

Enter the percentage of **Allowed N's** that will be allowed to occur before they are counted as errors when determining the alignment accuracy. The range is 0.0 to 100.0% and the default is 2.0%.

> *If a value is outside of an expected range, a message box will be displayed requesting that a valid value be entered.*

Advanced Alignment Parameters

When the **Perform Alignment** checkbox is selected, the **Advanced Alignment Parameters** button becomes active. Click the **Advanced Alignment Parameters** button to access the feature described below.

Figure 97: Advanced Alignmant Parameters
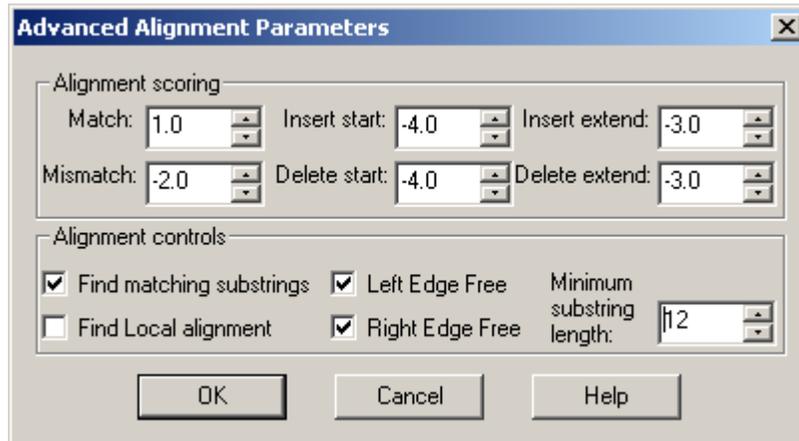
The information on this tab is used to define the parameters used to perform alignments with the active sequence. The system uses a scoring matrix to perform the alignment. The alignment that produces the highest total score is the one that will be displayed. In general, matches should be given higher scores than non-matches. The definitions of the scoring values are described below. Clicking the **Alignment Parameters** tab will provide the options described below.

The **Match** text box is used for the value of a match between a base position of the sequence and the alignment sequence. The range is -1000.0 to 1000.0. A value of 1.0 is the default value.

The **Mismatch** text box is used for the value of a mismatch between a base position of the sequence and the alignment sequence. The range is -1000.0 to 1000.0. A value of -2.0 is the default value.

The **Insert start** text box is used for 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. A value of -4.0 is the default value.

The **Insert extend** text box is used for 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. A value of -3.0 is the default value.

The **Delete start** text box is used for 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. A value of -4.0 is the default value.

The **Delete extend** text box is used for 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. A value of -3.0 is the default value.

Check **Find matching substrings** to optimize the alignment. When the alignments are optimized, the system will find matching substrings first, then run the alignment algorithm on the bases remaining outside of the substrings, reducing the execution time and storage space.

Check the **Find Local alignment** to use only the substrings with the highest score, ignoring the rest, as opposed to using every character from both strings to compute the similarity score.

Check **Left Edge Free** and/or **Right Edge Free** to specify that overhanging bases on the template or sequence will not be counted in the alignment score.

Enter the number of bases needed to constitute a substring in the **Minimum substring length** text box to specify a minimum length for detected substrings. The range is 5 to 2000. A value of 30 is the default value.

> **Note** *If a value is outside of an expected range, a message box will be displayed requesting that a valid value be entered.*

Quality-based Trimming Tab

Use Quality-based trimming to trim poor quality data from the ends of the sequence. The resulting Base Sequence and Trace Views display the base letters of the poor quality data as strikethrough.
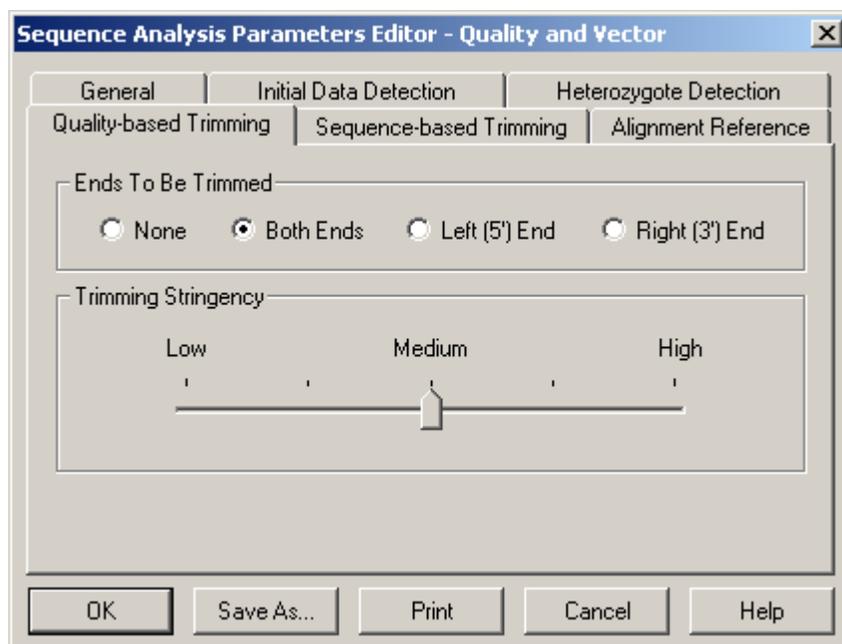
Figure 98: Quality-based Trimming tab

Ends To Be Trimmed

Click the radio button to select the end(s) 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 or select **Right (3') End** to trim to the right of the 3' end. If **None** is selected, no quality-based trimming will be conducted.

Trimming Stringency

The slider control sets 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, 0.01. The default value is Medium (5%).

Sequence-based Trimming Tab

The Sequence-based trimming function allows for contaminant (sequence-based) trimming. This can be done in Batch Analysis mode or in Sequence Analysis mode. The resulting Base Sequence and Trace Views display the base letters of the vector and other subsequences as underlined. If multiple vector or subsequences matched, they will appear as multiple underlined in the Trace View.
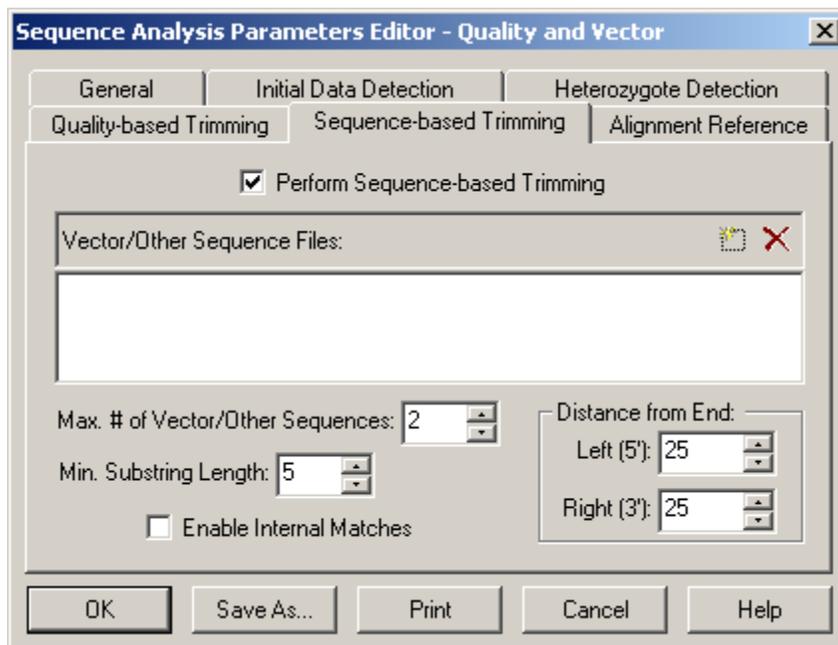
Figure 99: Sequence-based Trimming tab

The most common sources of contamination are accessory DNAs that have been deliberately attached to the DNA under investigation. These include:

- Vector Sequences such as plasmid, phage, BAC, PAC and cosmids
- Primer Sequences
- Restriction Enzyme Sites

Perform Sequence-based Trimming

Select this check box to enable Sequence-based trimming.

Vector/Other Sequence Files

Press ⬜ to select a reference sequence. It must be in the .fasta format and stored in an accessible folder. Press ✖ to remove a previously selected reference sequence.

> **Note** *There is no practical limit on the number of .fasta reference sequences that can be used. However, larger files (i.e. greater than 200 Kbase) will increase processing time.*

Max. # of Vector/Other Sequences

Set the number of contaminant sequences to be reported in the result. This determines the number of subsequences with the highest goodness of alignment scores that will be reported.

Min. Substring Length

Set the minimum substring length. Only the subsequences that meet or exceed this length will be trimmed from the result.

Distance from End

Set the distance from the end of the sequence and contaminant for automatic removal. As shown below, the Distance from End was set to 5. So, the entire subsequence, from the start of the sequence to the end of the contaminant sequence, was trimmed.

AACG<u>ACGGCCAGTGCCAAG</u>CTTGGGGAGATTAATCGTGAGCGCCTAT

Enable Internal Matches

Enable this check box to allow trimming within the sequence.

Quality Values

Quality Values are another measure of the probability of correctness of a called base and are used specifically for sequences assembled in *PHRAP*. They allow for more discriminating assessment of the bases called in the higher quality portion of the data (data with an error rate of *less* than one in one hundred). Quality Values aid in the assembly of multiple sequences in *PHRAP* or any program that performs quality-weighted sequence alignment or primer design.

Preventing Alignment Problems

To prevent alignment problems caused by occasional high quality bases appearing in the lower quality 3' end of a called sequence, it is suggested that you trim the 3' end of the sequence.

Bases at the 3' end of the sequence should be trimmed when:

- the average Call Score/Quality Value in a window of 25 bases falls below 0.75 - 0.8 on a linear scale and 6 - 7 on a log scale, **and**
- there are at least 5 bases within that 25 base window whose Call Score/Quality Value are less than 0.75 on a linear scale and 6 on a log scale.

Performing Quality-based Trimming

To setup Quality-based trimming, open the *Sequence Analysis* module and follow the steps below.

1. Open a result and select the **Analysis | Working Analysis Parameters** menu item.
2. Click the **Edit** button to open the *Sequence Analysis Parameters Editor*.
3. Select the *Quality-based Trimming* tab.
4. Select the **Ends To Be Trimmed** and the **Trimming Stringency**.
5. Click **Save As** and save the new parameters.
6. Click **OK**.

At this point, the result can be trimmed.

1. Select the **Analysis | Trim Based on Quality** menu item.
2. The Analysis Parameters, created in the steps above, should be listed. If not, click **Use Stored Parameters** and select the appropriate Analysis Parameter.
3. Click **OK**.

The resulting Base Sequence and Trace view displays the base letters, if poor quality, as strikethrough after a Quality-based trim has been performed as shown in Figure 100.

When performing Quality-based and Sequence-based trimming, Quality-based trimming is always performed first.

Figure 100: Base Sequence and Trace Views after Quality-based Trimming

Apply Quality Trimming

If the trimming is acceptable, select the **Edit | Apply Quality-based Trimming** menu item. The resulting Base Sequence and Trace view after a Quality-based trimming has been applied is shown in Figure 101.
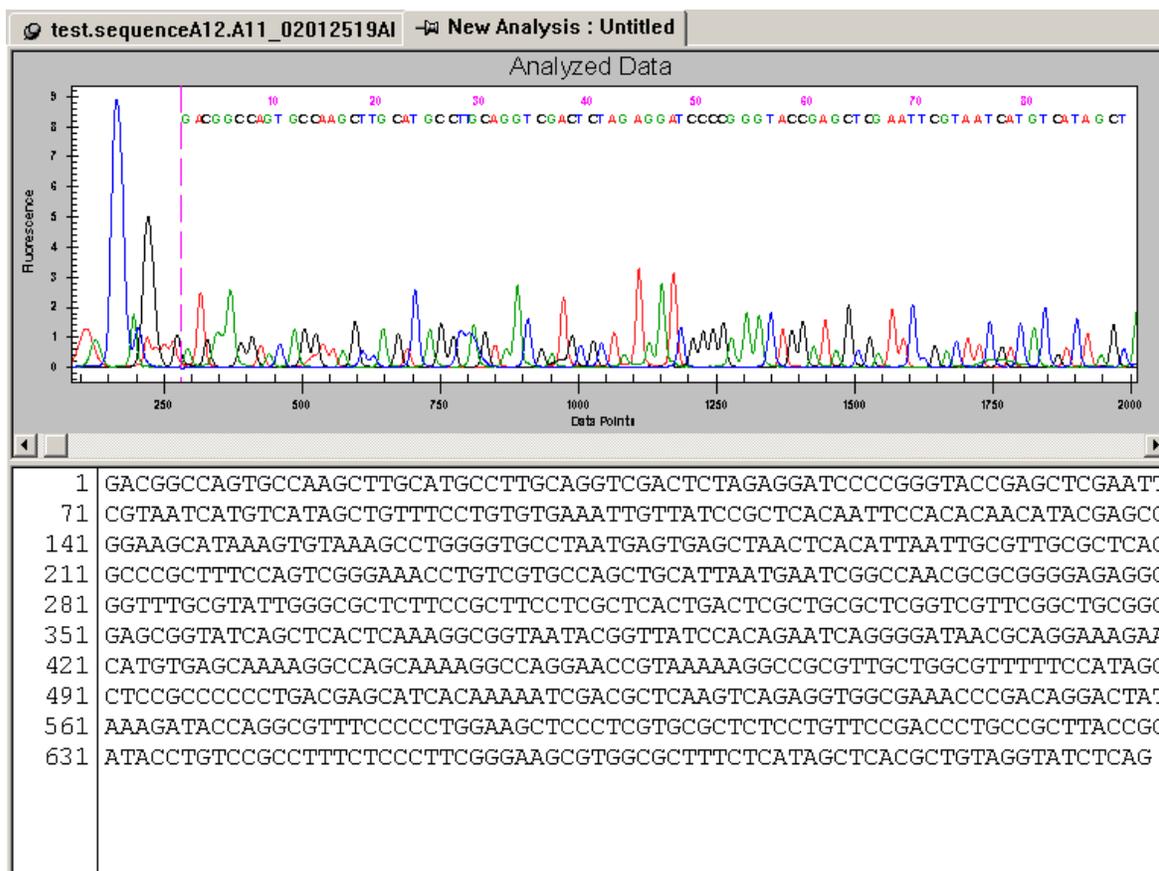
Figure 101: Base Sequence and Trace Views after Quality-based Trimming – Quality-based Trimming Applied

Undo

The original sequence can be recovered after **Apply Quality-based Trimming** has been performed by selecting the **Edit | Undo** menu item.

Performing Sequence-based Trimming

To setup Sequence-based trimming, open the Sequence Analysis module and follow the steps below.

1. Open a result and click the **Analysis | Working Analysis Parameters** menu item.

2. Click the **Edit** button to open the *Sequence Analysis Parameters Editor.*

3. Select the *Sequence-based Trimming* tab.
4. Click the *Perform Sequence-based Trimming* check box. And make all the needed selections discussed above.
5. Click **Save As** and save the new parameters.
6. Click **OK**.

At this point, the result can be trimmed.

1. Select the **Edit | Trim Based on Sequence** menu item.
2. The Analysis Parameters, created in the steps above, should be listed. If not, click **Use Stored Parameters** and select the appropriate Analysis Parameter.
3. Click **OK**.

> **Note** ***When performing Quality-based and Sequence-based trimming, Quality-based trimming is always performed first.***

The resulting Base Sequence and Trace view after a Sequence-based trim has been performed is shown in Figure 102.
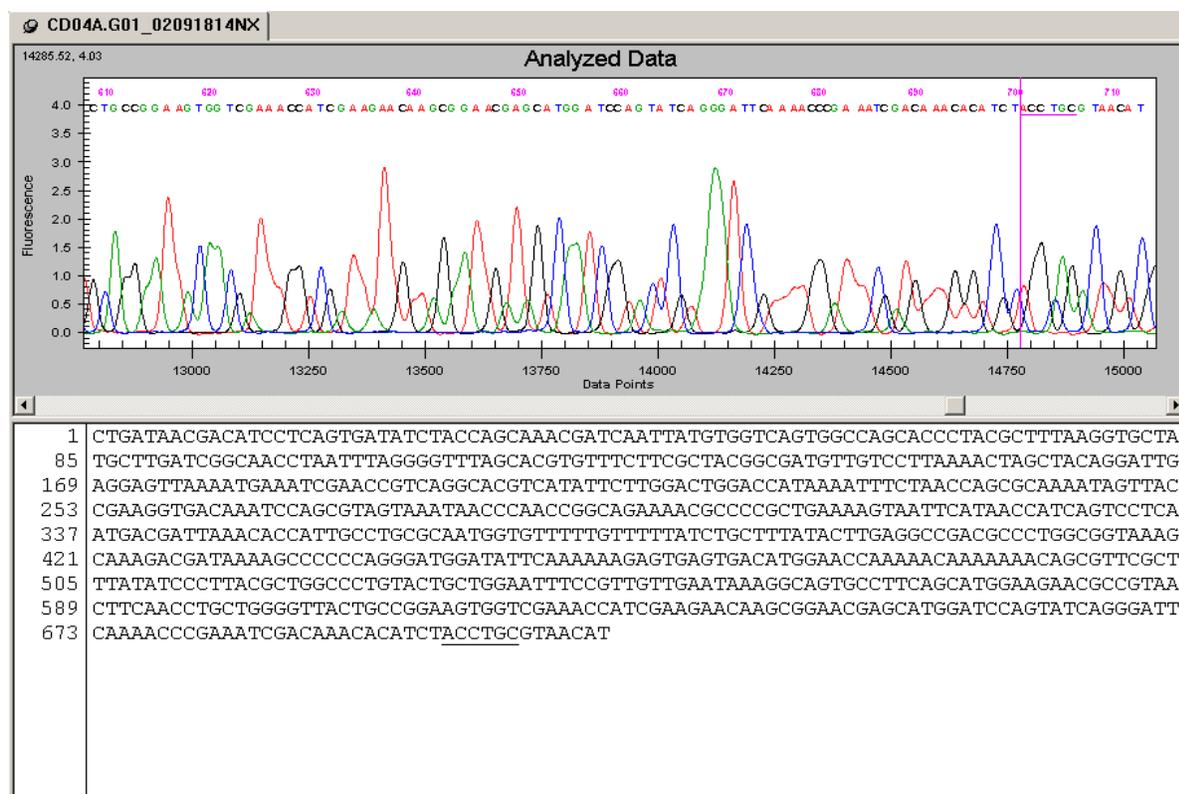
Figure 102: Base Sequence and Trace Views after Sequence-based Trimming

Apply Sequence-based Trimming

If the trimming is acceptable, select the **Edit | Apply Sequence-based Trimming** menu item to remove the trims from the sequence. The resulting Base Sequence and Trace view after a Sequence-based trimming has been applied is shown in Figure 103.

> *When selecting applying sequence-based trimming, quality-based trimming will automatically be applied first if quality-based trimming was performed.*
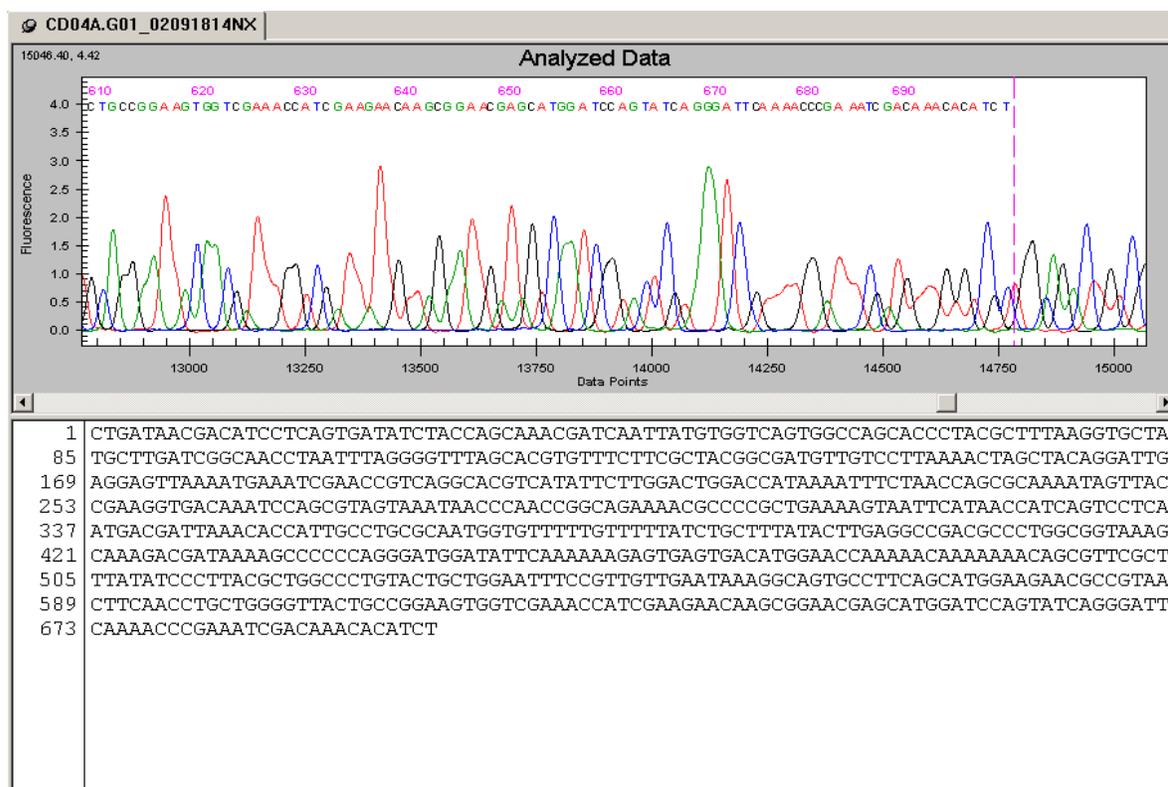
Figure 103: Base Sequence and Trace Views after Sequence-based Trimming – Sequence-based Trimming Applied

Undo

The original sequence can be recovered after **Apply Sequence-based Trimming** has been performed by selecting the **Edit | Undo** menu item.

Internal Trimming

Select the **Enable Internal Matches** checkbox from the *Sequence-based Trimming* tab to trim subsequences from within the sequence. To exclude a subsequence from final removal, right-click on the subsequence and select **Vector/Other Subsequences** and select the subsequence. Only those subsequences marked with a check (Figure 104) will be removed from the sequence when trimming is applied.

Figure 104: Base Sequence and Trace Views after Sequence-based Trimming – Enable Internal Matches

The internal matches in the resulting Base Sequence and Trace view, after a Sequence-based trimming has been applied, are replaced by the letter "x", as shown in Figure 105.
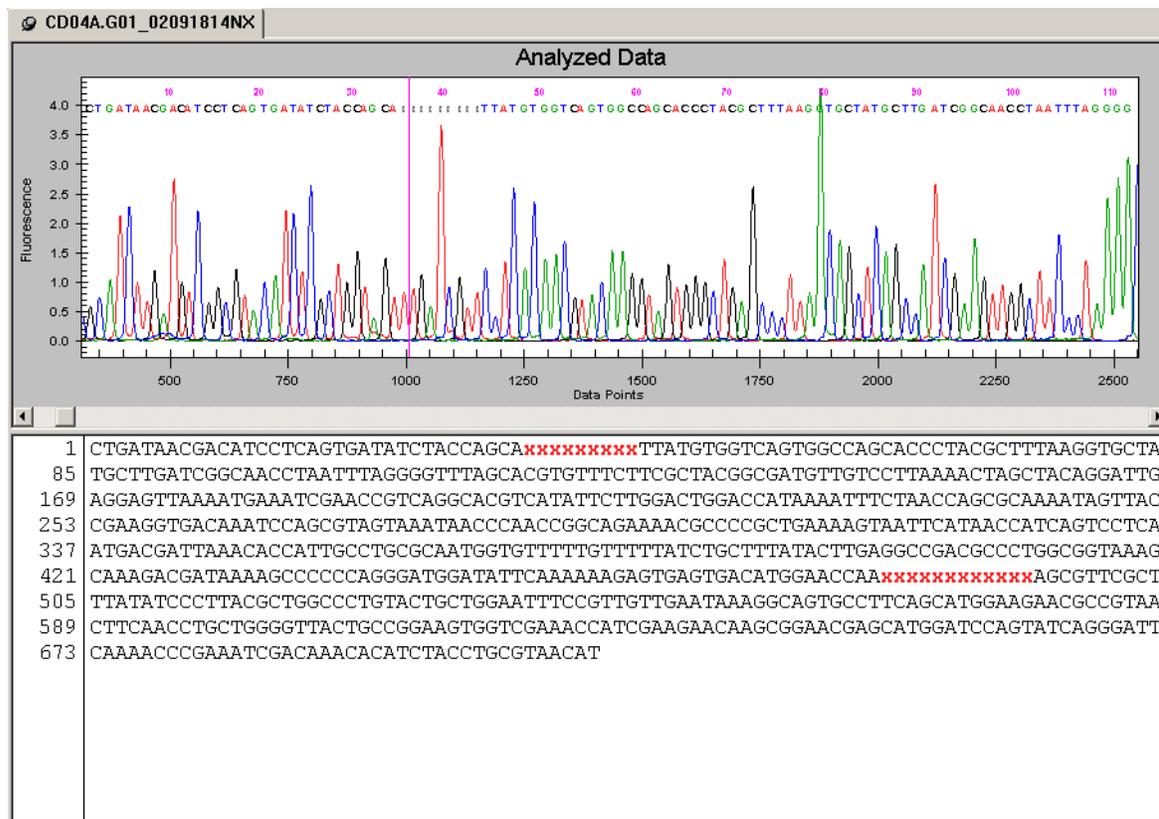
Figure 105:Base Sequence and Trace Views after Sequence-based Trimming – Enable Internal Matches

Batch Trimming

Batch trimming has been added to enable the automatic trimming of a large number of results. To perform Batch Trimming, open the *Sequence Analysis* module and follow the steps outlined below.

1. From the *Analysis Menu* select **Batch Trimming**.
2. Select the *Sequence Results* or *Sample Plate Results* tab.

3. Select the results to be trimmed and click the right-arrow to move them to the "selected" list box.
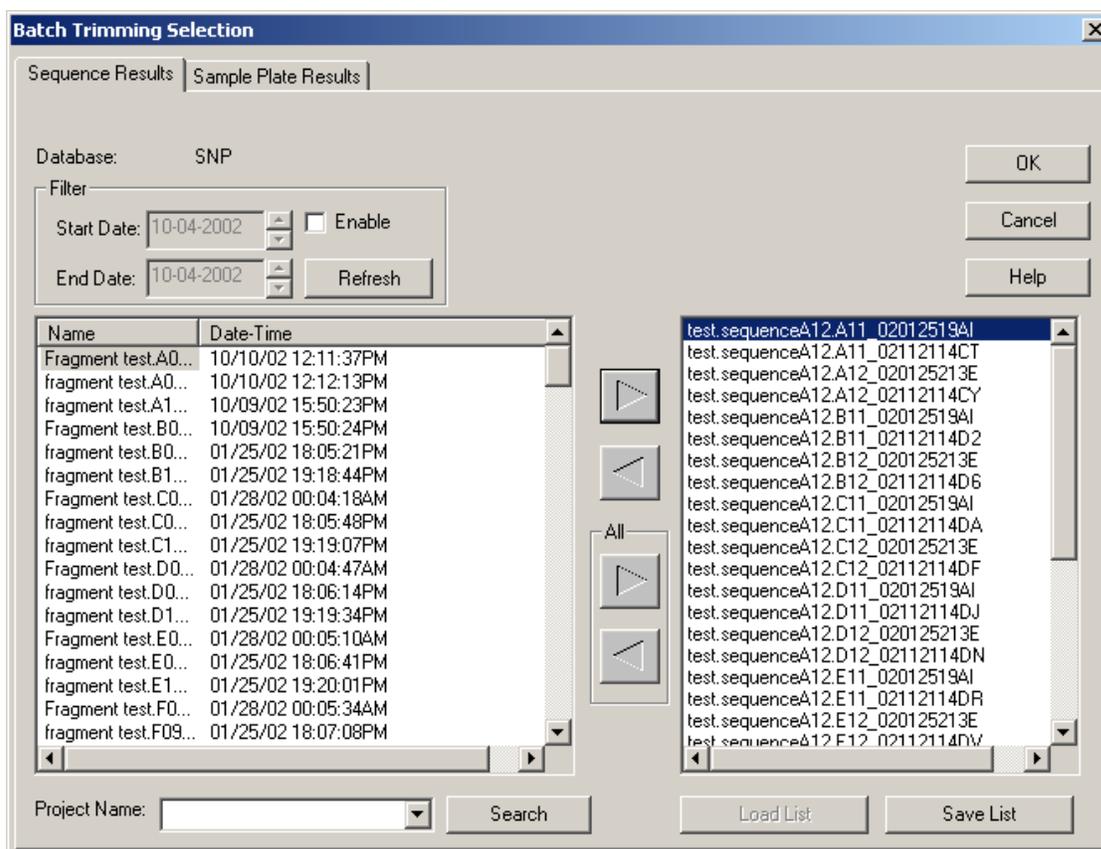
Figure 106: Batch Trimming Result Selection

4. Click **OK**.
5. Verify that the required Analysis Parameters are selected in the Working Parameters dialog. (If not, click **Use Stored Parameters** and select the appropriate Analysis Parameter).
6. Select the Export Results check-box if necessary.
    *   The Edit Export Options button will become available (Figure 107). See "Export Options" on page 175. for more information on additional options.
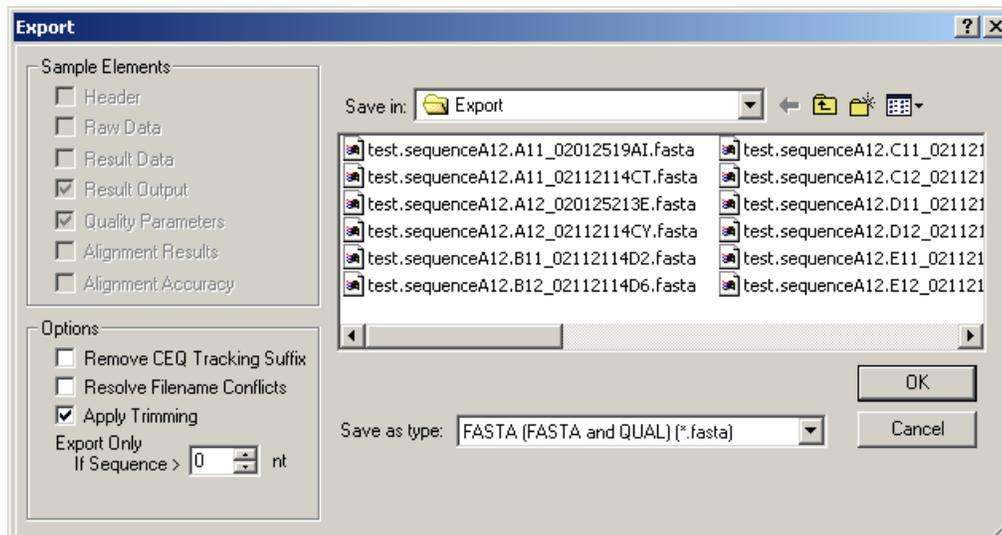
Figure 107: Export Options

7. Click **OK**.

A **Trimming Report** is created that can be viewed and printed. To view the Trimming Report select the **View | Trimming Report** menu item. The new pane will open at the bottom of the screen.

Items displayed include:

- Result Name
- Original Sequence Length
- 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 print the grid data, export the data as comma separated (.csv) or tab delimited (.txt) text files and you can format the grid. Access these options by right-clicking over the Trimming Report pane.

Print Grid Data

Select **Print Grid Data** to print the entire grid as it is represented on the screen.

Export Grid Data

Select **Export Grid Data** to save the grid data as a comma separated (*.csv) or tab delimited (*.txt) text file.

Format Grid

Format Grid allows the user to customize the look of the data grid. If rows or columns are hidden, Print Grid Data will omit them. However, this will not affect the Exported Grid Data (all data will be exported).
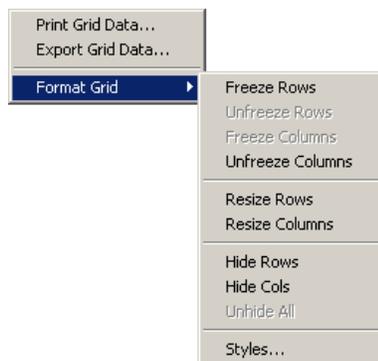
Figure 108: Format Grid Options

- Freeze/Unfreeze Rows
- Freeze/Unfreeze Columns
- Resize Rows/Columns
- Hide/Unhide Rows/Columns
- Styles
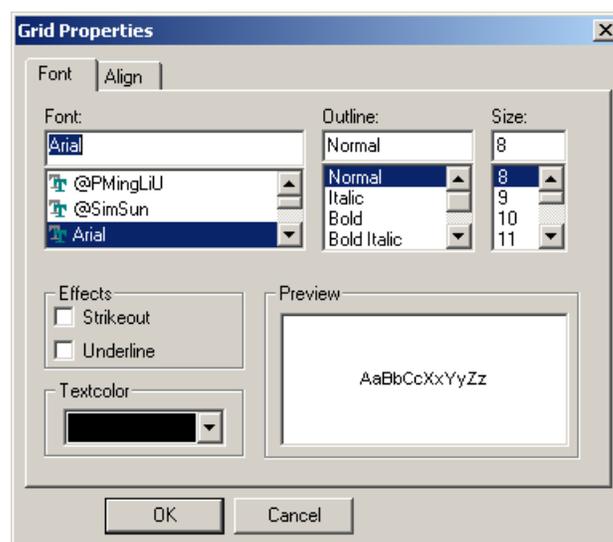
Figure 109: Grid Properties

Export Options

Two tools are available in the export dialog: *Apply Trimming* and *Export Only If Sequence > X nt*.

Apply Trimming

If the Export option was selected during sample setup or batch analysis, selecting the **Apply Trimming** option (Figure 110) will remove the trims from the sequence before it is exported. All internal trims will be replaced by the letter "x".

Export Only If Sequence > X nt

The resulting sequence, after trimming, may be to small to be useful. Use the **Export Only If Sequence > X nt** option to specify a minimum size that the sequence must be when exporting. Only those sequences greater than the set number of nucleotides will be exported.
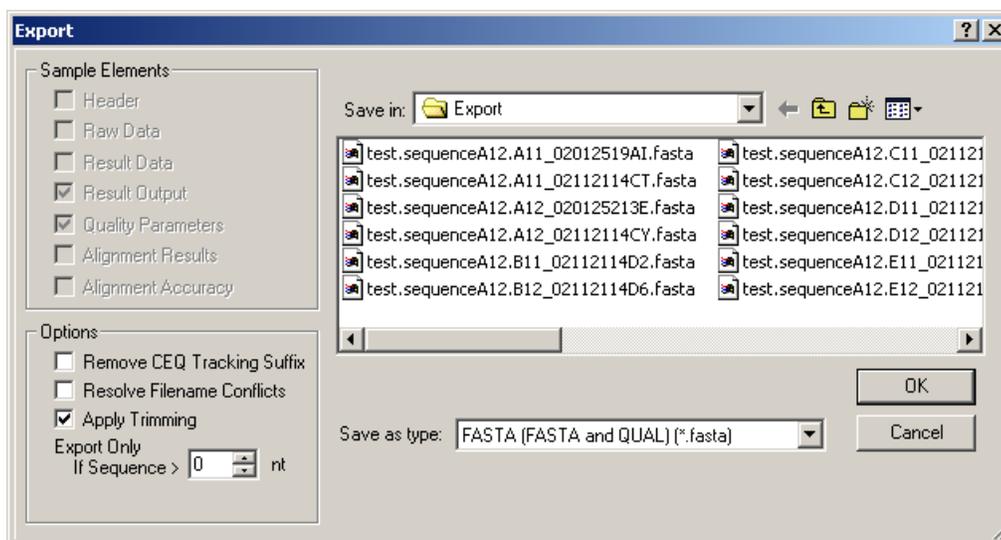
Figure 110: Export Options

Editing Bases

Inserting Bases in the Analyzed Data Pane

To insert bases in the Analyzed Data pane, perform the following steps.

1. Select **Tools | Edit** from the menu.
2. Click on an area *between* two bases.

3. From the **Insert Base** dialog box, select the desired base and then click **OK**. (The lower-case base is inserted in both the Analyzed Data and the Base Sequence pane.)

Inserting Bases in the Base Sequence Pane

To insert bases in the Base Sequence pane, perform the following steps.

1. Select **Tools | Edit** from the menu.
2. Place the mouse cursor *between* the two bases where you wish to insert the base, then type in the new base. (The lower-case base is inserted in both the Base Sequence and Analyzed Data pane.)

Changing Bases in the Analyzed Data Pane

To change bases in the Analyzed Data pane, perform the following steps.

1. Select **Tools | Edit** from the menu.
2. Click **on** a base in the analyzed data pane.
3. From the **Change Base** dialog box, select the desired base and then click **OK**. (The base is changed in both the Analyzed Data and the Base Sequence panes and will be lower-case to identify it as "edited."

Changing Bases in the Base Sequence Pane

To change bases in the Base Sequence pane, perform the following steps.

1. Select **Tools | Edit** from the menu.
2. Highlight the base(s) to change in the base sequence text and then type in the new base(s). (The base[s] is changed in both the Base Sequence and Analyzed Data panes and will be identified as "edited" by being lower-case.)

> **Note** *The left and right arrow keys will move the cursor through the data in the Base Sequence pane. Bases can be highlighted by holding the* **Shift** *key down while using the left and right arrow keys to select the desired bases.*

Deleting Bases in the Base Sequence Pane

To delete bases in the Base Sequence pane, perform the following steps.

1. Select **Tools | Edit** from the menu.
2. Highlight the base(s) in the base sequence text to delete and then press the **Delete** key (or use the Cut icon). (The base[s] is deleted in both the Base Sequence and Analyzed Data panes.)

Specifying Base Grouping

To specify base grouping in the Base Sequence pane, perform the following steps.

1. Select **Tools | Base Spacing** from the menu.
2. From the pull-right, select the desired group spacing.

Analysis Log

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

The Analysis Log may be viewed to see if the system was unable to find the end of PCR data. The following message will appear in the Analysis Log: "Analyzed Data: Cannot compute stop time. Using data stop time of xxx.xx minutes", as shown in Figure 111.

Figure 111: 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 minutes  ◄──
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 will not be able to find the end of PCR product data, even if the PCR Product option is selected. You will be able to determine if this is the case by reviewing the analysis log, and you can 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. Make sure that the PCR Product check box is selected and that 0.0 is entered for both the Analysis Start and Stop times.*

2. Select **View | Analysis Log** to view the analysis log for the sequence result.
3. The Analysis Log will be displayed in a pane at the bottom of the window.

Sequence Result Properties

The sequence result properties are used to view Method, Analysis, Alignment, Consumable, General information, Notes and Property set information.

Open the Properties dialog box by selecting **File | Properties** when a sequence result is open. See Figure 112.

> *If no alignment has been performed on the open sequence result, the Alignment tab will not be shown.*

Figure 112: General Tab, Properties Dialog
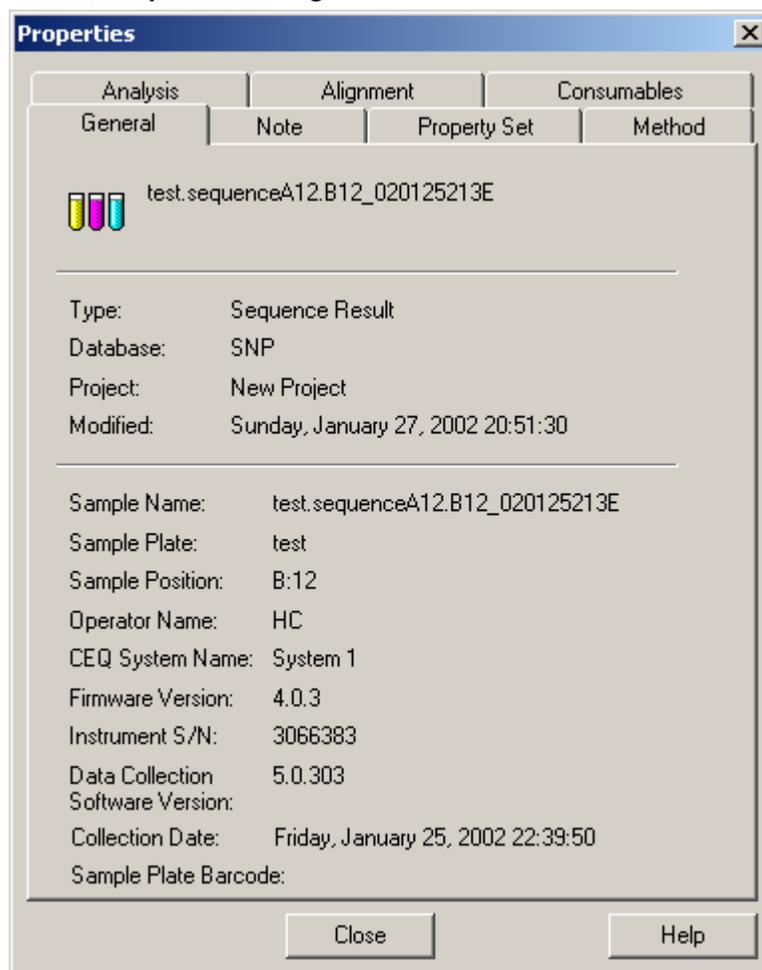
Notes Tab

Any notes associated with the sample well for the open sequence result can be viewed. These notes are entered in the *Sample Setup* module on the *Notes* tab at the bottom of the main window. See Figure 113.

Figure 113:Note Tab, Properties Dialog
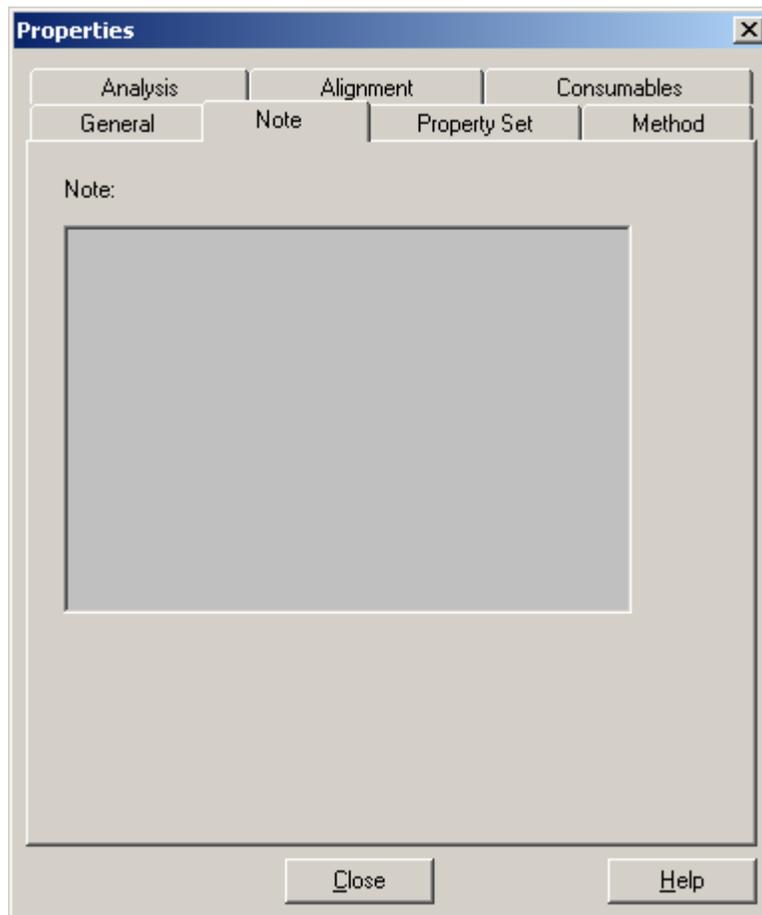
Property Set Tab

The user can view any property sets associated with the sample well for the open sequence result. These Property Sets are defined in the *Sample Setup* module on the *Notes* tab at the bottom of the main window. See Figure 114.

Figure 114: Property Set Tab, Properties Dialog
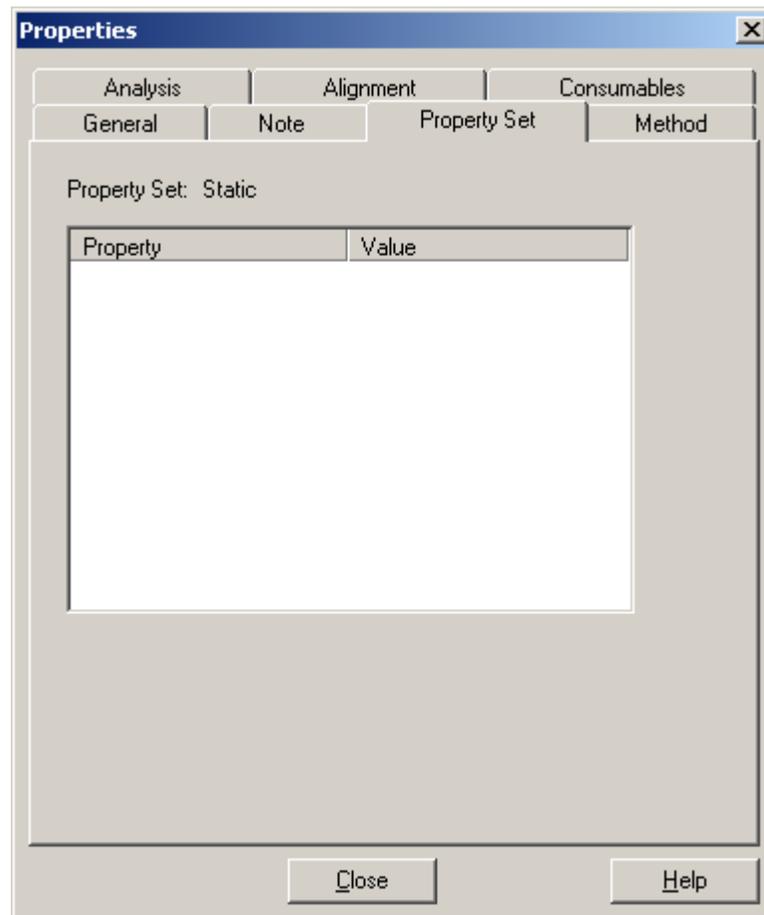
Method Tab

The method event parameters used to collect the raw data for the open sequence result are shown here. These parameters are defined on the *Method* tab in the *Sample Setup* module, prior to running the sample plate. See Figure 115.

Figure 115: Method Tab, Properties Dialog
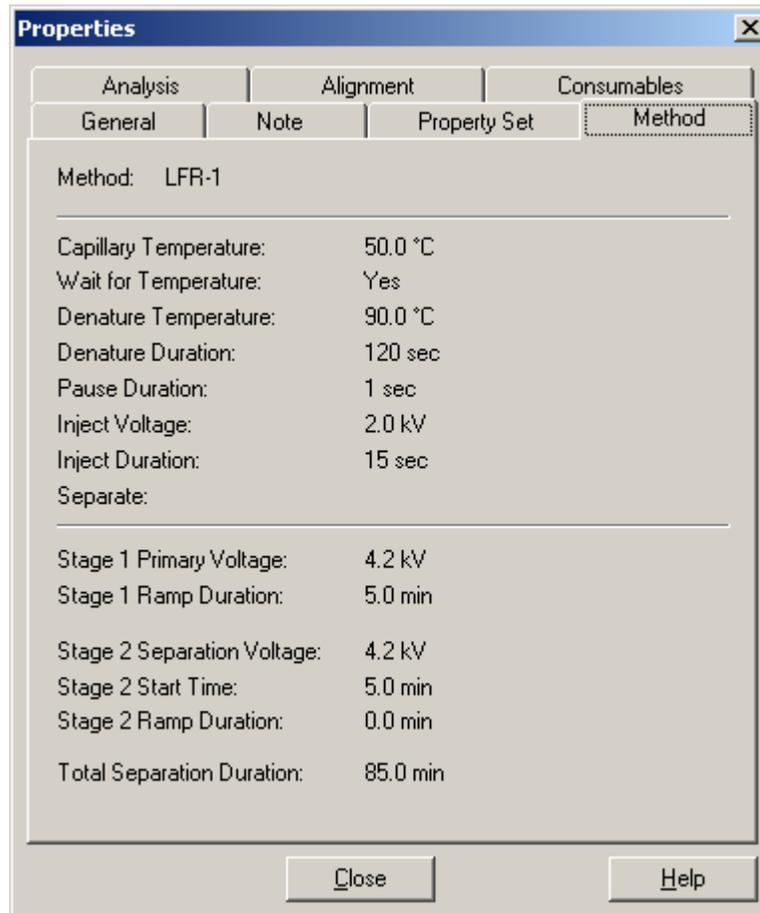
Analysis Tab

The user can view the sequence analysis parameter set used to analyze the open results. The *Analysis* tab lists the Analysis Parameter Set name and the defined values for that parameter set, as shown in Figure 116.

Figure 116: Analysis Tab, Properties Dialog
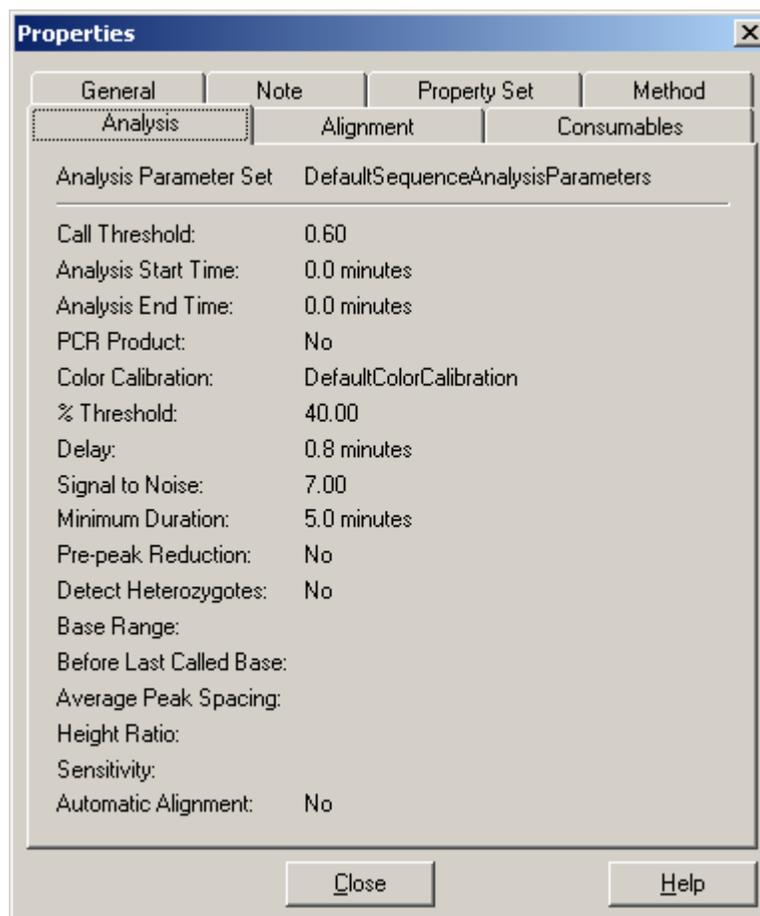
Alignment Tab

The user can view the alignment template information and the alignment parameters used to create the alignment of the currently open result. See Figure 117.

Figure 117: Analysis Tab, Properties Dialog
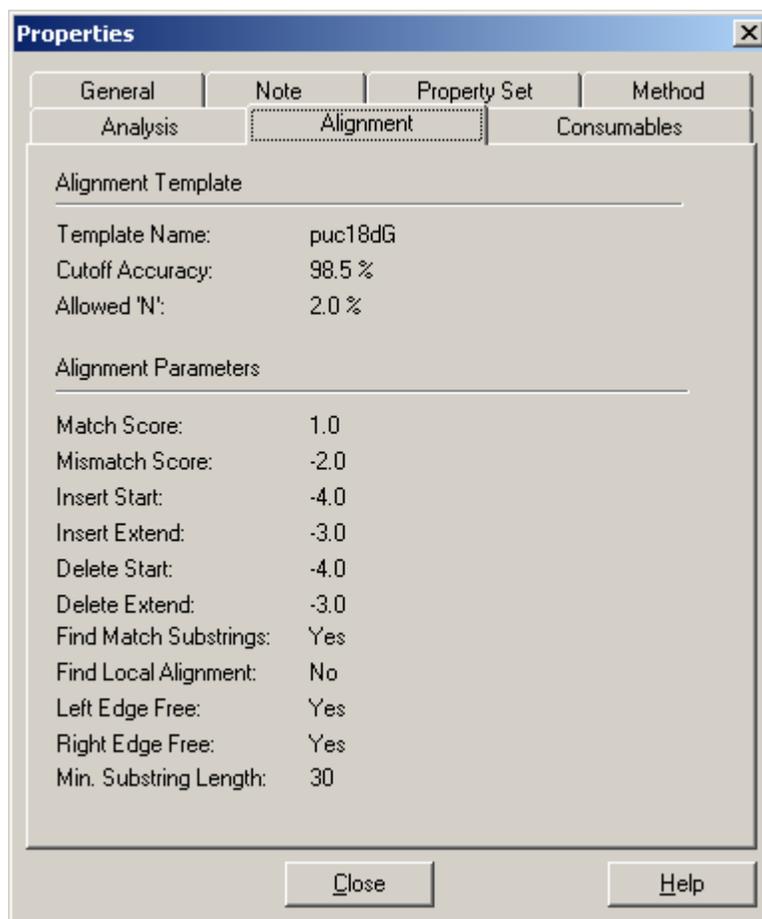
> *This tab will only be visible if an alignment has been performed on the sequence result.*

Consumables Tab

Information for all of the consumable products used to collect raw data associated with the currently open sequence result are presented in the tab. The capillary array information, the gel information and the buffer information are shown here, as defined in the *Run* module. See Figure 118.

Figure 118:Consumables Tab, Properties Dialog
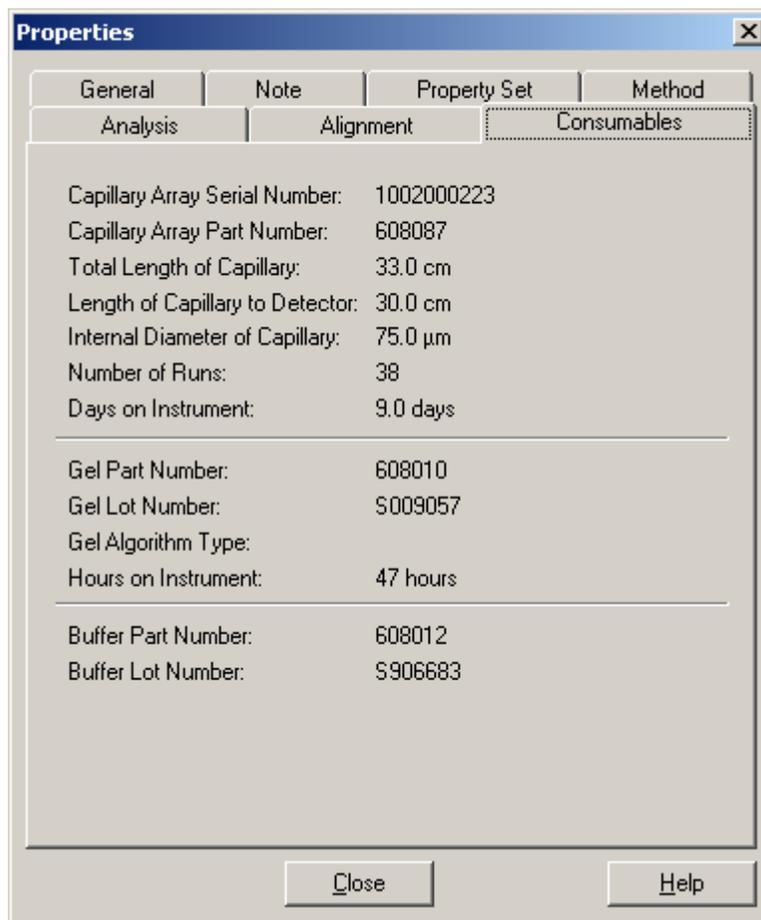
Setting or Changing Display Options

Setting or Changing Title Properties

To set or change the title of the displayed data panel, perform the following steps.

1. Double-click the left mouse button in any data pane (excluding the Base Sequence pane). The Display Options dialog box will open
2. In the **Display Options** dialog box, select the **Title** tab.
3. Change any item as necessary, then select **Apply** to continue or **OK** to close the **Display Options** dialog box.

Setting or Changing X Axis Options

To define the X Axis properties of the displayed data panel(s), perform the following steps.

1. Double-click the mouse button in the desired pane.
2. In the **Display Options** dialog box, select the **X Axis Options** tab.
3. Change any item as necessary, then select **Apply** to continue or **OK** to close the **Display Options** dialog box.

Setting or Changing Y Axis Options

To define the Y Axis properties of the displayed data panel(s), perform the following steps

1. Double-click the left mouse button in the desired pane.
2. In the **Display Options** dialog box, select the **Y Axis Options** tab.
3. Change any item as necessary, then select **Apply** to continue or **OK** to close the **Display Options** dialog box.

Setting or Changing Dye Trace Displays

To set or change the dye trace properties, perform the following steps.

1. Double-click the mouse button in the Raw or Analyzed data panes.
2. In the **Display Options** dialog box, select the **Dye Traces** tab.
3. Select the traces, to display or not display, under **Show Dye Traces**.
4. To change a color of any dye trace, click on the appropriate **Dye Colors** dye, select a color from the **Color** dialog box and then click **OK**.
5. When finished with Dye Traces, select **Apply** to continue or **OK** to close the **Display Options** dialog box.

Setting or Changing Current Trace Displays

To set or change the current trace options, perform the following steps.

1. Double-click the mouse button in the Current or Voltage data panes.
2. In the **Display Options** dialog box, select the **Current Traces** tab.
3. Select the radio button for the current type to display under **Data**. Click **OK**.

From the **Run** module:

1. Select **Tools | Display Options**.
2. Select the **Current Traces** tab.
3. To view the total current for the run, check the **Total Current** check box. To display current for individual capillaries, check the appropriate check boxes. Click **OK**.

Setting or Changing Color Options

To define the color options of the window, the current traces, the voltage trace or the optical scan trace, perform the following steps.

1. Double-click the mouse button in the Instrument Data or Data Monitor panes.
2. In the **Display Options** dialog box, select the **Colors** tab.
3. Change any item as desired and then select **Apply** to continue or **OK** to close the **Display Options** dialog box.

Changing Raw Data Colors

> **Note** *To display the Raw Data Colors toolbar, select View | Toolbars from the menu. Select the desired toolbar options and click Close.*

To change the colors of the raw data traces, perform the following steps.

1. On the **Raw Data Colors** toolbar, click on the trace color to change (**A**, **C**, **G** or **T** for Sequence Analysis and **D1**, **D2**, **D3**, and **D4** for Fragment Analysis).
2. In the **Color** palette dialog box, select the new color and then click **OK**.

Changing Heterozygote Display Color

The heterozygous bases will be bold and red, by default. You can change the color of the heterozygotes in the base sequence pane by selecting **Tools | Heterozygotes Display Color**. Select a new color and click **OK**. The color of the ambiguity codes will be changed in the base sequence text.

> Note — *You can search for heterozygotes by using the Heterozygote Backward Search* H *and the Heterozygote Forward Search* H *icons in the Standard toolbar.*

Pinning Results

Several analyses (single or batch) may be necessary before a given result is satisfactory. The CEQ System Software provides the ability to either overwrite a previous result, or produce additional result tabs.

1. To overwrite a previous result, click the pin in the left-hand corner of the result tab to "unpin" the result. A reanalysis of that sample will overwrite the existing result.
2. To produce a new results tab, click the pin to "pin" the result. Upon reanalysis, a new tab containing the reanalyzed results will be created.

Performing a Batch Analysis

To analyze more than one sample simultaneously, perform the following steps.

1. Select **Analysis | Batch Analysis** from the menu.
2. In the **Batch Analysis Selection** dialog box:

   a. Select a **Project** name from the drop-down menu at the bottom of the dialog box.
   b. Select the appropriate tab: **Sample Data** or **Sample Plate Results**.
   c. Select **Enable** and enter a filtering start and stop date, if desired.
   d. Highlight a sample name and click the right arrow to move the sample name to the right-side listbox. Continue highlighting sample names and moving them to the right-side listbox until you have selected all the samples for analysis.
   e. Select **OK**.
6. In the **Working Analysis Parameters** dialog box, edit the parameters, if necessary, then select **OK**.

Reanalyzing a Batch

To reanalyze a batch, perform the following steps.

1. After performing a batch analysis, select **Analysis | Reanalyze Batch** from the menu.
2. Select a new parameter set or edit the currently selected set from the **Working Analysis Parameters** dialog box to obtain different results.

3. Select **OK** to start the reanalysis.

> | Note | ***To keep the results from the previous analysis, "pin" the results tab by clicking on the pin icon*** ⊣▣ ***. Otherwise, the results will be overwritten by any subsequent reanalyses.***

Viewing the Selected Batch Result

To view the selected batch result after performing a batch analysis, click the desired sample in the upper pane of the Batch Analysis window and then select **Analysis | View Selected Batch Sample Result** from the menu.

Skipping the Current Sample Analysis in a Batch

To skip the currently executing analysis of a *sample*, while a batch analysis is running, select **Analysis | Skip Current Sample** from the menu.

Skipping the Current Sample Set Analysis in a Batch

To skip the currently executing analysis of a *sample set*, while a batch analysis is running, select **Analysis | Skip Current Sample Set** from the menu.

Skipping the Current Sample Plate Analysis in a Batch

To skip the currently executing analysis of a *sample plate*, while a batch analysis is running, select **Analysis | Skip Current Sample Plate** from the menu.

Restoring the Original Sequence Data

With a Results file open, select **Analysis | Restore Original Base Sequence** from the menu. This option will return the result data to its original state, losing all edits.

Compare Mode

In the Sequence Analysis module, you can select up to 15 results to compare against the open result, enabling the comparison of a maximum of 16 sequence results at one time.

If you select more than 16 results from the Compare dialog box you will be presented with the following warning.
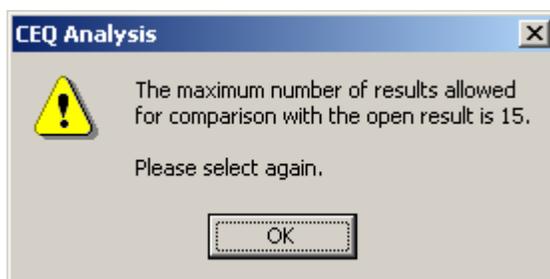
Comparing Sequence Results

1. Launch the Sequence Analysis application.
2. Select **File | Open**.
3. Click on the **Sequence Results** tab and select one sequence result and click **OK** to open the result.
4. Click on the **Compare** button on the Sample View toolbar to open the Compare dialog box.
5. Select up to fifteen results and click **OK**.
6. The system will process all fifteen results and the original result, and will display the results on the screen.

> *The originally opened sequence result will be displayed in the top pane.*

7. Enable Compare Synch by selecting **Tools | Compare Synch**.

Zooming Results

8. Click on the Zoom Mode button and zoom in on a region in the original sequence result and the other fifteen results zoom into the same region.
9. Select **Tools | Unzoom All** and all sixteen results zoom out so you can view the entire result.

Paning Results

10. Click on the Pan Mode button.
11. Pan through the data in the original sequence and all sixteen results will pan together.

12. Pan through the data in a result other than the original one and all sixteen results will pan together.

13. Use the horizontal scroll bar to move the data along the X-axis.

14. The data panes will scroll synchronously.

Aligning Results

15. Click on the Align Mode icon.

16. Select a point in the original result.

17. Select points in the other results you wish to align with the point in the original result.

18. Access the Display Options dialog box by double clicking in the original result pane. If you choose the X axis or the Y axis range, the range will change in all of the panes.

19. Disable Compare Synch by selecting **Tools | Compare Synch**.

20. Check zoom, align and pan and the panes will function independently of each other.

21. Close the Compare tab by selecting **File | Close Tab**.

Sequence Result Report

The sequence result report includes additional information when certain Sample Elements are selected in the Report Format dialog box. The following table describes what each sample element includes.

Header	The header contains summary information for the sample such as sample name, position, the method under which it was run, the sample plate name, the 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 box.*
Raw Data	The raw data is the unanalyzed data from the sample run.
Result Data	The result data is 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	The result output is 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	Current is the trace associated with the current output of each capillary or the total current of the sample set run.
Voltage	Voltage is the trace associated with the voltage output of the system during the sample set run.
Analysis Log	The Analysis Log contains all of the steps used to perform the base-calling routine on the raw data.
Run Log	The Run Log contains all of the actions performed and instrument messages for the sample run.
Method Summary	The Method Summary contains the details of the method used to perform the sample run. Note: *This is the information found on the Method tab of the Properties dialog box.*

Analysis Parameters	The Analysis Parameters are 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 is the information found on the Analysis and Alignment tabs of the Properties dialog box. No alignment information will be included if an alignment was not performed.*
Quality Parameters	Quality parameters are 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:	This log details the parameters used in *Quality-based* and *Sequence-based* trimming. It includes the original sequence length, the length after Quality-based sequencing, the length after sequence-based trimming, the vectors trimmed and the base sequence in its original and trimmed forms. For sequence-based trims, an "x" will replace 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	The alignment results are 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	The alignment accuracy is the statistical analysis of the accuracy of the sample sequence. The errors in the alignment are grouped into 50-base regions and the number and type of error for each region is displayed.

To define the format and contents of the report, select **File | Report Format**. Select **File | Print Report** to print the report without previewing or formatting prior to printing.

Select **File | Print Preview** to the view the report, shown in Figure 119.

Figure 119: Sequence Results Report

Project : Sequence   System : CEQ 8080   Operator :
Sample : testseq.A04_03030518NG   Instrument : CEQ 8080 (Ver. 7.0.26)
Result : testseq.A04_03030518NG

Sequence Results: testseq.A04_03030518NG

Created:	03/05/03 20:33:42	Project:	Default
		System:	CEQ 8080 [Rev 0]
Modified:	03/05/03 20:33:42	Project:	Default
		System:	CEQ 8080 [Rev 0]

Sample Name: testseq.A04_03030518NG
Sample Plate: testSeqLong
Sample Plate Barcode: 234567891000
Sample Position: A04
Method: LFR-a

Instrument: CEQ 8080 (Ver. 7.0.26)

Analysis Parameters: DefaultSequenceAnalysisParameters

Note:

Properties:

Consumables:

Capillary Array Serial Number:
Capillary Array Part Number: 608087
Total Length of Capillary: 33.0 cm
Length of Capillary to Detector: 30.0 cm
Internal Diameter of Capillary: 75.0 µm
Number of Runs: 9
Days on Instrument: 2.0 days

Gel Part Number: 391438
Gel Lot Number: CEQ Sequencing Gel
Gel Algorithm Type: 0
Hours on Instrument: 49 hours

Buffer Part Number: 608012
Buffer Lot Number: CEQ Sequencing Separation Buffer

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Displaying Quality Parameters in a Report

1. To display the Quality Parameters in a report, go to the **File** menu and select **Report Format**.
2. In the **Sample Elements** section enable **Quality Parameters** then click **OK**.

Figure 120: Report Format Dialog Box
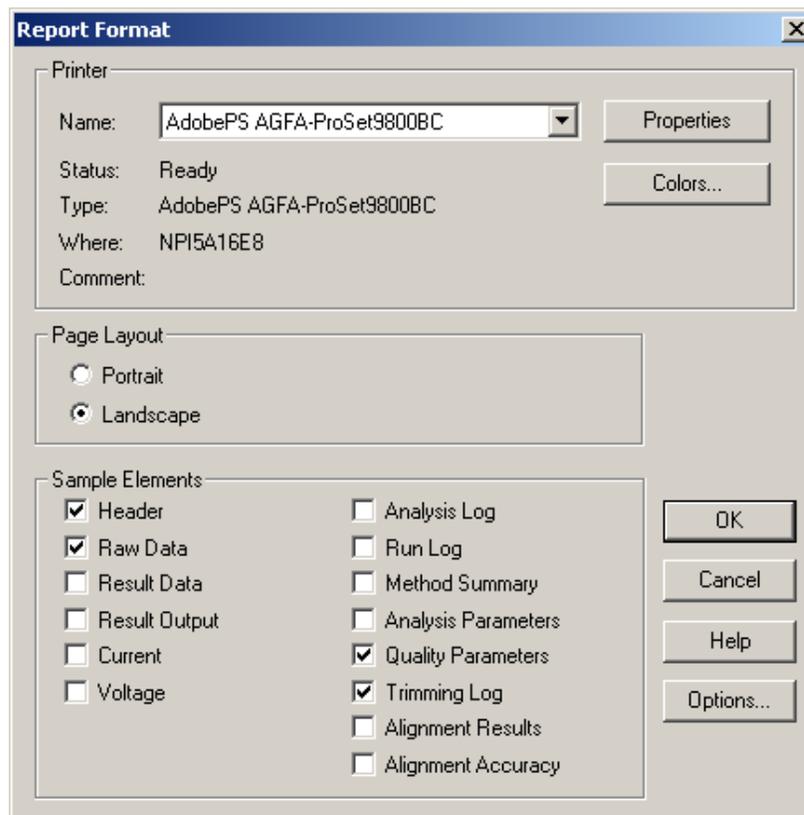
3. To view the Quality Parameters of an analyzed data set prior to printing, go to the **File** menu and select **Print Preview**. To exit the Print Preview option, click **Close**.
4. To print the Quality Parameters for that data set, go to the **File** menu and select **Print Report**. The Quality Values will be printed as numerical values and will appear as shown in Figure 121.

Figure 121: Quality Parameters Report

The categories on the Quality Parameters Report are described in Table 70:

Table 70: Quality Parameters Report Categories

Number	the number of the called base
Base	the single letter designation of the called base (A, C, G, T or other IUB code)
A	base identity score assigned by the base-caller, ranging from 0 - 255
C	base identity score assigned by the base-caller, ranging from 0 - 255
G	base identity score assigned by the base-caller, ranging from 0 - 255
T	base identity score assigned by the base-caller, ranging from 0 - 255
Call Score	the calculated probability of correctness indicated on a linear (0.00 - 1.00) scale
Quality Value	the probability of correctness presented on a logarithmic (1 - 100) scale
Migration	the actual migration time (in minutes) in the raw data corresponding to the called base(s)

Data Point	the actual number of the data points in the analyzed data corresponding to the called base(s)
Insert	indicates a base that has been manually added to the base sequence
Edit	indicates that the base call has been manually changed

Magnifying (Zooming) Data

To magnify data, perform the following steps.

1. Click on the upper left-hand point of the area to be magnified, drag the mouse cursor down to the lower right-hand point of the area and then release the mouse button.
2. Repeat this procedure to increase magnification.

> *Note* *Use the Unzoom* 🔍 *or Unzoom All* 🔍 *icons to zoom out.*

Panning Data

To pan back and forth in the data pane, perform the following steps.

1. Select **Tools | Pan** from the menu.
2. Click anywhere in the Raw or Analyzed Data panes and drag the mouse cursor left or right to move the display.
3. Deactivate the Pan Mode by selecting **Tools | Zoom** from the menu or by clicking on the **Autoscale** button.

Synchronizing Result Data with Result Output

To synchronize the Analyzed Data with the Base Sequence, perform the following steps.

1. Access the Sequence Analysis module.
2. Open the desired analyzed data.
3. Select **Tools | Base Synch (Peak Synch)** from the menu, or click the **Synch** ⬆ icon.
4. Select a base or range of bases in the base sequence pane. The corresponding peak(s) will appear between two hairline markers in sequence analysis.

Aligning Bases and Peaks

To align the bases in the Base Sequence pane with the peaks in the Analyzed Data pane perform the following steps.

1. Select **File | Open** from the menu.
2. In the **Open** dialog box, select the **Results** tab, highlight the desired sequence results and then select **OK**.

3. To visually align the peak with the base call, click the **Align Mode** icon, then click down on the apex of the peak for which you wish to view the alignment. (A hairline cursor is displayed through the apex of the peak to its corresponding base.)

4. Click the **Zoom, Pan** or **Edit** button to exit from the Align mode.

Specifying the Base Call Location

To specify the base call location, perform the following steps.

1. Open a Sequence Results file.
2. Select **Tools | Bases on Top** from the menu to toggle the bases from the top to the bottom of the Analyzed Data pane or vice versa.

Using Audio Enable

To enable the audio feature of the software, perform the following steps.

1. With a Sequence Results file open, select **Edit | Audio Enable** from the menu.
2. Enter text into the Base Sequence pane to hear the letter bases announced as they are typed.

> **Note** *To enter text in the Base Sequence pane,* **Edit** *mode must be enabled.*

Using Audio Playback

To enable the audio playback feature of the software, perform the following steps.

1. With a Sequence Results file open, highlight a series of base sequence text.
2. Select **Edit | Audio Playback** to hear the announcement of the highlighted base sequence text.

> **Note** *To use this feature,* **Edit** *mode must be enabled.*

Viewing Color Calibrations

To view a color calibration for a sequence result, perform the following steps.

1. Select **File | Open** from the menu.
2. In the **Open** dialog box, select the **Sequence Results** tab, highlight the desired result name and then select **OK**.
3. Select **View | Parameters Used to Compute Sequence** from the menu.
4. In the **Parameters Used** dialog box, select **Color**.
   • To view the color calibration prior to the run, select the **Initial Values** radio button.
   • To view the color calibration after the run, select the **Final Values** radio button.

> **Note** *This is a read-only dialog box. To make changes to the color calibrations, select* **Analysis | Working Analysis Parameters | Edit | Color.**

5. To add a successful color calibration to the list of available color calibrations, click **Save As** in the **Color Calibration Editor** dialog box. Enter the desired name and click **OK**.

Computing a New Color Calibration

To compute a new color calibration, perform the following steps.

1. Select **Analysis | Working Analysis Parameters**.
2. In the **Working Parameters** dialog box, select the **Edit** button.
3. Click the **Color** button in the **Sequence Analysis Parameters Editor** dialog box.
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).

Sequence Result Export

To export from the Sequence Analysis Module, follow the steps below:

1. Launch the Sequence Analysis application and open a sequence result.
2. Select **File | Export** to open the Export dialog box.
3. Select a target folder and file name.
4. Select the desired format in the Save as type text box as shown in Figure 122.

Figure 122: Sequence Analysis Export Dialog
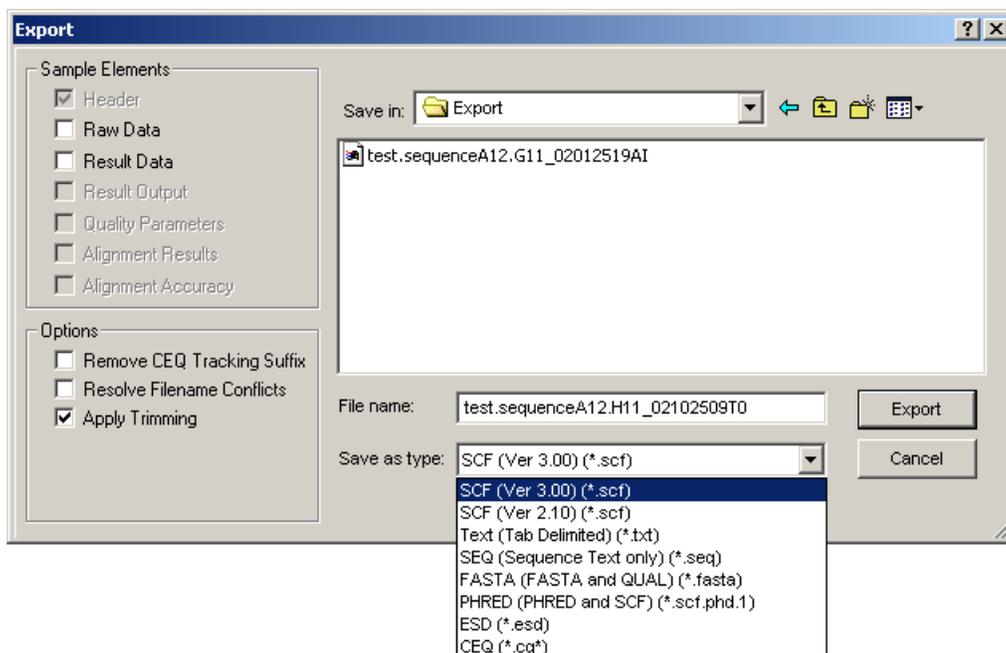
5. Click **Export**.

Sequence Export File Types

Listed in Table 71 are the available export file types and options.

Table 71: Sequence Export Options

Format	Description
SCF (.scf)	The SCF (Standard Chromatogram Format) format is used 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 trace data points, the called sequence, the positions of each called base within the trace data, and the call scores for each called base. A public comment section is also defined. The "private" section can be used to store other information that will generally be ignored by most software packages. The CEQ System uses the private data section to store sample data (including raw data along with current and voltage data). Sample data from either sequencing or fragment analysis samples can be stored. The notes and property sets for the sample are stored in the comment section. The CEQ 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 Inc.). 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 CEQ Sequence Analysis module. If you use SCF to exchange data between instruments you will lose informational data such as sample plate name, capillary array serial number, etc. If you wish to maintain exact copies of data including header information, use the CEQ format and import and export these data via the Data Manager.
Tab-Delimited Text (.txt)	The Tab-delimited format is valid for raw data, sequence results and fragment results. Check the Raw Data check box to include raw data, as well as current and voltage data. Check the Results Data and Results Output check boxes to include the sequence results. The data points will be expressed as text values and can be viewed in any text editor or in Excel.
SEQ (.seq)	The Seq format only applies to the sequence results portion (e.g., text bases) of analyzed sequence data. This format is used, for instance, to export data into Lasergene's SeqMan from DNASTAR Inc. If the SEQ format is selected, the Result Output (Base Sequence) will be exported 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 will be exported.

Table 71: Sequence Export Options (Continued)

Format	Description
FASTA (.fasta)	The FASTA format only applies to the sequence results portion (e.g., 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 will have a value of 99. You would use this format, for instance, to export data into PHRAP. If the FASTA format is selected the Result Output (Base Sequence) and the Quality Parameters will be exported 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 will be exported. *Note* ***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 CEQ System, we bypass PHRED and export directly into PHRAP.***
PHRED (.phd.1)	The PHRED format will produce 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 and "99" respectively for CEQ 2000. The TIME stamp is the current time at the time of export. The DNA section is composed of the base-call (always lower-case), PHRED Quality Value, and the Peak Index (data point) of the analyzed data contained within the associated SCF file. Values are separated by spaces. If the PHRED format is selected the Result Data, Result Output (Base Sequence), and Quality Parameters will be exported 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 will be exported.
ESD (.esd)	The ESD, electropherogram sample data, format is used to export raw data from the CEQ System to a third-party software package for analysis. When you select this option, on the raw data will be exported, and no other information is included.

Table 71: Sequence Export Options (Continued)

Format	Description
CEQ (.cq*)	If you select the CEQ format, the format native to our database, you will export 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 box, such that all of the associated information listed above will be exported. This format essentially takes a "snapshot" of the file and is able to retain all of the information belonging to that file. This format can only be used when importing data into the Data Manager. This format can also be used when exporting data from the Sequence and the Fragment Analysis modules and the Data Manager. When exporting as CEQ data, the various data types are exported to files with the following extensions: Sequence Analysis Parameter CQA CEQuence Investigator CQC Fragment Size Standard CQD Fragment Analysis Parameter CQF Galvo Scan Data 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 may automatically remove the plate position coordinates (*.A01, *.B02, etc.) and the time/date stamp from the sample name which is normally generated by the CEQ software on export. To do so, check Remove CEQ Tracking Suffix.
Resolve Filename Conflicts	If the system encounters the same sample name at the destination, the name will normally be overwritten (this may happen if you choose to remove the plate position coordinates and the time/date stamp which ensures a unique name). If you check Resolve Filename Conflicts the system will increment the sample name(s) on export when it encounters a sample(s) with the same name(s). For example, if the system encounters the duplicate sample name of Fred on export, the system will rename the file exported to Fred_1.
Apply Trimming	Selecting the Apply Trimming option will remove the trims from the sequence before it is exported. All internal trims will be replaced by the letter "x".

Customizing Filename Extension

To supply your own custom filename 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 and the Export section will be generated. Then modify as needed.*

1. 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 ".phd.1"

2. To have no file extension added, set the entry to nothing (make sure there are no spaces after the "=")

    SCF_EXT=

    Example Output FileName: SpaSample

3. To use a unique file extension, add or modify the entry with the desired file extension (do not place the leading dot ".", the system will add it).

    SCF_EXT=scf v3

    Output FileName: SpaSample.scf v3

4. To hide an entry and make the system use the default, either delete or place an ";" before it

    SCF_EXT=SCFV3

    Output FileName: SpaSample.scf

Exporting Data to a Third Party Package

> **!** **CAUTION** **Verify that the third party analysis package will accept the data transferred, via a command-line, and that it is compatible with the file types provided by CEQ System Software before attempting to perform this procedure.**

To export sequence data to a third party analysis package, perform the following steps.

1. Select **File | Open** from the menu.
2. In the **Open** dialog box, select the appropriate tab, highlight the desired data and then select **OK**.
3. Select **Analysis | Third Party Analysis Setup** from the menu.
4. In the **Third Party Analysis Setup** dialog box:

    a. Specify the path and executable file of the third party analysis package.

   b.   Select the appropriate **Export Data Format** radio button and then click **OK**.

3.   Select **Analysis | Third Party Analysis** from the menu.

4.   In the **Export** dialog box, enter the filename and then click **OK**. The data will be exported and the third party package launched with the exported data open.

CEQuence Investigator Procedures

Creating a Reference File

The reference file is a text file that can be created 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. The sequence text may be broken up 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, perform the following:

1. On the line below the reference sequence, type an open bracket, then "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.

A sample reference is shown below.

```
reference1.txt - Notepad
File  Edit  Search  Help
TGGGTAAAACGACGGCCAGTGCCAAGCTTGCATGGCCTGCAGGTCGACTCTAGAGGATCCCCGGGKACCGAGCTCGAATT
CGTAATCATGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAA
AGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGA
AACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTC
CTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTAT
CCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCG
TTGCTGGCGTTTTTTCCATAGGCTCCGCCCCCCTGGACGAGCCATT

[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
```

Reference Sequence	TCCTGTC
Amino Acid Mutations	[KNOWN AA MUTATIONS]
Known Amino Acid Notation	Q2K
Base Mutations	[KNOWN BASE MUTATIONS]
Known Base Notation	A30C

> **Note** *A default Reference folder is created upon installation of the software. 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.*

Selecting a Reference and Sequence Results

Open the **CEQuence Investigator** application and select **File | New** or click the New ▦ button on the CEQuence Investigator Standard toolbar. An Open dialog appears listing the contents of a reference folder. Select the sample reference and press the Open button.

Figure 123: Open Reference Dialog
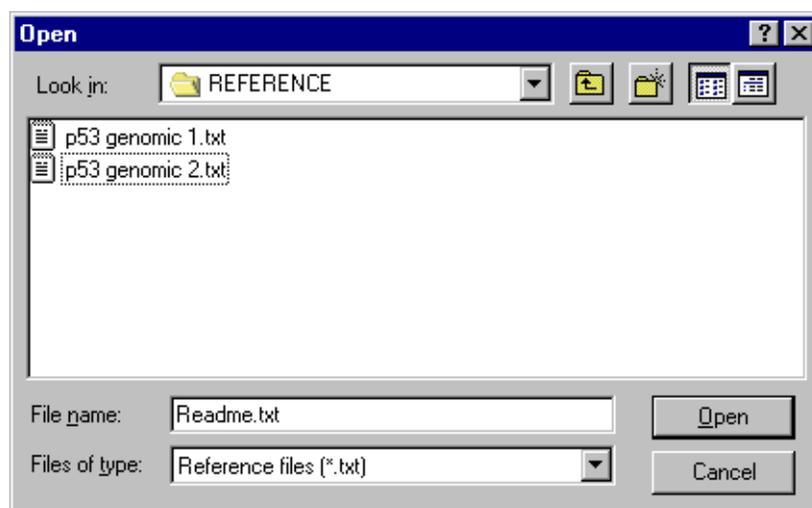
The Open Sequence Results dialog will then be displayed. Select one or two results and press **OK**.

> *To select two results, highlight the first result, hold the* **Ctrl** *button down and use the left mouse button to highlight second result.*

Figure 124: Open Sequence Results Dialog
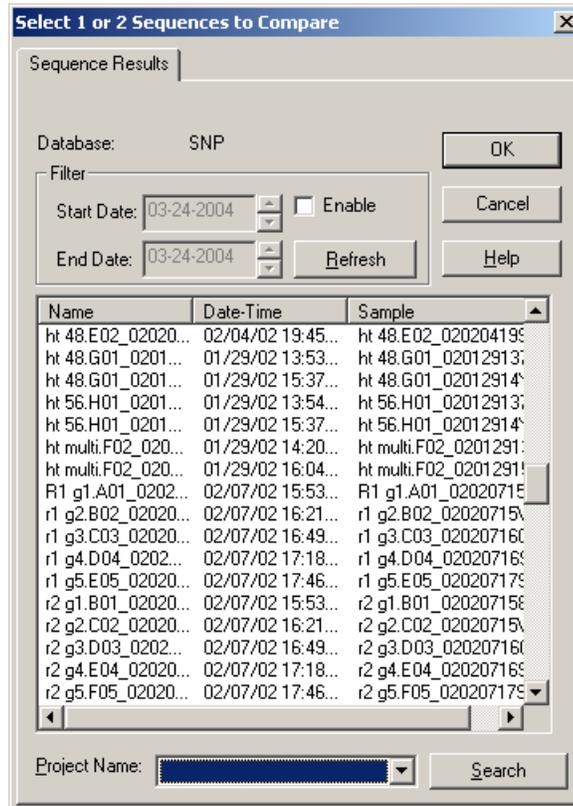
If two results are in different projects, select "(none)" as the Project Name to display all available results files for a given database.

A new window will appear as shown in Figure 125.

Figure 125: CEQuence Investigator Main Screen
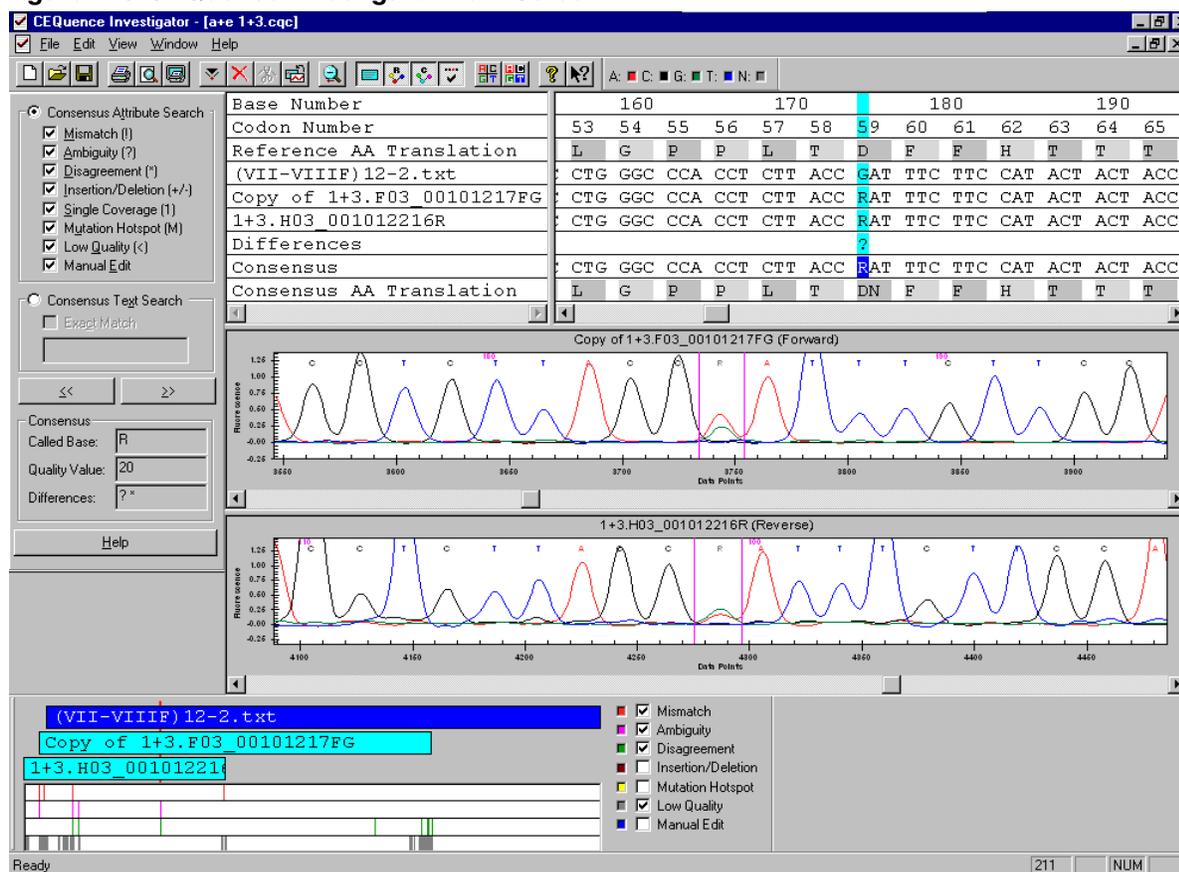
By default the Standard, Navigator, Dye Color, and Discrepancy Map toolbars are displayed and docked. If they are not displayed, select **View | Toolbars** and enable the desired toolbar.

Numbering

Base and codon numbering start at the beginning of the reference sequence. They are used 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 is displayed in the lower right-hand corner of the Status bar. All edits made in CEQuence Investigator, and their column number, are recorded in the History log.

Viewing 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 displays 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 displayed above the codon. Each codon will be shaded to show its boundaries. (If an inserted space exists, signified by a dash "–," the space will be 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 will be displayed. If more than two possible amino acids are detected at any location, an asterisk is displayed instead.

You may show or hide the reference amino acid sequence by clicking on the **View** menu, and then on **Reference Amino Acid Translation**. The reference amino acid translation will be shown or hidden, depending on whether the item is checked or unchecked.

Viewing Sequence Traces

When you first perform a comparison, you select one or two sequences to form the consensus. Once the comparison is performed, the traces for these sequences are displayed. When you click on any position in alignment view, the corresponding peak(s) in the traces will move to the center of the trace view and will be framed by two vertical lines.

When you first open or create a comparison, the traces will be scaled for ease of readability. You may zoom each sequence pane individually and repeatedly by clicking down on the left-hand mouse button, dragging the mouse pointer to form a box around the desired area and then releasing the mouse button.

To unzoom both traces, click the **Unzoom All** button . The traces in both panes will revert to a completely unzoomed state, displaying the entire range of the sequence result(s).

To unzoom one of the two open data traces, right-click in the desired trace pane and select **Unzoom Pane**. The selected trace will be unzoomed to display the entire range of the result.

You cannot edit sequence traces in Sequence Investigator. However, sequence traces can be edited in the Sequence Analysis module. If you use an edited result for your comparison, your edited bases will appear in lower case.

Viewing Consensus Amino Acid Translations

When the system generates the comparison, it translates the consensus nucleotide sequence into an amino acid sequence and displays 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 re-translation of the consensus amino acid translation occurs automatically and the Discrepancy Map and the Differences line are updated automatically.

Each codon will be shaded to show its boundaries. If a heterozygote occurs, the system will note up to two possibilities; if more possibilities exist, an asterisk is shown instead.

The consensus sequence is editable within Sequence Investigator. Your edited bases will appear in lower case.

Quality Values

If you are using a single sequence, the quality value assigned to each base in the consensus will match each base in the sequence. If you are using two sequences and the bases agree, one of two calculations will occur: If the orientation of the two sequences is opposing, the two quality values will be added. If the orientation is the same, the higher quality value will be used. If the bases disagree, a calculation will be performed to determine the probability of error for that position, providing a quality value that will be the difference between the higher and lower value.

To set the value at which bases in the consensus will be considered low quality, select **Edit | Quality Threshold**. Low quality bases will be identified in the Differences line, the Navigator toolbar and the Discrepancy Map.

Viewing CEQuence Investigator Differences

The system uses codes to denote the type of discrepancy encountered when the comparison was performed. These codes are shown in the Differences section of the Alignment pane. You may turn the Differences line on or off by clicking the **View** menu, and checking or unchecking **Differences**. The highest level code will appear 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 been assigned a hierarchy; the code with the highest precedence will be shown. To view all difference codes associated with any position, ensure the Navigator toolbar is displayed by clicking on the* **View | Toolbars | Navigator.** *The Differences text box in the Navigator toolbar will display the difference code(s) in order of precedence.* |

Navigation and Editing

The Navigator toolbar allows you to search for locations of interest in the consensus based on attributes. You may also search for specific base(s) and view their associated quality values and difference code(s).

To use the Navigator toolbar, click **View | Toolbars** menu option, then check the **Navigator** option. The Navigator toolbar is displayed.

Figure 126:Navigator toolbar
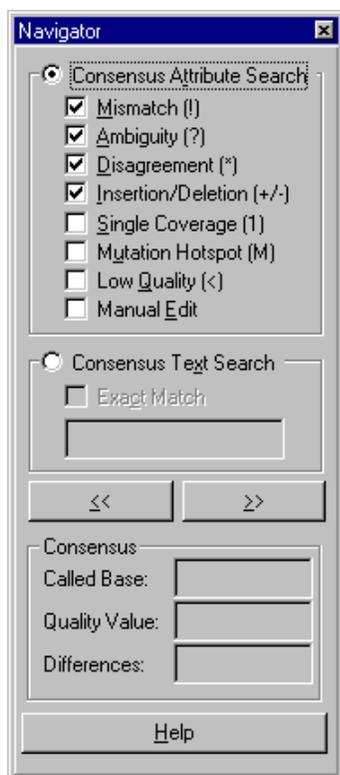
> *Trim operations must be completed before any other editing. After any other editing, the trim feature will be disabled.*

> *You may insert and delete spaces in the Reference Sequence, but no bases may be changed.*

> *Base editing can only occur when a single consensus base is highlighted.*

> *All edit operations, including Restore to Original, are added to the history log along with the date, time and position of the operation. These operations will be retained in the history log.*

Complementing the Sequence

When the system performs a comparison it will determine which orientation, forward or reverse, provides a better fit with the reference. If the system determines that the sequence(s) will generate a better comparison in the opposite orientation, it will automatically reverse complement the sequence(s). You can manually reverse complement a sample by right-clicking on the desired Trace Data pane, and then selecting Reverse Complement from the menu. The trace and associated bases will be reverse complemented.

Flipping the Traces

You may want to switch the order of sequence presentation. To move the sequence trace shown in the top trace pane and exchange its position with the bottom trace, click **Edit | Flip Traces** or right-click in one of the trace data panes and select flip traces from the menu. The position of the sequences is exchanged in both the alignment pane and the trace panes.

Trimming the Bases

When you first open or create a compared sequence result, the sequence(s) used in the comparison will appear in the alignment pane. You can then trim the ends of the sequences.

If sequences are not shown, select **View | Sequences** to display the sequences in the alignment pane.

To trim the sequence, select a group of sequence bases at either end of the sequence and then click on the Trim button to remove unwanted bases from the alignment. The bases will be deleted 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.*

After each trim, the software will automatically align and redisplay the alignment text and traces.

> **Note** *Trim operations are added to the history log along with bases trimmed and date and time of trim operation. The history log is accessed by selecting* **View | History.**

Searching the Consensus for Specific Attributes

You may search the consensus for specific attributes. To do so, click on the **Consensus Attribute Search** radio button and check the attributes of interest that you wish to define as the search criteria. Then click on the forward >> or reverse << buttons to search the consensus in the specified direction. You may also press the **Ctrl** and the **arrow** (←, →) keys to perform the search. Consensus bases that conform to the search criteria will be highlighted when encountered by the search.

Searching the Consensus for Specific Bases

To search for a specific base or contiguous multiple bases, click on the **Consensus Text Search** radio button and enter the bases in the text box.

To search for the exact match for text, check the **Exact Match** check box. Use the forward and back arrow buttons or press the **Ctrl** and **arrow** (←, →) keys to search backwards and forwards though the base sequence for more text searches.

To search for a base without requiring an exact match, uncheck the **Exact Match** check box. The searching result will be all exact matches as well as all IUB codes with an overlapping base. That is, a search for the ambiguity code **K** will not only result in the bases **G** or **T**, but will also result in any ambiguity code that *contains* **G** or **T**. In fact, the search would stop 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 will match any ambiguity codes containing the base. For example, searching for **A** will result in any ambiguity code containing **A** (specifically, M, R, W, U, H, D, N).

## Editing the Consensus

You may edit the base selected by the search by typing the desired base, or by clicking on the **Edit** menu, then on **Replace**, and then selecting the desired base or ambiguity code. You may also automatically jump to the next base that matches your search criteria once you have completed the edit by selecting Forward or Backward on the Edit tab of the Preferences dialog box.

Displaying 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 sequence(s). You may also view the color-coded locations in the experiment of any type of attributes of interest that you specify.

Figure 127:Discrepancy Map toolbar
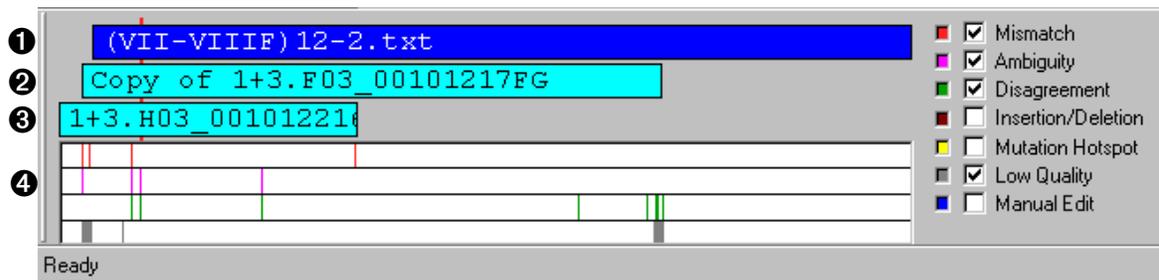
The top bar, ❶, in the discrepancy map represents the reference sequence.

The second ❷ and third ❸ bar (if two sample sequences were selected) displays the coverage of the sample sequences in relation to the reference.

The next area, ❹, 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 will refresh and display that area.

For a global view of the desired attributes, check the check box(s) located on the right side of the map. For each attribute checked, a new bar will be added to the consensus area showing the location(s) where the selected attribute exists. To change the color-code of the Attribute Display, click on the color well at the left of the attribute. The color palette is displayed. Select a new color, then click on OK. The new color will be used for that attribute in the Discrepancy Map.

Printing and Reports

You may print a report for the active compared sequence result. To set up your report click **File | Report Format** and select the default printer to use by clicking on the **Printer Name** down-arrow and selecting the desired printer. Set your preferred page layout by clicking on **Portrait** or **Landscape** and select the following option elements for your report:

Element	Description
Header	The Header portion of the report 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	The Alignment Results portion of the report prints the reference amino acid translation, the reference, the sequence(s), the difference codes, the consensus and the consensus amino acid translation.
History Log	The History Log portion of the report will print a history of changes you performed in the alignment.
Statistics	The Statistics portion of the report prints the statistics of the report, e.g., the number of discrepancies before and after editing.
Mutations	The Mutation section of the report will print the known base mutations and amino acid mutations and the novel base mutations and amino acid mutations.

Exporting

You may export the active compared sequence in either of three formats; text (*.txt), tab delimited text (*.txt) and protein (*.pro). The text file will export the base sequence of the consensus. In tab delimited text files, data appears in a single line and all carriage returns (↵) are replaced with tabs (→). The protein file will export the amino acid sequence. You may specify one of two protein formats; Condensed or Expanded, in the Preferences dialog box under the File menu.

If you specify Condensed, all protein translations will be provided in the same text line. Heterozygotes will be designated within brackets.

If you specify Expanded, all possible translations will be exported on different lines.

Figure 128: Comparison of condensed and expanded protein translations
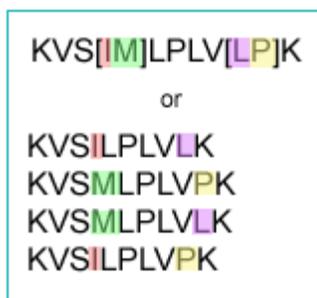
The export formats available are text files (**.txt**) and protein files (**.pro**). Click **OK** to export the data. An hourglass will be displayed while the data is being exported.

If a file with the same name exists in the selected directory, you will be given the option to overwrite the existing file or rename the new file.

Saving

To save a new document, select **File | Save** from the menu toolbar. A **Save As** dialog will be displayed prompting you to enter a file name and folder location. Press **Save** and the data is saved to the flat file for later retrieval.

If the document has previously been saved, select **File | Save** from the menu toolbar and the document will be saved immediately.

To change the name or location of an existing file, select **File | Save As** from the **Menu** and a **Save As** dialog will be displayed prompting you to enter a new file name and folder location. Press **Save** to execute the command.

Fragment Analysis Procedures

The CEQ System Fragment Analysis module estimates DNA fragment sizes and amounts and identifies alleles represented by specific DNA fragments. Parameters used to identify alleles are organized as locus tags that can be customized by the user. The results of the analyses may be viewed, exported and printed.

Analyzing fragment or allele data with the CEQ System software is readily carried out with the use of "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 which are analyzed and exported together. The user defines a Study by selecting the samples to be included and then applying the parameters used to analyze the fragment data or allele data.

Performance Safeguards

Available controller resources are checked before result processing begins. If it is determined that the resources needed to complete the analysis are not available, a warning dialog (see Figure 129) will open. If this occurs, it is recommended to close *all* applications, re-start the Fragment Analysis module and attempt the analysis again.
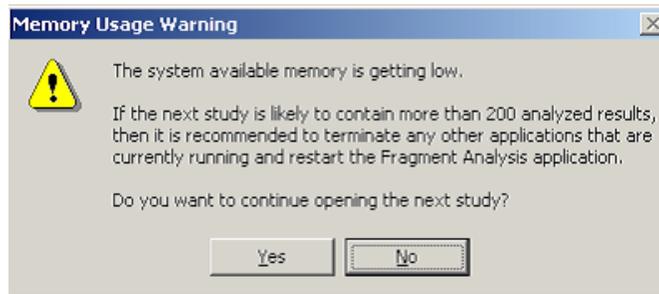

Figure 129:Memory Usage Warning

Working with Studies in the Fragment Analysis Module

A "**Study**" is a set of multiple sample results together with the results of analyses performed on the group of sample results.

Creating a new Study can be easily carried out with the help of the **Study Wizard**. 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 is automatically displayed when the Fragment Analysis module is launched. A new or existing Study may be opened immediately from this window or at any time while working within the Fragment Analysis module. To access the Study window, open the **File** menu and select the **New Study** or the **Open Study** command.

Figure 130: Study window - Creating or Opening a Study
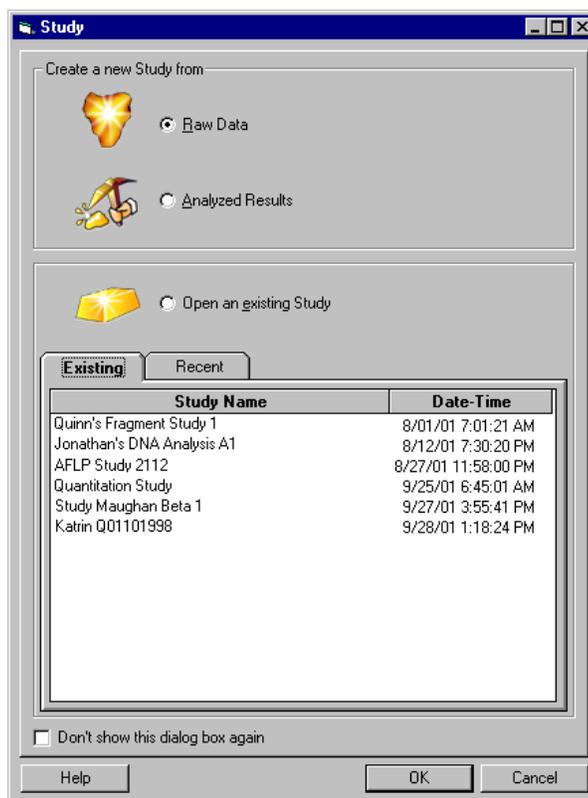
A new **Study** can be created from either **Raw Data** or previously **Analyzed Results**. Make your selection using one of the option buttons and click **OK** to continue. The Study Wizard will be launched to guide you through the steps of creating a Study. After the samples in your Study have been analyzed, you will have the opportunity to include additional results in the new Study.

To open an existing **Study**, select it from the list of Study names available under either the **Existing Studies** tab or the **Recent Studies** 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.

Selecting 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 the *first level of filtering*.

Following analysis of these samples, you will have the opportunity to further "filter" the sample results (*second level filtering*) in the **Result Set View**.

Finally, you will "filter" the generated fragment and allele data (*third level filtering*) in the **Fragment List**. What remains will be a collection of identified fragments and alleles that exactly match the requirements of your Study.

Selecting Raw Data for the New Study

When you select the **Raw Data** option button and click **OK**, the **Study Wizard** will be launched and the **Select Raw Data** window will be displayed.

Figure 131: Select Raw Data window - Selecting the Components of the New Study
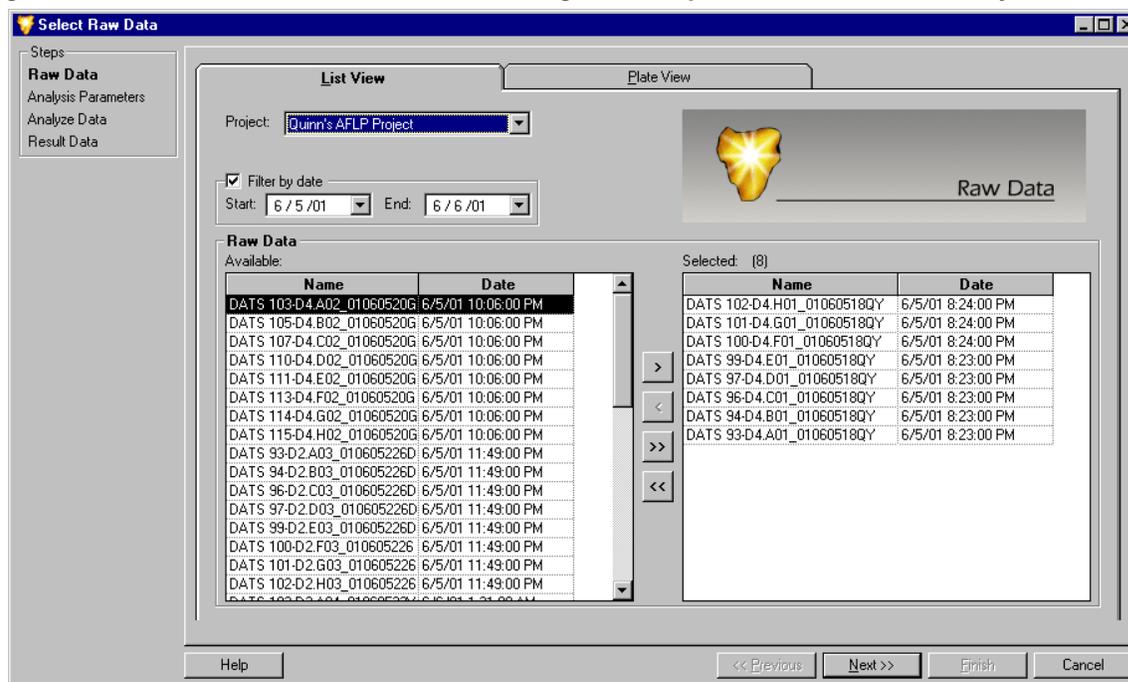
This window is used to select the raw data to be analyzed from a database of samples that have been "run" in a particular project(s). The raw data selected in this window will be included in your Study and analyzed in the subsequent steps.

> **Note**
> *You may view an individual sample electropherogram and other collected parameters prior to adding the sample to your Study by right-clicking on a highlighted sample and selecting the Show Single Sample View command from the menu displayed.*

The **List View** and **Plate View** tabs provide you with two methods of making your raw data selections. Each selection method contains the same sample information presented in different ways. Which method you use is a matter of personal preference.

Selecting Raw Data from the List View tab

The **List View** tab allows you to make your selections from a list of samples contained in a specific database and/or collected during a particular time frame.

1. Click the **List View** tab available 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** drop-down list box.
   - Click on the down-arrow located on the right side of the **Project** list box to reveal the available projects in the working database.

- If the project you are looking for is not displayed, it may be located in a different database.
- To enter a different database, all applications must be closed. See the topic "Set Working Database" in the Online Help for more information.

3. 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 will display only those samples that were run during the specified time period.

4. Once you have selected a project, a list of all of the samples located in the project will be displayed in the **Raw Data / Available** area.

5. 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.

6. To select all of the displayed samples to be included in your Study, click the right-double-arrow button to move them to the **Raw Data / Selected** area.

7. To remove samples from your Study, click to highlight the sample(s) in the **Raw Data / Selected** area and click either of the left-arrow buttons to remove them from your selected samples.

> *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.*

8. When you have finished making your selections, click **Next** to proceed to the **Analysis Parameters** window.

## Selecting Raw Data from the Plate View tab

The **Plate View** tab allows you to make your selections from a visual display of the sample plate (see the following figure).

Click the **Plate View** tab available in the **Select Raw Data** window.

Figure 132: Selecting Raw Data from the Plate View tab
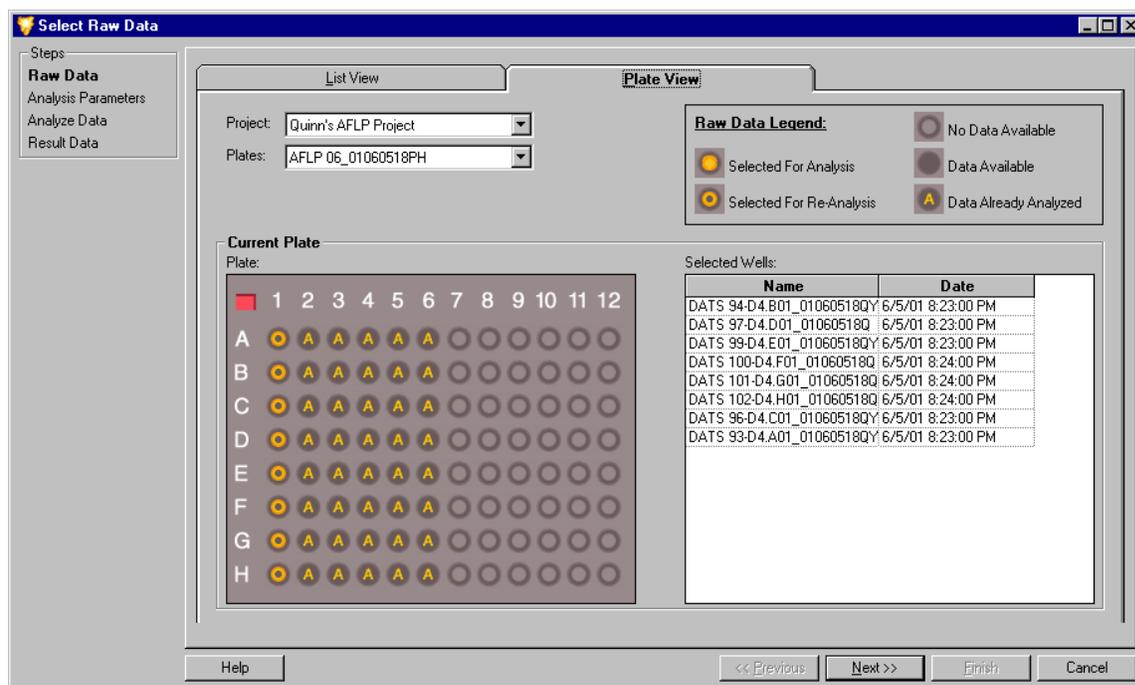
1. Select the Project folder that contains the sample data that you wish to include in the Study from the **Project** drop-down list box.
    - Click on the down-arrow located on the right side of the **Project** list box to reveal the available projects in the working database.
    - If the project you are looking for is not displayed, it may be located in a different database.
    - To enter a different database all applications must be closed. See the topic "Set Working Database" in the Online Help for more information.
2. Once you have selected a project, the **Plates** list box will contain all of the sample plates available for that project.
    - Click on the down-arrow located on the right side of the **Plates** list box to display the plates available for selection.
    - When you highlight your plate selection, a visual representation of the plate will be displayed in the **Current Plate** area of the window.
3. To select the samples to be included in your Study, click on the well location of the sample of interest.
    - The sample ID will be moved to the **Current Plate / Selected Wells** area of the window, indicating that the sample will be included in the Study.

> *Note* *See the Raw Data Legend area of the window for information regarding sample well selection.*

4. To remove samples from your Study, click on the well location of the sample you would like to remove.

- The sample ID will be moved to the **Current Plate / Selected Wells** area of the window, indicating that the sample has been removed from the Study.

5. To select or remove entire rows or columns of wells, click on the corresponding row letters or column numbers.

6. To select the entire plate to be included in your Study, click on the red box in the upper left corner of the plate.

> *Note* *You may select data from multiple projects and/or multiple plates to be included in your Study. Continue making selections from the list of available projects and plates until all of the desired samples have been added to the Raw Data / Selected area.*

7. When you have finished making your selections, click **Next** to proceed to the **Analysis Parameters** window.

Selecting an Analysis Parameter Set

The second step in creating a new Study from raw data involves defining the parameters that will be used to analyze the selected samples. Analysis parameters are used to process the fragment data to estimated fragment sizes and to identify alleles.

Figure 133: Analysis Parameters window - Selecting a Fragment Analysis Parameter Set
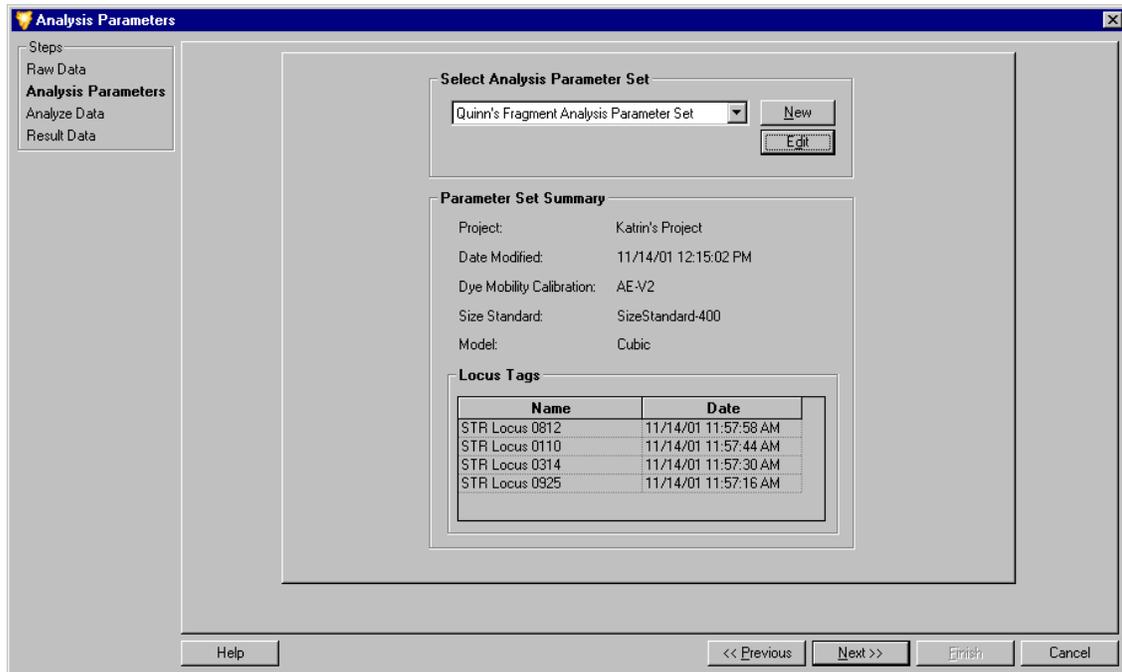
The **Analysis Parameters** window can be used to create, select or edit the analysis parameter set used to analyze the data which has been selected for the new Study. Your choice of a particular set of analysis parameters will depend upon the particular fragments you are attempting to identify.

The CEQ System Software is equipped with its own set of default analysis parameters. These are used to estimate unknown fragment lengths from a calibration curve based on the migration times of the size standard fragments. The default set of analysis parameters cannot be edited.

Selecting a Saved Analysis Parameter Set

1. In the **Select Analysis Parameter Set** area of the window, select the parameter set from the choices available in the drop-down list box.

2. The **Parameter Set Summary** area of the window will display summary information about the selected analysis parameter set.
   These include:
   - The **date and time** the analysis parameter set was created or last modified.
   - The selected **size calibration standard**, **calibration curve** and optional **dye mobility correction**. These parameters will be discussed in the following sections.
   - The currently assigned **locus tags** that have been selected for this Study.

3. When you have made your selection, click **Next** to proceed to the **Analyze Data** window.

   | Note | *If the available parameter sets are not applicable to your Study, you will need to create a new set of analysis parameters or edit an existing set to meet the requirements of your Study. Refer to the "Defining the Fragment Analysis Parameters" section of this manual or the Online Help for more information.* |

Analyzing the Data

With the new data selected and the method of analysis defined, the third step in creation of the Study is to analyze the data.

The **Analyze Data** window will display all of the samples that have been selected for analysis, the date each was collected, the status (unanalyzed) of each sample, and the parameter set being applied to the Study (see the following figure).

1. To begin the analysis, click the **Analyze** button.

2. As the system proceeds with the analysis, the progress bar will be active and the lower dialog box will record and display the analysis log for each sample as it is analyzed.

3. To stop the analysis while it is in progress, click the **Stop** button. The system will stop the analysis when it has finished with the sample in progress.

Figure 134: Analyze Data window - Analysis in Progress (shown)
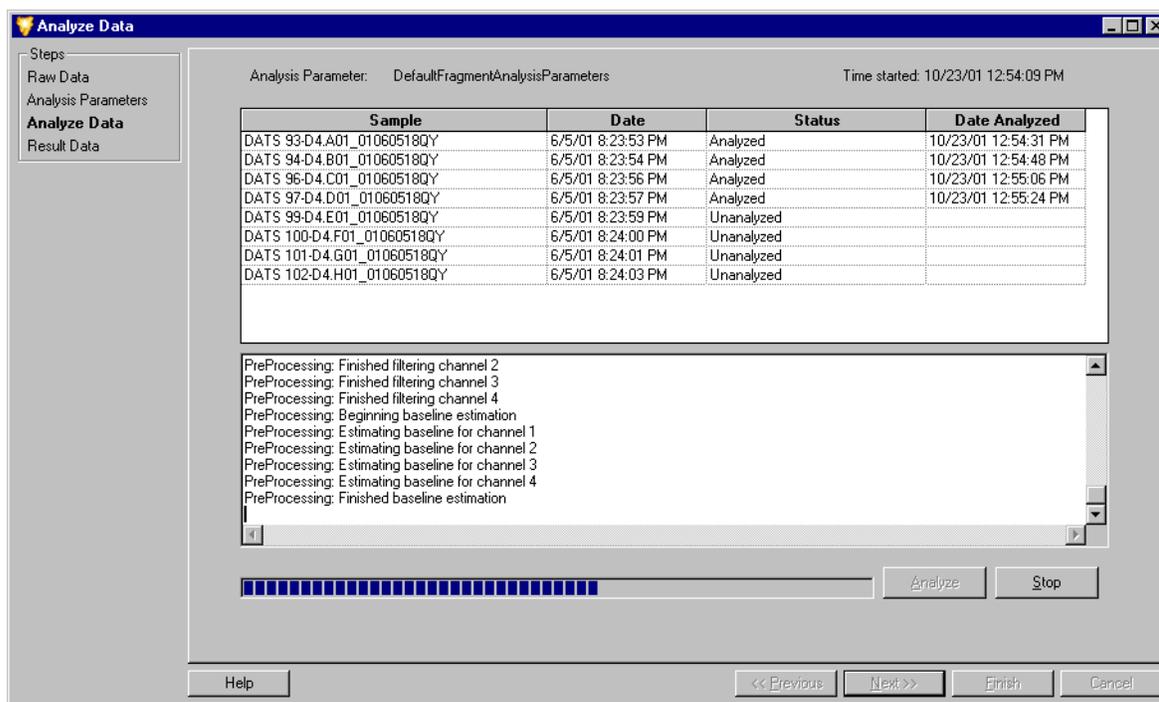
4. When the analysis is complete, the status column will indicate if the analysis succeeded or failed for each sample included in the Study.

5. 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.

6. When you have finished, click **Next** to proceed to the **Select Result Data** window.

Selecting Additional Results to be added to the Study

The fourth step in the development of your Study gives you the option to add additional result data to your new Study. 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 displayed in the first step of the Study Wizard.

> **Note** *Refer to the "Selecting Raw Data from the List View and Plate View tabs" sections located on the preceding pages for an explanation of the features available in the Select Result Data window.*

Select any additional results that you would like to add to the new Study and click **Finish** to complete the Study.

To save your new Study, open the **File** menu and select the **Save / Save As...** command.

Defining Fragment Analysis Parameters

The previous sections on **Creating a Study** described the application of saved parameter sets to analyze raw sample data. The following sections will describe how to edit a parameter set or create an entirely new set of analysis parameters to meet the specific requirements of your samples.

Parameter sets are created or edited in 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 to be used, assign quantitation values, and define several other factors that will affect how your data will be analyzed.

The Fragment Analysis Parameters window can be accessed while developing a Study from within the Study Wizard (see the previous sections on Creating a Study). It can also be accessed at any time while working within the Fragment Analysis module by selecting the Analysis Parameters command from the Analysis menu.

In either option, the **Analysis Parameters** window is first displayed showing the currently saved parameter sets available for selection. To create a new parameter set or to edit an existing set, click on either the **New** or **Edit** buttons, respectively.

The **New** or **Edit Fragment Analysis Parameters** window will be displayed.

Figure 135: Fragment Analysis Parameters window - Creating or Editing a Parameter Set
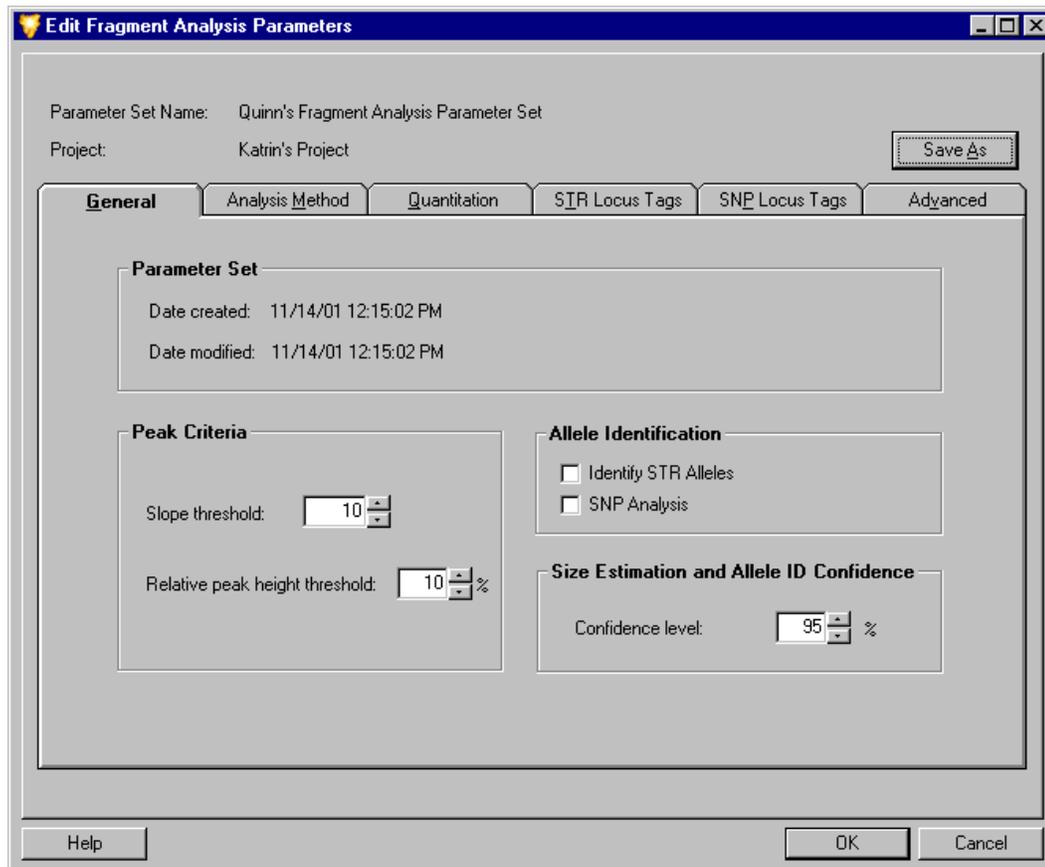
Fragment Analysis Parameters - General tab

By default, the **General** tab is the first tab to be displayed when opening the **Fragment Analysis Parameters** window. This tab contains general parameter set information and peak identification properties.

Parameter Set Information

1. If you are creating a ***new parameter set***, input a descriptive name in the **Parameter Set Name** field and select the project under which the parameter set will be located.
2. If you are editing an ***existing parameter set***, you may choose to save the edited parameters under a different name. Use the **Save As...** button and input 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.
3. The date and time the parameter set was created and modified is displayed in the **Parameter Set** area.

Peak Criteria

4. The **Slope Threshold** field allows you to 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 - 1000. The default value is 10.
    - The slope is proportional to the height of the peak and also dependent on the width of the peak. This parameter can be set 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 will vary for each dye trace.
        - Although only one slope threshold value is defined, the system will make adjustments for each dye, depending on the noise detected for that dye trace.
        - The default value should be appropriate for most samples, however, if peaks are "missed", the threshold can be lowered.

5. The **Relative Peak Height Threshold (%)** field allows you to set a minimum height criterion which unknown peaks must meet before being displayed in the fragment list or annotated in fragment (trace) data.

    - Enter a value between 0 - 100%. The default value is 10%.
    - The unknown peaks above this set value (%) relative to the second highest peak in each dye trace will be displayed in the fragment list and the sizes will be displayed in the fragment (trace) data.
    - This value does not apply to standards.
    - To size the peaks only, make sure that the **Allele Identification** boxes are **not checked**.

Allele Identification

6. If you would like the parameter set to include allele identification as specified in the allele lists of the selected locus tags, select the **Identify STR Alleles** check box.

7. If you would like to include Single Nucleotide Polymorphism (SNP) analysis in the parameter set, select the **SNP Analysis** check box.

> Note: *You may choose to identify STR Alleles <u>or</u> perform a SNP Analysis <u>or</u> to do neither, but not both in the same analysis.*

Confidence Level

8. Set the confidence level 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.

Fragment Analysis Parameters - Analysis Method tab

This tab allows you to specify the size standard and model to be applied to the calibration curve. The model selected should be the best fit for the standard, as it is used to generate the calibration curve, which estimates sizes for the unknown fragments.

Figure 136: Fragment Analysis Parameters window - Analysis Method tab
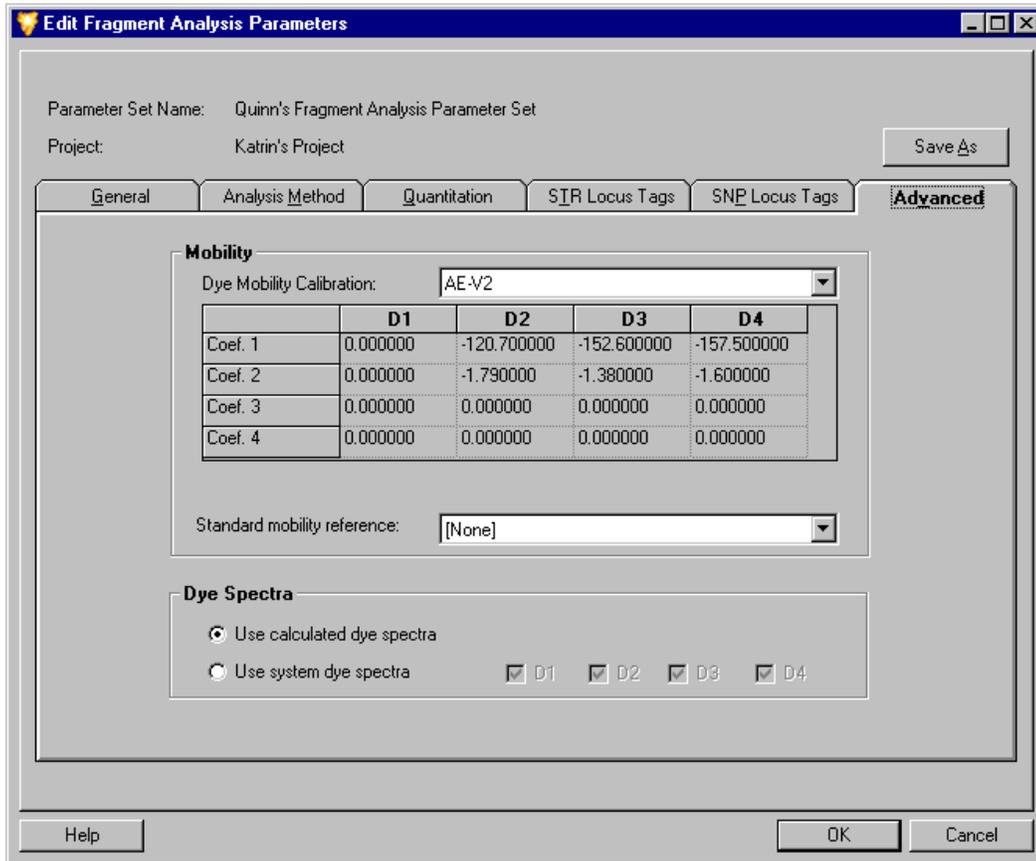
Size standards are sets of DNA fragments of known sizes that are run with a sample and are used to create the calibration curve. This calibration curve is then used to relate the known fragment size to either migration time or mobility in order to estimate the size of the unknown fragment(s).

When creating a new size standard, there are two requirements that help the system to find the standards and make correct associations between the fragment peaks and the fragment list. Refer to Online Help for information on creating size standards.

1. 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.
2. 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.

Setting the Analysis Method

1. Select the standard to be run with the sample from the **Size Standard** drop-down list box.

    - The size standards available are: **SizeStandard-400**, **SizeStandard-600**, & **SizeStandard-80**.
    - Your selection in this list box will make available various size standards to be selected in the **Standard Fragments** frame to the right of the window.
    - To use all fragments in the selected standard, click the **Select All** button.
    - 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 no standard is to be run with the sample, click the **Deselect All** button.

    > **Note** *If you do not use a size standard when analyzing the data, the unknown fragments will not be sized.*

2. The **Dye** field displays the dye label on the size standard fragments.

3. The **Size Standard Fit Coefficient** field displays 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 "lack-of-fit".

4. Select the empirical model that will be used to generate the curve that best fits the identified standard fragments from the **Model** drop-down list box.

    > **Note** *The relationship between fragment size and mobility (or migration time) in CE with replaceable gels is non-linear, 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 non-linearity.*

The model applied to the calibration curve can be either linear, quadratic, cubic, quartic or Local Southern, depending on the standard used.

- A **Linear Fit** will result in a straight line model which will only be useful for a set of size standards spanning a very small range.
- A **Quadratic Fit** will adequately account for the non-linear dependence of mobility on size under some conditions, but usually over relatively short size ranges which are well separated from the size limits of the separation system.
- Generally, a **Cubic Curve** will represent the curvature in a time or mobility versus size plot well enough that errors in estimated sizes are dominated by other factors, e.g., effects of primary structure on mobility. *This is usually the best choice for routine use with the SizeStandard-400 set*.

- For calibrations that span a long size-range or extend into size regions where the size selectivity of the separation system is very non-linear, a fourth degree polynomial (**Quartic Curve**) may result in more accurate size estimates than a cubic curve. ***This is usually the best choice for routine use with the SizeStandard-600 set***.
- The **Local Southern** method estimates the size of a sample fragment from its mobility and the mobility's of the four standard fragments closest in size. If this method is selected, the **X Variable** options are grayed-out and mobility is automatically selected.

5. The **Migration Variable** fields are used to select either **migration time** or **mobility** as the independent variable for the calibration curve. Size is the dependent variable.
    - Selecting migration time will use the average migration times and sizes of the standard fragments to construct a size calibration.
    - Selecting mobility will use fragment velocity (cm/s) divided by the separation potential (V/cm) with the sizes of the standard fragments to construct a size calibration.

Fragment Analysis Parameters - Quantitation Tab

This 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.

Once you have entered the information for the known peak into this window, the software will look for the reference peak in each trace using the specified size or time. Based on this information, the software can then calculate the quantity of each of the remaining peaks in the sample.

ID Standard

1. Within the **ID Standard** area of the window, use the option buttons to select the method which the software will use to identify your reference peak.
    - By selecting the **Size** option, the software will identify your reference peak based upon the fragment size specified in the **Size** field listed for each of the four dyes.
    - By selecting the **Time** option, the software will identify your reference peak based upon the migration time specified in the **Time** field listed for each of the four dyes.

Figure 137: Fragment Analysis Parameters window - Quantitation tab
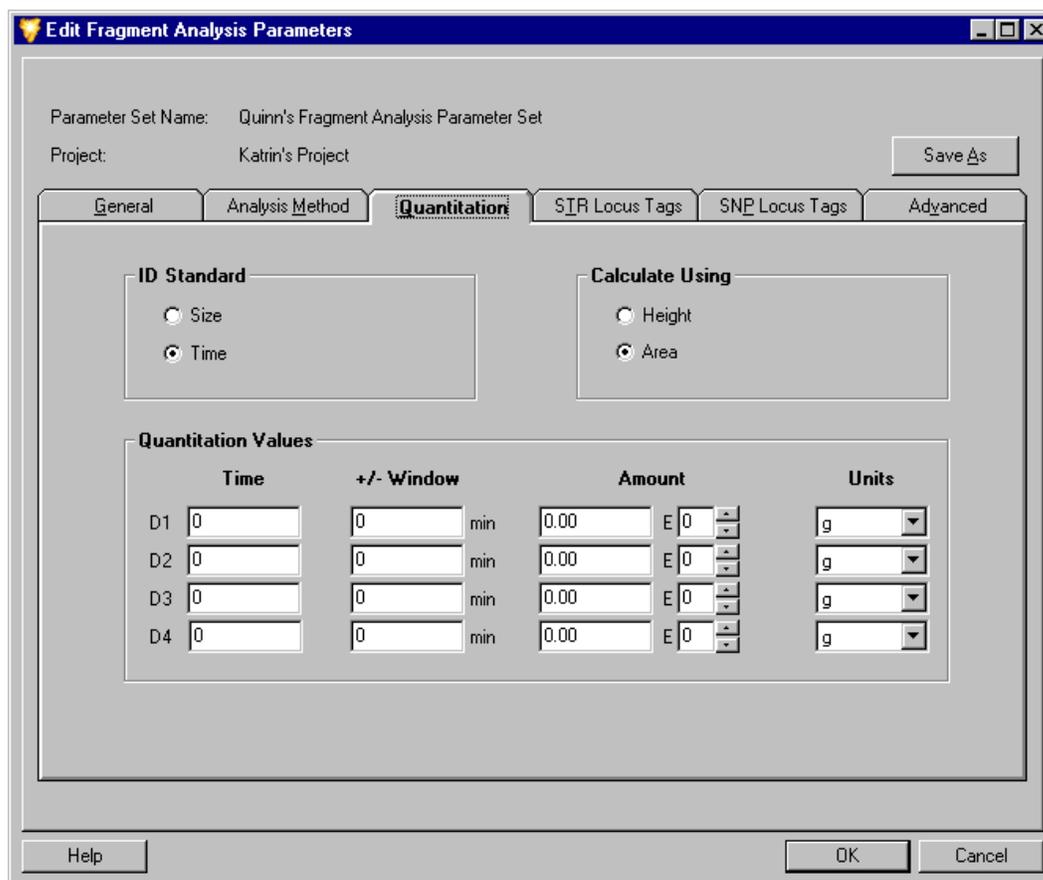
Calculate Using

2. Within the **Calculate Using** area of the window, use the option buttons to select the method which the software will use to calculate the quantity of your reference peak.

   - Select either **Height** or **Area**.
   - By selecting the **Height** option, the software will calculate the quantity of your reference peak based upon its peak height.
   - By selecting the **Area** option, the software will calculate the quantity of your reference peak based upon its peak area.

Quantitation Values

3. Select the check box for each dye with which you are using a reference peak. For each dye selected, you must input the appropriate values into the fields listed.

4. When you have selected **Size** in the **ID Standard** area of the window, the first field next to the dye selection check box requires you to input the size of the reference peak (nucleotides) for that dye.

5. When you have selected **Time** in the **ID Standard** area of the window, the first field next to the dye selection check box requires you to input the migration time of the reference peak (minutes) for that dye.

6. The **+/- Window** field allows you to input a variable quantity to be considered 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 will compensate for any variability that might arise in identification of the reference peak.
     - This field can be either **+/- nucleotides** (when Size is selected) or **+/- minutes** (when Time is selected).

7. Enter the known quantity of your reference peak in the **Amount** field.

   - This value must be entered into **exponential notation**.

8. Select the **units** for the quantity entered into the **Amount** field from the drop-down list box.

   - Options are **moles (mol)**, **grams (g), molarity (M)** or **(g/L)**.

Fragment Analysis Parameters - STR Locus Tags tab

This tab allows you to select the *locus tags that will be used for allele identification* with the parameter set being configured. Selections can be made 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.

Locus tags are used to define a particular locus of interest. Each available locus tag has been configured with a specific set of parameters that define the manner in which the system will identify detected fragments as allele fragments. These parameters include the fragment size range, repeat unit length, the dye to be used and an allele list associated with the locus tag.

When a locus tag is selected and an analysis performed, the system identifies the alleles present within the locus region using the estimated size of the fragments and the allele list.

> **Note** *The system will estimate sizes only if a size standard is used (see the "Analysis Method tab" section) and will call alleles only if an allele list exists for the selected locus tag (see the "Locus Tag Editor" section).*

> **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. Additional information can be found on the following pages and in Online Help.*

Fragment Analysis Parameters window - Selecting STR Locus Tags
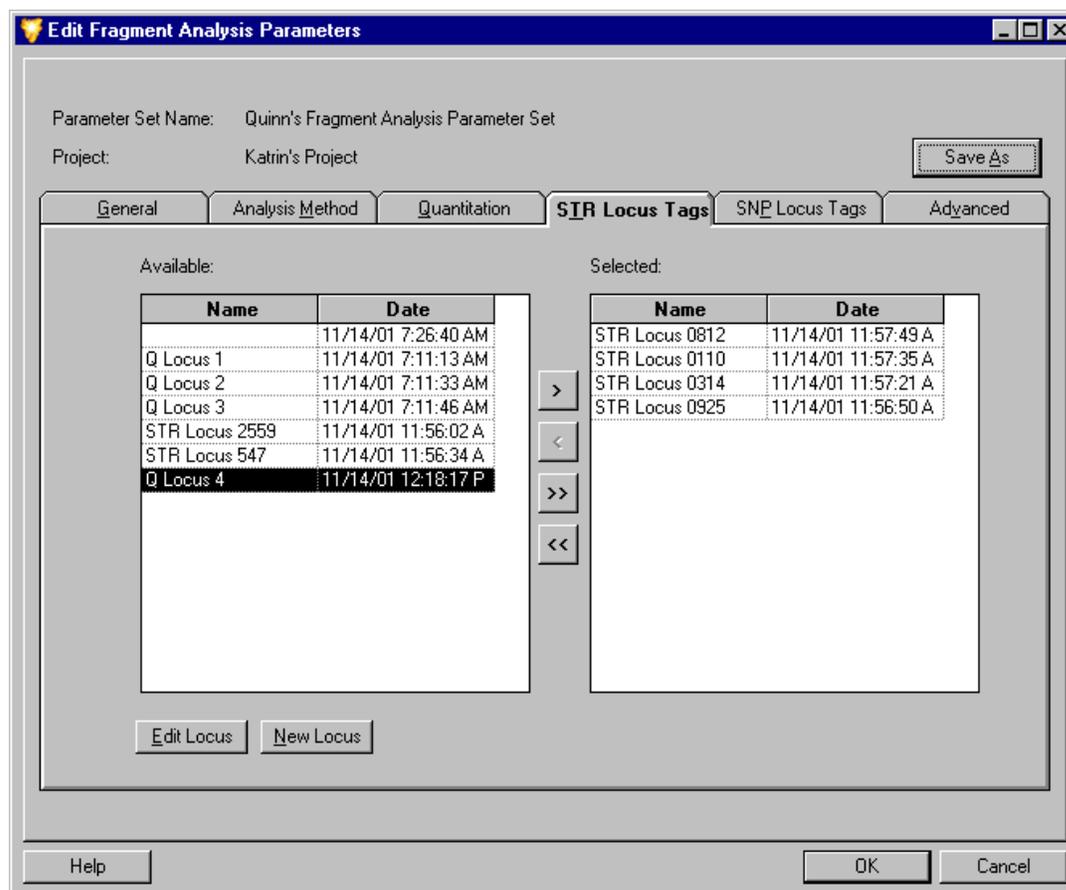
Selecting Locus Tags

1. To select a locus tag to be included in your parameter set, click to highlight the locus in the left "available" frame, then click the right-arrow button to move the selection to the right "selected" frame.

2. To select all available locus tags, click the right-double-arrow button to move the selections to the right "selected" frame.

3. To remove locus tags from your parameter set, click to highlight the locus tag(s) in the right "selected" frame and click either of the left-arrow buttons to remove them from your parameter set.

4. To edit a locus tag, highlight the selection and click the **Edit Locus** button to display the **STR Locus Tag Editor** window.

5. To create a new locus tag, highlight the selection and click the **New Locus** button to display the **STR Locus Tag Editor** window.

> **Note**
> *Refer to the "STR Locus Tag Editor" section of this manual or the Online Help for information about creating or editing locus tags.*

Fragment Analysis Parameters - SNP Locus Tags tab

This tab allows you to select the *locus tags that will be used for SNP analysis* with the parameter set being configured. Selections can be made 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 samples.

SNP locus tags are used to detect the presence of specific Single Nucleotide Polymorphisms (SNP's) in the group of sample data being 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 software 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 will look the same as those produced by a STR analysis. Fragments identified as alleles of a Locus will be 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).

Fragment Analysis Parameters window - Selecting SNP Locus Tags
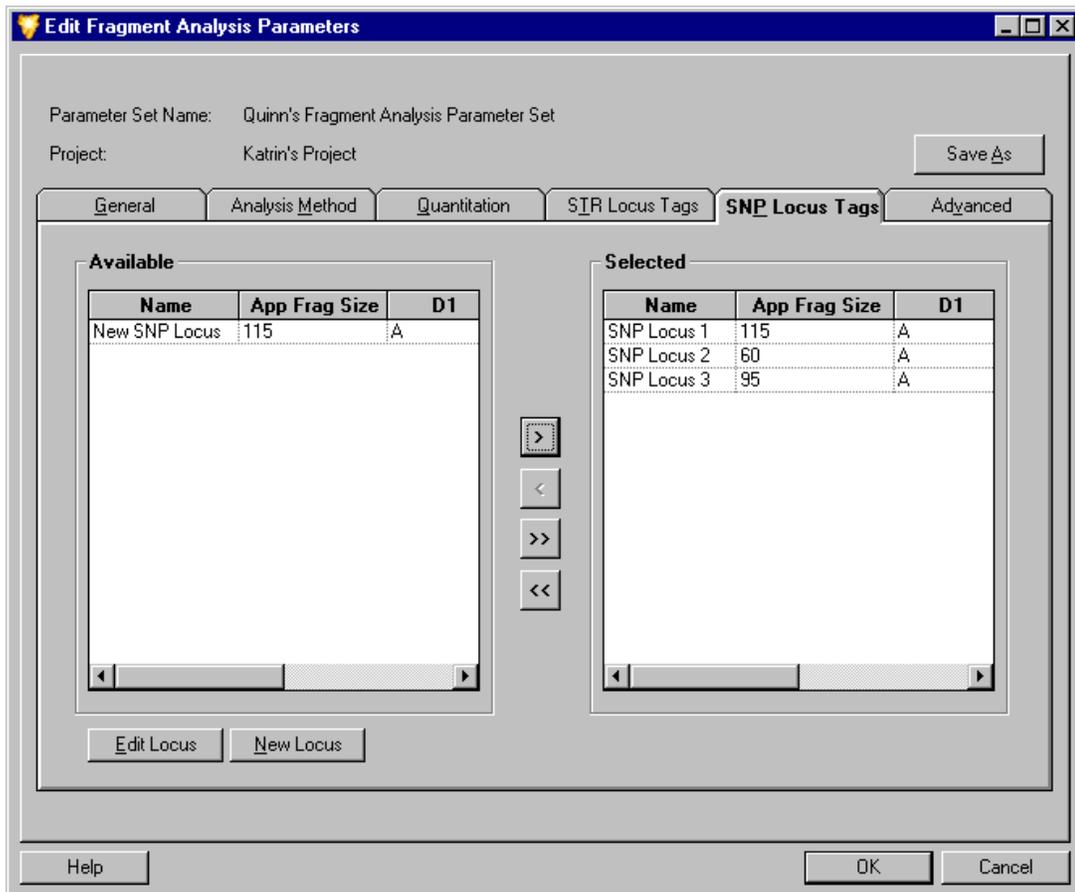
Selecting Locus Tags

1. To select a locus tag to be included in your parameter set, click to highlight the locus in the left "available" frame, then click the right-arrow button to move the selection to the right "selected" frame.

2. To select all available locus tags, click the right-double-arrow button to move the selections to the right "selected" frame.

3. To remove locus tags from your parameter set, click to highlight the locus tag(s) in the right "selected" frame and click either of the left-arrow buttons to remove them from your parameter set.

4. To edit a locus tag, click the **Edit Locus** button to display the **SNP Locus Tag Editor** window.

5. To create a new locus tag, highlight the selection and click the **New Locus** button to display the **SNP Locus Tag Editor** window.

> *Refer to the "SNP Locus Tag Editor" section of this manual or the Online Help for information about creating or editing locus tags.*

SNP analysis will only take place if the following 3 criteria are met:

1. **SNP Locus Tags** have been selected as part of the analysis in the **SNP Locus Tags** tab of the **Analysis Parameters** window (see above).

2. The **SNP Analysis** check box has been selected in the **General** tab of the **Analysis Parameters** window (see previous).

3. An appropriate **SNP Reference Standard** has been selected in the **Analysis Method** tab of the **Analysis Parameters** window (see previous).

   • A size standard with exactly 2 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 2 fragment standard is the Linear model.

Additionally, you should select the **SNP ver.1 Dye Mobility Calibration** in order to correct for the mobility differences between the reference fragments and each of the four possible dye-labeled SNP fragments. This selection is made in the **Advanced** tab of the **Analysis Parameters** window (see the following section).

The analysis will still proceed if the mobility correction is not selected, but the values obtained may vary slightly.

Fragment Analysis Parameters - Advanced Tab

The Advanced tab is used to 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 dye spectra used should be estimated from sample data or from saved system spectra.

Figure 138: Fragment Analysis Parameters window - Advanced tab

Mobility

1. Select the desired **Dye Mobility Calibration** from the drop-down list box.
   - The Dye Mobility Calibration is used to select a specific calibration from the options available in the list box and to adjust migration times or mobility to compensate for the varying effects of different dyes on fragment mobility.
   - Select **PA ver.1** if analyzing samples made from primers labeled with the phosphoramidite dyes D2-PA, D3-PA, D4-PA.
   - **AE ver.2** provides an improved calibration for primers labeled with the active ester dyes (D1, D2, D3 or D4) and should be selected to obtain the most accurate size estimates for these primers.

- **AE ver.1** is the calibration used for the CEQ Fragment Analysis test sample.
- **SNP ver. 1** is used when performing a **SNP analysis**. The SNP analysis will still proceed if the mobility correction is not selected, but the values obtained may vary slightly.
- **No Correction**, which is the default dye mobility calibration, should be used when no dye mobility calibration is desired. With this selection, the system will not make any compensation for dye effects on fragment mobility.

> *Note* *The CEQ Fragment Analysis test sample must be analyzed with the AE ver.1 dye mobility calibration for correct sizing of the fragments.*

2. Select the desired **Standard Mobility Reference** from the drop-down list box.
- The standard mobility reference is a calibration curve from an existing analysis run under similar conditions.
- Use of a standard mobility reference provides information which will assist in the identification of size standards when spurious peaks or other factors make size standard identification problematic.
- Once a calibration curve has been generated and the coefficients determined, it can be saved as a standard mobility reference to aid in the identification of standard fragments in subsequent analyses of different samples using the same standard.

> *Note* *If you have an existing set of results with good resolution, you may want to save the parameters of the standard curve as a Standard Mobility Reference to be used in subsequent analyses.*

- In order for the curve to be saved as a standard mobility reference, the X variable must be mobility.
- If the system fails to identify standard fragments during analysis, try creating a new standard mobility reference, run under separation conditions that more closely resemble those used in the failed analysis

Dye Spectra

Dye spectra values are calculations of the relative emission intensities of each dye in each of the four detection channels. These values are calculated during each analysis, and are used to transform the four electropherogram channels to dye signal traces for each of the dye labels present in the sample.

When a dye spectrum cannot be calculated from the sample, the analysis process will terminate unless system dye spectra are available.

The **Dye Spectra options** allow you to select an existing set of dye spectra to be used instead of estimating the dye spectra from each sample.

> *Note* *This option can shorten the analysis time by up to 50%.*

Only one set of system spectra is stored. This set of system spectra is automatically used if one or more of the needed spectra cannot be estimated from the sample data.

- The **Use calculated dye spectra** option button is the default setting. This selection uses the set of dye spectra obtained from the sample data.
- Select the **Use system dye spectra** option button and the **Dye** check boxes for each dye trace you would like to be generated. With this option selected, no attempt will be made to estimate the dye spectra from the sample being run, and only the selected system dye spectra will be applied to the data.
- The system dye spectra can be established when the fragment test sample is run during the installation process by viewing the Dye Matrices window for a sample result in which spectra for all four dye labels were estimated. This set of dye spectra can then be saved by the system by clicking on the *Save as System Dye Spectra* button.

STR Locus Tag Editor

The **STR Locus Tag Editor** allows you to create a new STR Locus Tag or edit an existing one. It can be accessed from the **STR Locus Tag** tab of the **Analysis Parameters** window (see previous section).

Figure 139: STR Locus Tag Editor window - Creating a New Locus Tag

The **Locus Tag Editor** contains two tabs for creating or editing locus tags; the **Locus Tag** tab and the **Allele ID Criteria** tab. The locus name and project location are displayed at the top of the window.

New or edited locus tags can be saved to a selected project location for use in additional analyses.

Locus Tag Editor - STR Locus Tags

The Locus Tag tab is where the general locus information and supplementary reporting information are defined. These parameters include the fragment size range, repeat unit length, and the 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 that are used to generate a list of expected alleles in your analysis.

1. You may input the identification of the locus in the **Locus Description** field. This is an optional field.
2. Select the **dye** to be used for the locus from the drop-down list box.
3. Input the **fragment length range** (the minimum and maximum fragment size associated with the locus).
   - This parameter defines the range over which the system will look for the particular fragment.
4. Input the **length** of the **repeat unit** that represents the allele.
5. Select the **number of repeats in the shortest allele**.
   - 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.

Allele Naming Convention

The **Allele Naming Convention** area allows you to select the method in which the system will label the identified alleles in the fragment list. For example, the alleles can be labeled 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.).

6. Select the method in which the alleles will be named in the fragment list.
   - Select the **nominal size** option button if you want the alleles to be labeled by their nominal fragment size.
   - Select the **alphabetic** option button if you want the alleles to be labeled in lettered order and select the "**Start at**" value from the drop-down list box.
   - Select the **numeric** option button if you want the alleles to be labeled in numeric order and select the "**Start at**" value from the drop-down list box.

> *Note* *With the exception of "ploidy", the following fields are not needed 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 be included with your locus tag parameters.

- Input the **name of the primer set**.
- Input the **GenBank Accession** Number.
- Input the **sequence of the repeat unit** associated with the locus tag.
- Input the number of alleles (**ploidy**) 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.

- Select the **forward** option button if the primer is dye labeled on the forward strand.
- Select the **reverse** option button if the primer is dye labeled on the reverse strand.
- Select the **none** option button 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.

- If the primer is labeled on the **forward strand**, input the sequence of the primer in the **5' to 3' direction**.
- If the primer is labeled on the **reverse strand**, input the sequence of the primer in the **5' to 3' direction**.

Generating and Viewing the Allele List

Once you have made your selections, you may choose to begin creating the allele list by clicking the **Generate Allele List** button.

The system generates an allele list from the information provided by the user. The allele list starts at the low end of the fragment length range and creates an allele at each perfect repeat length until the end of the fragment length range is reached.

The generated fragment lengths are displayed in the **Nominal Size** column as the initial integer values. These values can be user-modified to represent the allele sizes that appear when the data is analyzed. The nominal sizes are also displayed in the apparent size column, but these will usually be edited or regenerated.

The **ID's** assigned to each allele in the list use the allele naming convention described in the section above.

The fragment lengths displayed in the **Apparent Size** column are either user-entered from the locus tag editor, generated by interpolation or regression from an incomplete list, or generated from the automatic binning analysis feature (see the "Bin Analysis" section).

When apparent fragment lengths are user-entered or are generated by interpolation or linear regression, the nominal sizes must have first been entered by the user. 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.

The **Standard Deviation and # of Points** columns describe the number of points included in each bin and the sample standard deviation. Both of these columns are generated as a result of the automatic binning feature (see the "Bin Analysis" section) and are empty for allele lists not based on a bin analysis.

User-comments can be written into the **Comment** column.

Allele List Generation using Interpolation and Linear Regression

An allele list can be generated from a list of nominal sizes and a partial list of apparent sizes using Interpolation and Linear Regression.

Interpolation and Linear Regression

Through **interpolation** the apparent fragment sizes between two selected points are calculated.

Linear Regression calculates the "line of best fit" for the selected alleles. The line is then used to determine the apparent sizes for all points not selected. This option requires that more than two apparent fragment sizes are selected.

1. Individually edit the apparent fragment sizes within the allele list from which interpolation or a linear regression line will be generated.
2. Select the rows within the allele list to be used to generate the allele apparent sizes list by clicking on the row header.

    > *Holding the* **Shift** *key and dragging through the headers will select multiple consecutive selections. To select non-consecutive rows, hold down the* **CTRL** *key while selecting each of the rows with the left mouse button.*

3. When the alleles have been selected, right click on the highlighted area and select the **Interpolate** or **Regression** command from the menu displayed.
4. Existing or missing apparent fragment sizes will be replaced or supplied by the values generated in the Interpolation or Linear Regression function.

STR Locus Tag Editor - Allele ID Criteria

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 is identified as representing a particular allele if its apparent size is found to be the very similar to the size of a particular allele fragment in the allele list. However, because the mobility of a fragment is affected by both its sequence and its size, 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. This is carried out by the system using a user-defined confidence level.

Confidence Interval

The confidence interval is automatically generated as a result of the bin analysis, but can also be entered manually by the user. When automatically generated, the value displayed is dependent on the overall standard deviation computed in the bin analysis and the user-specified confidence level (default = 95%). When the confidence interval is manually entered by the user, the system uses the entered value and the confidence level to estimate an overall standard deviation.

Increasing the confidence interval will have the effect of increasing the width of the bins. *The confidence interval should be set to a value less than 0.5 nt.*

If a value of zero is entered into 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 the confidence interval is user-entered, the allele identification will not correspond to an error probability estimated from actual data but will still be a useful relative indicator of the "goodness" of the match.

1. Select the **Overwrite system generated value** check box if would like to overwrite the confidence interval value generated by automatic binning.
2. If you are overwriting the system generated value, specify a confidence interval (number of nucleotides (+/-)) in the **Allele confidence interval** field.

Figure 140: Locus Tag Editor window - Allele ID Criteria tab
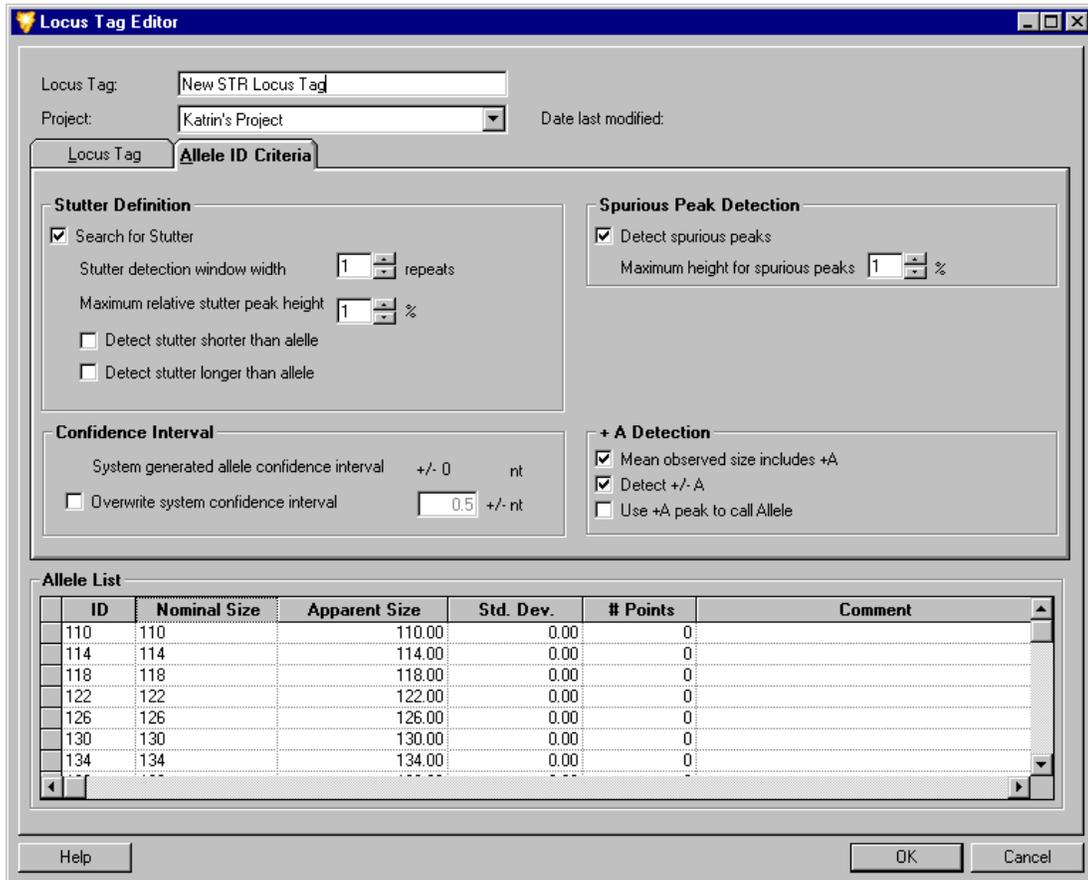
Stutter Definition

Stutter are usually small peaks, generally DNA amplification artifacts that form as a result of halted polymerase activity. When this happens, allele fragments are formed that differ in length from the true allele by an integral number of repeats (i.e., n-1, n-2, etc.). The absolute size of a fragment is a function of the variety of repeats (i.e., dinucleotides, trinucleotides, etc.).

The **Stutter Definition** area of the window allows you to set the parameters for which the system will identify these peaks as stutter and not as true alleles.

1. Select this check box if you want the system to **search for stutter**. When selected, you must also choose at least one of the options in steps 4 and 5 below.

2. Select the number of repeats to specify the **width of the window** around each allele in which stutter will be detected.

3. Specify the **maximum height percent of the stutter** relative to the true allele below which detected peaks are considered stutters. Peaks above this threshold will be considered unknown alleles.

4. Selecting this check box enables the system to **detect stutters shorter than (occurring prior to) the allele**.

5. Selecting this check box enables the system to **detect stutters longer than (occurring after) the allele**.

Spurious Peak Detection

Spurious peaks are generally small trace artifacts that are 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 will identify a peak as contaminant and not include it in your allele list.

1. Select the **Detect spurious peaks** check box if you want the system to remove any peaks which are below the maximum height threshold for spurious peaks.

2. Specify the **maximum height 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 which is used to compensate for a common DNA polymerase phenomenon of adding a "non-templated" Adenine to the ends of PCR products. What results is a mixture of PCR products of different lengths from a single allele. Some of these products are templated "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 are often 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 artifact.

> **Note** *More than one of these check boxes can be selected at a time. Read through the following descriptions or refer to the tables below to see how these selections will affect +A Detection.*

Apparent size includes +A

Selecting this check box indicates to the system that the sizes entered into the Apparent Size column of the allele list were for the +A fragments. The parameter affects where the software looks for peaks when identifying alleles.

Detect +/- A

If the Detect +/- A box is <u>checked</u>, the software will identify in the fragment list either the +A or -A peaks. The software will then identify one of them as the true allele based upon your selection in the **Use +A peak to call allele** check box. The other peak will then be designated as either +A or -A accordingly.

If the Detect +/- A box is <u>not checked</u>, the software will identify one of the two alleles as the true allele based on the **Use +A peak to call allele** check box. Other peaks will be included in 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 <u>checked</u>, the software will identify the +A peak as the true allele in the fragment list when the **Detect +/- A box is <u>also checked</u>**. The other peak will then be designated as either -A accordingly.

If the Use +A peak to call allele box is <u>not checked</u>, the software will identify the +A peak as the true allele in the fragment list when the **Detect +/- A box is <u>also not checked</u>**. The other peak will be included in the fragment list as an **unknown allele**.

If the **Detect Spurious Peaks** check box has been 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**.

Size Adjustments

+A Detection	Use +A Peak to Call Allele **Not Checked**	Size Includes +A **Checked**
Use +A Peak to Call Allele **Checked**	No Size Adjustment	One nucleotide is subtracted from the size of the allele in the allele list.
Size Includes +A **Not Checked**	One nucleotide is added to the size of the allele in the allele list.	No Size Adjustment

Truth Table for Setting +A Allele Identification

+A Detection	Detect +/- A **Checked**	Detect +/- A **Not Checked**
Use +A Peak to Call Allele **Checked**	All fragments <u>assumed to be</u> +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 <u>assumed to be</u> +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.

Use +A Peak to Call Allele **Not Checked**	All fragments <u>assumed not to be</u> +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 <u>assumed not to be</u> +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.

> **Note** *The only time that "Use +A peak to call allele" is checked while the "Detect +/-A" is not checked is when there is complete conversion of the PCR product to +A. When neither "Use +A peak to call allele" nor "Detect +/-A" are checked, no +/-A labeling will 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 software 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 software will correctly categorize peaks. If the option is incorrectly configured, the alleles will still be identified, but the +A and -A peaks are frequently categorized as unknown alleles and the software will report "Too many alleles" in the Comment column of the fragment data table.

SNP Locus Tag Editor

The **SNP Locus Tag Editor** allows you to create a new SNP Locus Tag or edit an existing one to be used for SNP analysis. It can be accessed from the **SNP Locus Tag** tab of the **Analysis Parameters** window (see previous section).

Figure 141:SNP Locus Tag Editor window - Creating a New Locus Tag
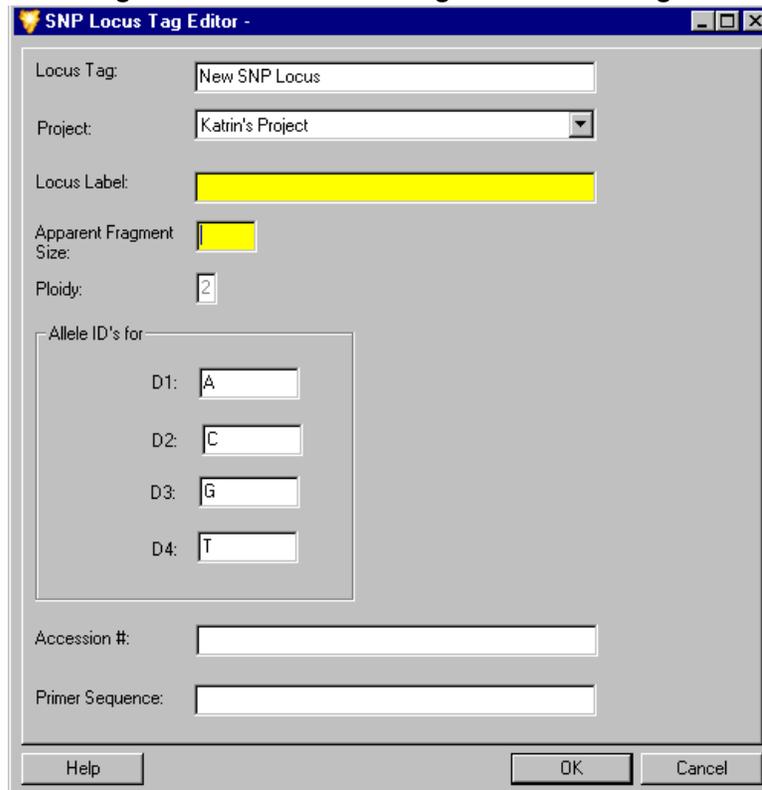
Input or edit the name of the locus tag in the **Locus Tag** field.

Select the **Project** to which the new or edited locus will be saved.

You may input the identification of the locus in the **Locus Description** field. This is the locus tag name that will be displayed in the fragment list for all fragments that are identified as belonging to this locus.

The **Apparent Fragment Size** must be 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.

The **Ploidy** field is inactive for this release of the software.

The **Allele ID's** are 8 character alphanumeric fields that are used to label the Allele ID's in the fragment list. The default values are A, T, G & C. These fields cannot be blank.

The **Accession #** and **Primer Sequence** fields are not used for analysis and are for supplementary reporting purposes only. These fields are optional.

Apparent sizes of SNP probe fragments as estimated with SNP reference fragments will often differ more from actual sizes than is the case with STR allele fragments. 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.

Additionally, because the effects of different dye labels are relatively greater on shorter fragments, it is essential that the **SNP ver. 1 Dye Mobility Calibration** is included in the parameter set for an SNP analysis.

Result Set Filtering

The first steps in the creation of a Study involve the application of 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.

Specific "filters" are then used to scrutinize and selectively isolate results based upon the parameters of the applied filters. In other words, the types of exclusions that you apply to the data will "filter-out" all unwanted results from your Study. This process is known as Result Set Filtering.

Accessing the Result Set View

The Result Set View can be viewed at any time while working with a Study by opening the **View** menu and selecting the **View Result Set** command or by clicking on the **Result Set** icon located on the **Data** tab of the **Study Explorer**.

Figure 142: Result Set View window - Second Level Filtering
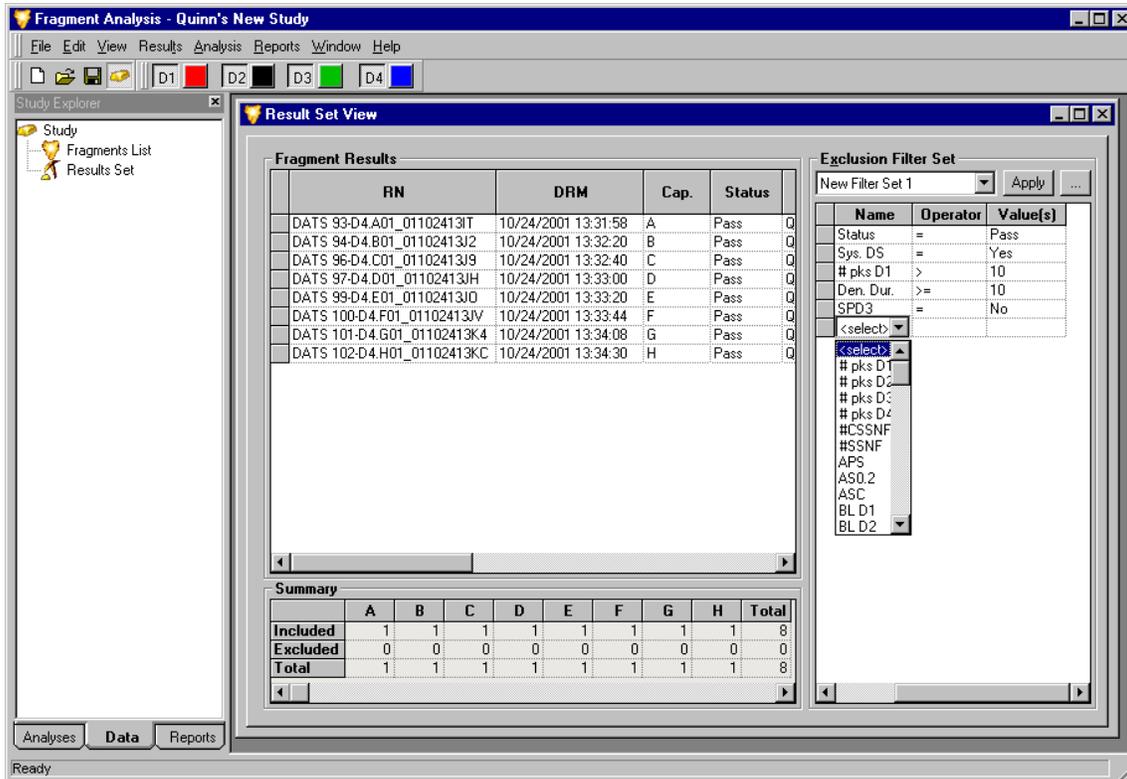
There are three key elements to the Result Set View window shown:

The **Fragment Results** table displays all analyzed samples, both included and excluded, that have been selected for the Study. The displayed columns are user-defined and can be modified to show a number of different system variables and information.

The Filter Set area is 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 two pre-defined sets of exclusion filters. Additional filter sets can be created, modified and saved.

The **Summary** area displays 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 upon your filter set parameters.

> *The Filter Sets applied to the Result Set data (2nd Level Filtering) are different from the Filter Sets applied to the Fragment List data (3rd Level Filtering). To the Result Set you apply filters to an entire sample which may contain numerous fragments. To the Fragment List you apply filters to individual fragments that have been identified within the various samples. See the "Fragment List" section of this manual or Online Help for more information.*

Applying 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.

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 drop-down list box in the **Name** column of the **Filter Set** area and select the **SS** (Size Standard) command.
2. Open the drop-down list box in the **Operator** column of the **Filter Set** area and select the "**=**" command.
3. Open the drop-down list box in the **Value(s)** 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.

There are many filters available and each must be applied using an operator and a value. Any number of filters can be applied to a result set and the filter sets can be saved 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.

The end result of this "second level" filtering is to leave only results that you are certain of and to save you the time of "weeding out" unwanted fragment data later.

Available Exclusion Filters

All of the exclusion filters can be found in the drop-down list box available in the **Name** column of the **Filter Set** area. Once you have selected the name of the filter to use, you must select an **operator** (usually +, <, >=, etc.) and a **value** to be applied to the filter.

> **Note** *Refer to the Online Help for detailed descriptions of the available exclusion filters and their use.*

Summary Area Information

The **Summary** area displays a table that identifies the number of samples 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 upon your filter set parameters.

- The number of results that are **included** in result set within each sample capillary (A-H) is displayed. 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 is displayed.
- The **total** number of all samples, both included and excluded, that are part of the result set is displayed.

Customizing the Result Set View

The components of the fragment results available in the Result Set View are user-defined and can be modified through commands available in the right-click menus. These commands allow you to select specific columns for display in the fragment results, customize the arrangement of fragment results information and modify several other parameters of the fragment results.

> **Note** *Information regarding use of the Column Selector window, the Column Sort window and other display parameters can be found in the "Customizing the Fragment Results" section of this manual or in the Online Help.*

Viewing Trace Data

Individual sample traces can be viewed at any time while working within the fragment analysis module. They can be viewed 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

The **Single Sample View** displays all of the available information for an individual data sample, including the collected trace views, fragment data, separation and analysis parameters, the calibration curve, the run log, and more.

To access this information, right-click on the highlighted sample (or multiple samples) from any of the data or fragment lists and select the **Show Single Result** command from the menu displayed.

Figure 143:Single Result View
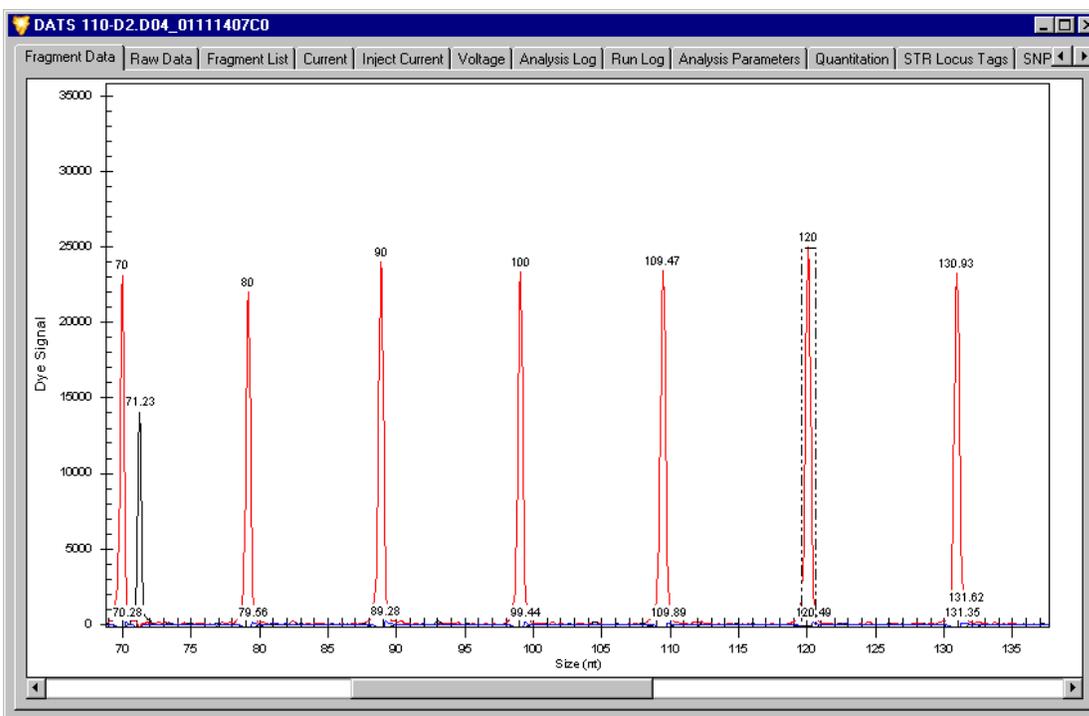
The top of the **Single Result View** window displays the name of the individual data sample selected. All of the information pertaining to the data sample is distributed under several different tabs within this window.

The table on the following page provides a brief description of the components of each of the tabs in the Single Result View.

Note *Refer to the Online Help for detailed descriptions of each of the available tabs in the Single Result View.*

Table 72: Information available in the Single Result View

Tab	Description
Fragment Data	Displays a plot of the dye signal traces.
Raw Data	Displays a plot of the raw, four-channel electropherogram.
Fragment List	Displays a list of all fragments that meet the minimum relative height criterion set in the parameter set. All available fragment properties are displayed in this view.
Current	Displays a graph of the separation current versus time for the duration of the separation event.
Inject Current	Displays a graph of the injection current versus time for the duration of the injection event.
Voltage	Displays a graph of the separation voltage versus time for the duration of the separation event.
Analysis Log	Displays the list of messages generated by the primary analysis program during the analysis of the selected result. The analysis log contains information that can be useful in determining the cause of an analysis failure.
Run Log	Displays the list of messages generated by the CEQ instrument during the pre-separation and separation events of the 8 samples that include the individual selected result. May be used to troubleshoot the data in the case of a run failure.
Analysis Parameters	Displays a summary of the fragment analysis parameters applied to the raw data in the selected result.
Quantitation	Displays a summary of the peak information used to estimate fragment amounts within the selected result.
STR Locus Tags	Displays a list of STR locus tags that have been applied to the raw data in the selected result.
SNP Locus Tags	Displays a list of SNP locus tags that have been applied to the raw data in the selected result.
Size Calibration	Displays a description of the size standards and calibration curve used to calculate the sizes of the unknown fragments in the selected result.
Dye Matrices	Displays the dye spectra and dye mobility calibration parameters used in generating the selected result.

Table 72: Information available in the Single Result View

Tab	Description
General/Notes	Displays a list of system and user-generated notes specific to the selected result (i.e., sample name, dates, database location, hardware information, etc.)
Property Set	Displays a list of user-defined property fields and the values associated with the selected result. These parameters may be edited.
Method/Consumables	Displays a summary of the separation methods, consumable parts and lot numbers that were used with the selected result.

Copying a Trace

A trace can be copied to the clipboard, as an image, by right-clicking over the trace and selecting the **Copy** option (see Figure 144).
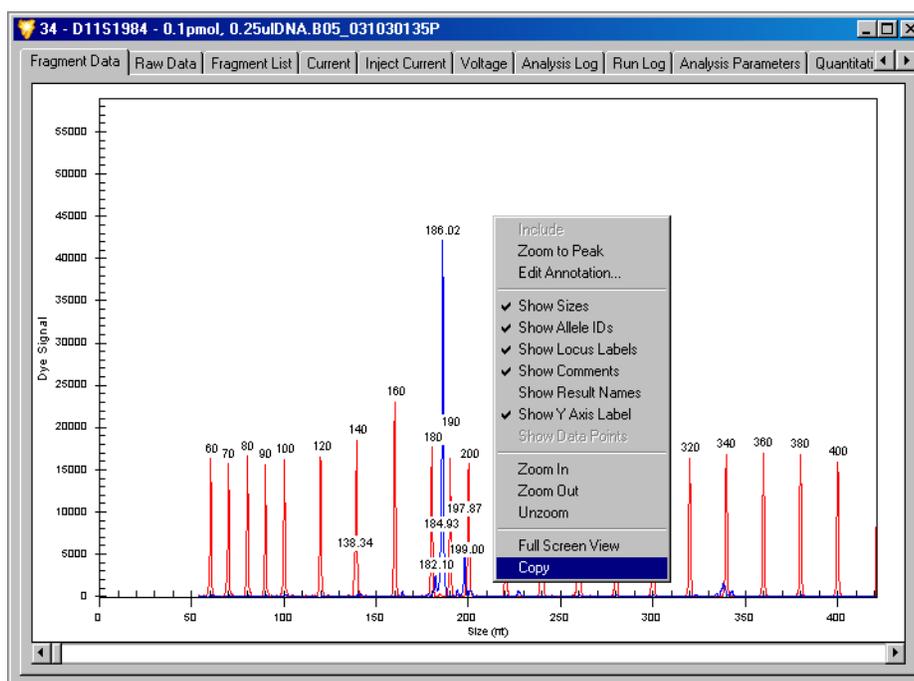

Figure 144:Copy Trace to Clipboard

Subtitles are copied with the image, as shown in Figure 145. To paste the image in other software, use its *Paste* command.
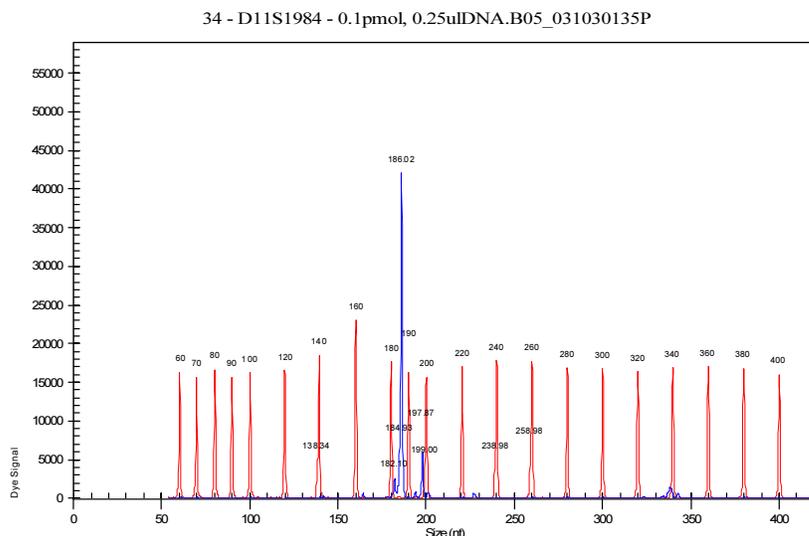
Figure 145:Trace with Labels

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, right-click on the highlighted sample(s) from any of the data or fragment lists and select the **Show Stacked Graph** command from the menu displayed.

Overlay Graph

The Overlay Graph function superimposes selected traces on a single x/y plot. This function has two different modes. The default mode allows you to draw a rectangular box around a selected area to zoom into. The second mode (toolbar buttons) allows you to select a group of traces and add them to a Stacked Graph view.

To access this function, right-click on the highlighted sample(s) from any of the data or fragment lists and select the **Show Overlay Graph** command from the menu displayed.

Including Results

Results that have been excluded may be re-included in the Study.

To access this function, right-click on the highlighted sample(s) from any of the data or fragment lists and select the **Include Results** command from the menu displayed.

Excluding Results

Results may be excluded automatically through the use of filter sets or manually through the **Exclude Results** command. To remove results through the application of filter sets, refer to the Filter Set area of this manual or the Online Help.

If selected for display, results that have been excluded through the application of filter sets will be shaded in a ***teal color***. Manually excluded results will be shaded in a ***maroon color***.

Results excluded with either of these methods may be re-included at any time through the **Include Results / Peak**s command available in the right-click menu. Results that have been re-included will be shaded in a ***light gray color***.

Both filtered and manually excluded results may be hidden from view by de-selecting the **Show Excluded** command available in the right-click menu.

> Note
> ***Refer to the Online Help for detailed descriptions of each of these and other display parameters.***

Re-Analyze Results

The Fragment Analysis module contains a Re-Analysis feature that allows you to select one or more samples from the Result Set view to be re-analyzed. This provides a useful method of applying a different set of fragment analysis parameters to a previously analyzed set of data to pick up missed peaks or to avoid picking up unwanted peaks. The data resulting from the re-analysis automatically replaces the older Result Set data, allowing you to quickly continue with your Study.

To access this function, right-click on the highlighted sample(s) from the Result Set view and select the **Re-analyze Results** command from the menu displayed. This command is also available from the **Analysis** menu.

Re-analyzing Results

1. Select one or more results from the Result Set View.
2. Right-click on the highlighted result(s) and select the **Re-Analyze Results** command from the menu displayed.
3. A message box will be displayed indicating that procedure will replace the selected results with re-analyzed results. Click **Yes** to proceed with the re-analysis.
4. The Fragment Analysis Parameters window will be displayed, allowing you to select a new or edited set of parameters to use for re-analyzing the data.

Figure 146: Selecting a Parameter Set to be used for Re-Analysis
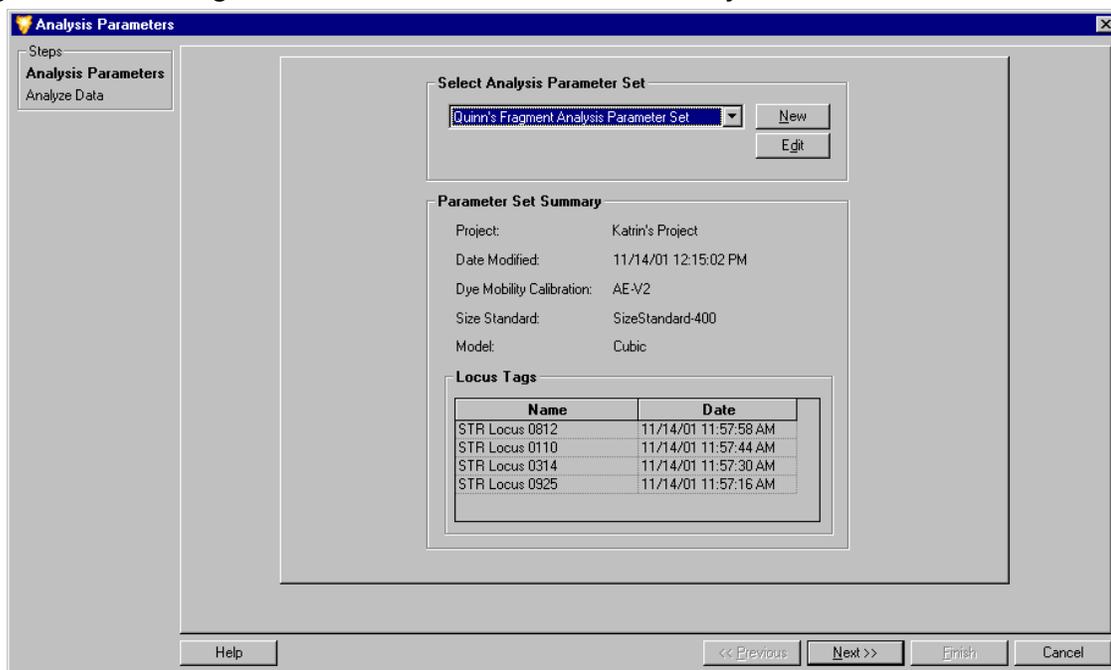
> _Refer to the Analysis Parameter Set area of this manual or the Online Help for information on this window and on selecting, creating, editing and saving Fragment Analysis Parameter Sets._

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5. When an Analysis Parameter Set has been selected, click **Next** to proceed to the **Analyze Data** window.

Figure 147:Re-Analyzing the Data (re-analysis shown in progress)
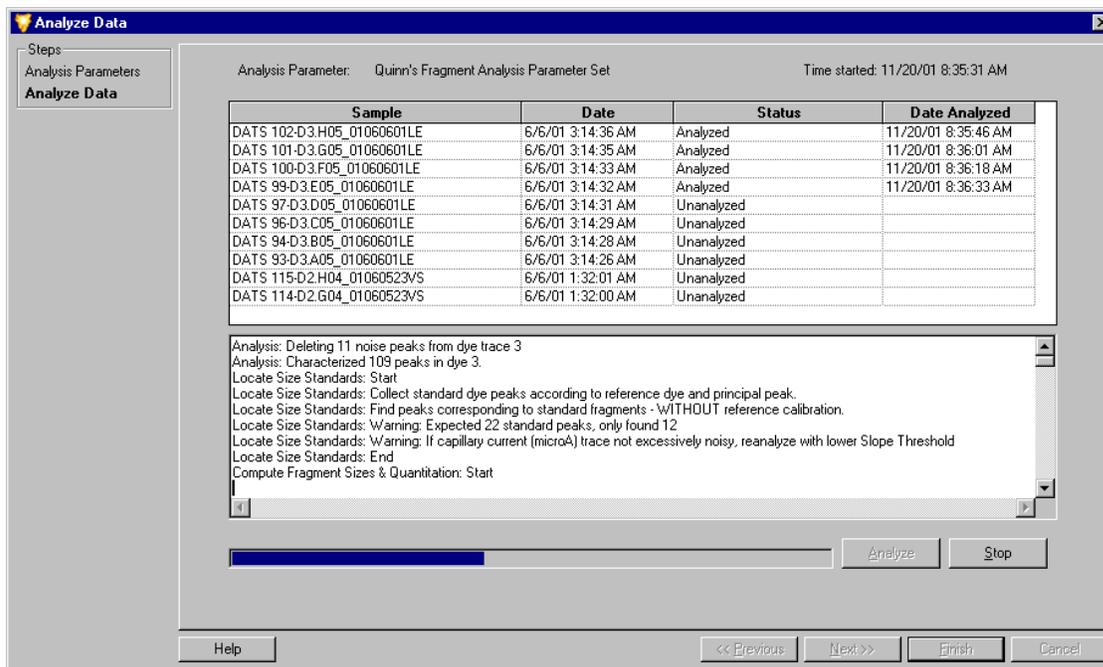
> *Note* *Refer to the Analyzing Data area of this manual or the Online Help for information on this window and the data analysis process.*

6. Click the **Analyze** button to begin the re-analysis.

7. As the system proceeds with the re-analysis, the progress bar will be active and the lower dialog box will record and display the analysis log for each sample as it is re-analyzed.

8. To stop the re-analysis while it is in progress, click the **Stop** button. The system will stop the re-analysis when it has finished with the sample in progress.

9. When the re-analysis is complete, the status column will indicate if the re-analysis succeeded or failed for each sample selected for re-analysis.

10. 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.

11. When you have finished, click **Finish** to return to the **Result Set View**.

When the re-analysis is complete, the initially selected results are removed from the Study and replaced with the re-analyzed results. The Result Set View, including the Summary area, are refreshed with the new data and the selected filters are re-applied to the entire set of data.

> *Note* *Refer to the Online Help for detailed descriptions of the Re-Analysis process.*

Fragment Lists

The Fragment List window allows you to view and modify the results of the fragment data identified in the Study. This fragment list can be subjected to a "3rd Level" of filtering through application of additional exclusion filters as defined in the filter set.

Accessing the Fragment List View

- Open the **View** menu and select the **View Fragment List** command.
- The **Fragment List** can also be accessed from the **Data** tab of the **Study Explorer**.
- The **Fragment List** window will be displayed.

Figure 148: Fragment List window

The **Fragment List** displays a table of all analyzed fragments that have been included in the Study. The displayed columns are user-defined and can be modified to show a number of different system variables and information.

The Filter Set area is where the user-defined exclusion filters are selected and applied to the fragment data. Each column contains a menu of parameters to be applied as exclusion filters. Filter sets can be created, modified and saved.

> **Note** *The Filter Sets applied to the Result Set data (2nd Level Filtering) are different from the Filter Sets applied to the Fragment List data (3rd Level Filtering). To the Result Set you apply filters to an entire sample which may contain numerous fragments. To the Fragment List you apply filters to individual fragments that have been identified within the various samples. See the "Fragment List" section of this manual or Online Help for more information.*

Applying Exclusion Filters to the Fragment List

The purpose of the 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 Online Help for information and examples regarding the application of various filters to the Fragment List.*

There are many filters available and each must be applied using an operator and a value. Any number of filters can be applied to a result set and the filter sets can be saved 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.

The end result of this "third level" filtering is to leave you only sample data with fragments that you are certain of and to save you the time of "weeding out" inaccurate fragment data later in the analysis.

Available Exclusion Filters

All of the exclusion filters can be found in the drop-down list box available in the **Name** column of the **Filter Set** area. Once you have selected the name of the filter to use, you must select an **operator** (usually +, <, >=, etc.) and a **value** to be applied to the filter.

> **Note** *Refer to the Online Help for detailed descriptions of the available exclusion filters and their use.*

Customizing the Fragment List

The components of the Fragment List are user-defined and can be modified through commands available in the right-click menus. These commands allow you to select specific columns for display in the fragment list, customize the arrangement of fragment list information and modify several other parameters of the fragment list.

> **Note** *Information regarding use of the Column Selector window, the Column Sort window and other display parameters can be found in the "Customizing the Fragment List" sections of the Online Help.*

Manually Selecting Peaks for the Fragment List

The system provides the option of manually selecting peaks to be added to a fragment list, or clearing the fragment list completely and building a new one "from scratch".

To access this function, right-click within the fragment list and select the **Manually Select Peak** command from the menu displayed. When selected, the system asks if you want to clear the existing fragment list. Select *No* if you want to keep the list but add or remove peaks from it. Select *Yes* if you want to discard the system-generated list and start over.

Export Fragment List to CSV File

The fragment list can be exported to other 3rd party spreadsheet programs. To export the fragment list, right-click on the highlighted sample(s) from any of the data or fragment lists and select the **Export to CSV File** command from the menu displayed. When selected, the Export window will be displayed where you will select a name and location to save the file. See the Export section of this manual or the Online Help for more information regarding data export.

Show 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, right-click on the highlighted sample(s) from the fragment list and select the **Show Stacked Graph** command from the menu displayed.

Including, Excluding & Resetting Peaks in the Fragment List

Peaks may be individually included or excluded from the existing fragment list. To include or exclude a peak in the fragment list, right-click on the highlighted fragment(s) and select the **Include Peak** or **Exclude Peak** command from the menu displayed. A stacked view of the data will be displayed and the peaks to be included or excluded can be selected from the displayed trace. Left-click on the peak of interest to select it. Right-click to display the peak menu.

Select the Show Excluded Fragments command to display all fragments that have been excluded from the fragment list. Peaks that have been modified in the fragment list may be reset to their original configuration through the **Reset Peak** command available in the right-click menu.

Single Sample View

The **Single Sample View** displays all of the available information for an individual data sample, including the collected trace views, fragment data, separation and analysis parameters, the calibration curve, etc. Peaks can be added, removed, included or excluded from the fragment data and list in the Single Sample View. Refer to the previous section on **Viewing Trace Data** or the Online Help for more information.

Bin Analysis

"Binning" is a one-dimensional clustering, by size, of fragments compiled from multiple samples. Bin analysis is used to generate an allele list that is representative of the population being studied. This is particularly useful when establishing a database of information on alleles and possible anomalies that are associated with them (such as imperfect repeats).

Binning of alleles from multiple samples allow the user to create a new allele list or update an existing allele list for a locus tag based on the collection of fragment results.

When bin analyses are performed, the system groups fragments by size to form "bins" characterized by an average apparent size and standard deviation of the fragments in the bin.

Setting up a Bin Analysis

A result set must exist in order to create a bin analysis. The result set could be a previously saved Study or a new Study created solely for the purpose of binning. As long as the result set is open you can begin a bin analysis.

- From the **Analysis** menu, select the **New Binning Analysis** command.
- **Bin Analysis** can also be accessed from the **Analyses** tab of the **Study Explorer**.
- The **New Binning Analysis** window will be displayed.

Figure 149: New Binning Analysis window - Bin Parameters
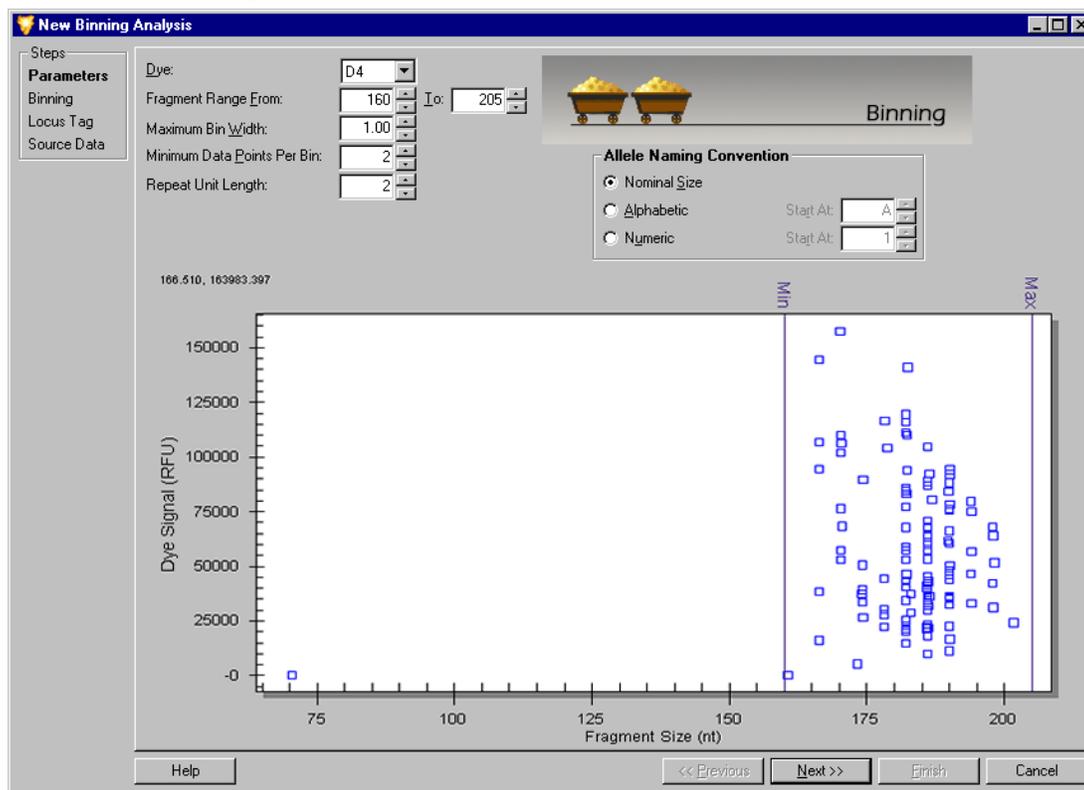
> **Note**: *A minimum of 10 samples is required to begin a Bin Analysis.*

Bin Analysis Parameters

The first step in creating a new bin analysis is to set the bin analysis parameters. These parameters identify which data in the fragment list will be included in the bin analysis and also define the appearance of the view generated. Once the bin analysis parameters have been set, the information from the selected result set will be used to generate a scatter plot. This plot consists of all of the analyzed fragment data displayed as a function of the peak height (y-axis) and fragment length (x-axis).

1. Select the **dye** that was used for the analysis of the raw data. Only one dye can be examined at a time during each bin analysis.
2. Input the **fragment range** (the minimum and maximum fragment size).
3. Input the **maximum bin width** (maximum difference between the smallest and largest fragments within a bin) in nucleotides.
4. Input the **minimum data points per bin** (the limiting number of points necessary to form a bin in the scatter plot).
5. Input the **length** of the **repeat unit** of the allele.
6. Select the preferred **allele naming convention** (see the "Locus Tag Editor" section for more information).

When finished setting the bin analysis parameters, click **Next**. The new parameters will be applied to the scatter plot and the bins will be assigned as specified.

Figure 150: New Binning Analysis window - Bin Analysis View
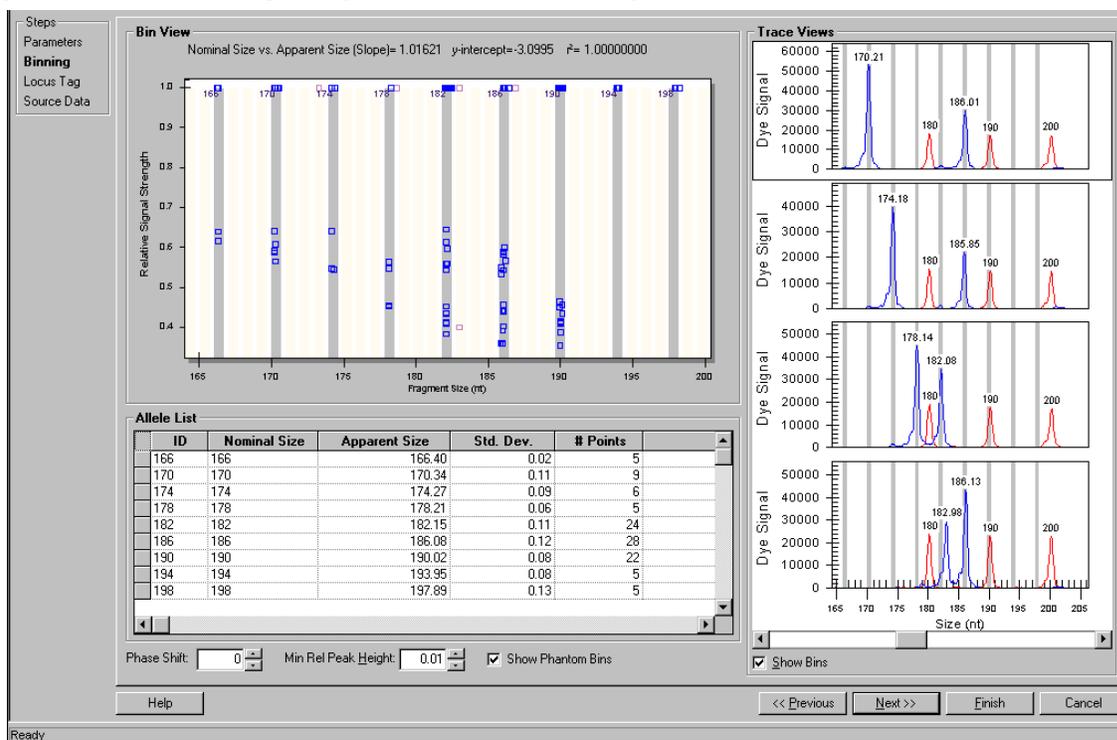
Bin Analysis View

Once the bin analysis parameters have been applied to the selected data, the system displays the results of the bin analysis. This bin analysis view is composed of three areas representing the selected results.

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 in the creation of the study. The bins are defined and visible with the majority of the result points within their borders.

Each trace from which the points in the bin were derived can be displayed in the **Trace View** area at the right of the window. Traces to be displayed are selected by clicking on a point in the bin view. The selections are stacked in the trace view area.

The **Allele List** is initially developed from the information provided in the first window of the Bin parameters plus the data from the fragment list (see previous). When the analysis is complete, the user can make corrections to the allele list using the tools described below.

A **Phase Shift** moves all identified bins by a user-selected integer (nucleotide) value in either direction. This feature can be used to override the system-determined position of perfect repeat alleles.

The **Minimum Relative Peak Height** value can be adjusted 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.

The system also 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.

Selecting the **Show Phantom Bins** check box will highlight the area of all system-generated phantom bins. Phantom bins can be converted to real bins (alleles) by right clicking on the desired bin location and selecting the **Create Allele** command from the menu displayed. A line will be added to the **Allele List** representing the new bin and should be checked for consistency.

Selecting the **Show Bins** check box will display the bins with each trace seen in the stacked trace views.

Investigating Points

Position your mouse-pointer over any point in the bin view plot and left-click to make your selection. The trace for the point will be displayed for visual comparison to other points.The visual comparison of the two traces will help you to confirm the assignment of the data point to an already defined bin, or to a new bin of its own.

The system will assign 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 on the system suggested ID and make changes. The final classification is up to the user.

Continue selecting points and viewing their traces until you are satisfied with all the points in the histogram.

The information for a new allele or the modified information of an existing one is stored in the system to be applied in later studies. The system automatically recalculates the statistics based on the edited results.

Updating the Locus Tag and Allele List

Once the bin analysis is complete, the system has generated an allele list based on the information gathered during the analysis. The list is updated, but at this point has not been saved to the database. The system prompts you for the locus tag to save to the generated allele list.

Figure 151:New Binning Analysis window - Updating Locus Tag

You may select an existing Locus Tag from the drop down menu by clicking on it and may also add any ancillary information to the selected locus tag at this time.

When you have finished editing, save the new data to the selected locus tag so that the information updates the allele list with regards to Dye, Size and Repeat Unit Length.

> **Note** *Refer to the "Locus Tag Editor" section of this manual or the Online Help for more information about locus tag parameters.*

The system saves the following information:

- Observed Size (bin mean)
- Bin Minimum & Maximum
- Standard Deviation
- # of Data Points
- Minimum Peak Height
- The *X* and *Y* coordinates of all the data points in the plot (stored as additional information).

The allele list is automatically updated with the new information that is obtained during the bin analysis.

Reviewing the Source Data

Once the Bin Analysis parameters have been applied to the data, the reference and trace points selected and the locus tag and allele list updated, you may 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 (see Single Result View, Stacked View & Overlay), manually include or exclude individual samples or groups of samples from the analysis (see Add or Remove Results) and Re-Analyze selected samples (see Re-Analyzing Results).

Figure 152: New Binning Analysis window - Reviewing the Source Data
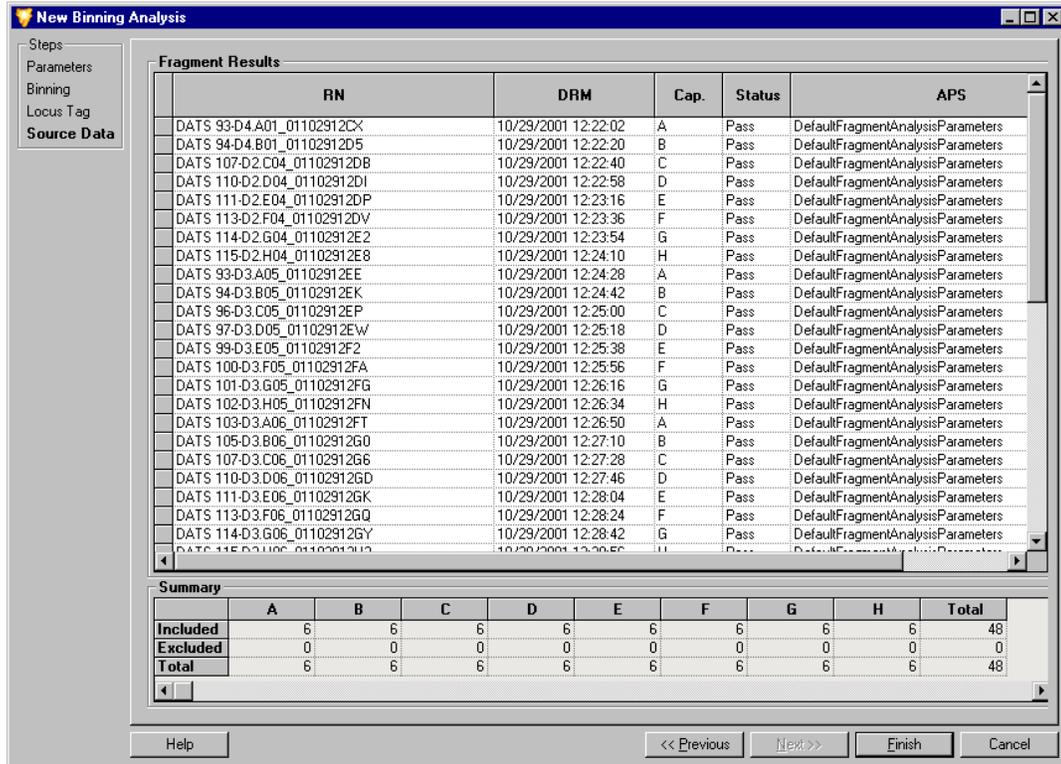
Click **Finish** to add the Bin Analysis to your Study.

Peak Ratios

Once a Study is created, you can begin Peak Ratio calculations. Peak Ratio calculations involve the analysis of a result from a study that quantifies the amount of DNA present in a fragment. The quantity of DNA is calculated by the system by relative comparison to a user defined reference peak.

Beginning a Peak Ratio Calculation

Once you have created a Study, peak ratios can be calculated.

- From the **Analysis** menu, select the **New Peak Ratio** command.
- The **New Peak Ratio** window will be displayed.

Figure 153: New Peak Ratio window
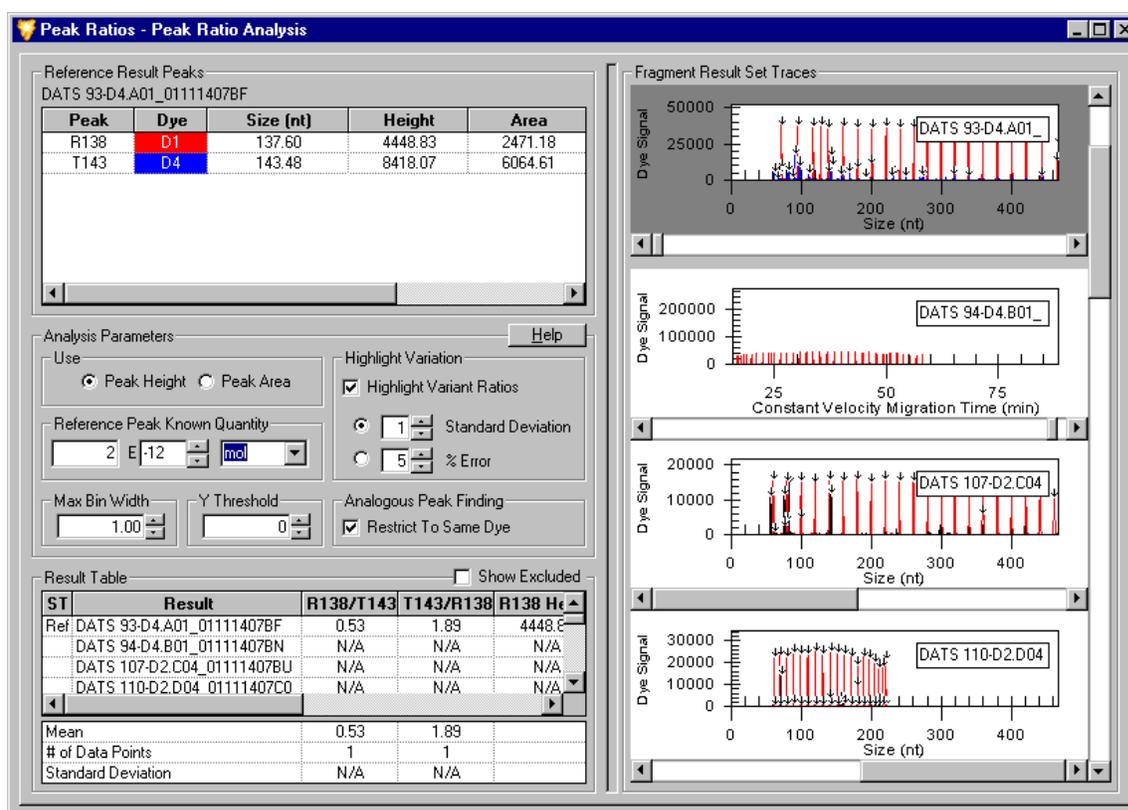
The Peak Ratio window displays all of the result data and the selected parameters used to quantify the amount of DNA present in your sample.

Reference Result Peaks

This area shows the selected reference and test peak information. The reference result is selected by the user and contains the best reference and test peaks for the system to use for a relative analytical comparison of trace peaks.

> *Note*   ***Any number of Test Peaks may be selected, but only one Reference Peak may be selected for each Peak Ratio Analysis.***

Peak Ratio Parameters

- Select the **Use Peak Height** option button to perform a quantitation based on peak height (Relative Fluorescence Units (RFU)).
- Select the **Use Peak Area** option button to perform a quantitation based on peak area (mm x RFU).
- (Optional) If desired, enter the known quantity of your reference peak in the **Reference Peak Known Quantity** field. This value must be entered into **scientific notation**. Select the **units** for the quantity from the drop-down list box. The options are **moles**, **grams**, **molarity or g/L**. The system uses this information to calculate the quantity of DNA in the other results.

Highlight Variation

- Select the **Highlight Variant Ratios** check box 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**.
- Select the **Standard Deviation** option button to enable the system to base its criteria for identifying any variant ratios on the number of standard deviations from the mean. Input the number of the standard deviation that the system will allow a ratio to reach before it considers the ratio to be variant.
- Select the **% Error** option button to enable the system to base its criteria for identifying any variant ratios on the percentage of error. Input the value of the percentage of error that the system will allow a ratio to reach before it considers the ratio to be variant.

Peak Ratio Result Table

The system will perform the analysis upon the selection of a reference and at least one test peak.

- Select the **Show Excluded** check box if you would like the result table to display peaks excluded from the analysis.
- The **Status** column displays an "R" if the result has been selected as a reference.
- The **Result** column displays the result name.
- The **Ratio R/T** column displays ratio of the reference peak to the test peak.
- The **Ratio T/R** column displays the ratio of the test peak to the reference peak.
- The **Timestamp** column displays time the quantitation was performed.
- The **Sample Name** column displays sample name.
- The **Reference Peak Height** column displays the height of the selected reference peak.
- The **Test Peak Height** column displays the height of the selected test peak.
- The **Test Peak Quantity** column displays calculated quantity of the test peak based on user-supplied reference values.
- The **Comments** column displays any of the user's comments that have been associated with the result.

Mean, Data Points & Standard Deviation

The lower left area of the Peak Ratio window displays 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 will calculate the **Mean**, the **# of Data Points** and the **Standard Deviation**.

Selecting 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. The reference trace selection can be made 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** on your selection to open the drop-down menu.
- Select the **Set Result As Reference** command.
- The fragment result will be marked with an "R" in the left-most column to distinguish it as the reference trace.
- The corresponding reference result trace will be highlighted with a dark background and moved to the top of the Fragment Result Set Traces area of the window to distinguish it as the reference result trace.
- **To de-select the reference result**, right-click on the reference result and select the Set Result As Reference command once again to toggle the command to the "off" position (unchecked).
- Once de-selected, the "R" will be removed and the trace will be returned 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** on your selection to open the drop-down menu.
- Select the **Reference** command.
- The result trace will be highlighted with a dark background and moved to the top of the Result Set Traces area of the window to distinguish it as the reference result trace.
- The corresponding result will be marked with an "R" in the left-most column to distinguish it as the reference peak.
- **To de-select the reference result**, **right-click** on the reference result trace and de-select the Reference command once again to toggle the command to the "off" position (unchecked).
- Once de-selected, the "R" will be removed and the trace will be returned to its original highlight status and position in the Fragment Result Set Traces area of the window.

Selecting a Reference Peak

- A reference peak is a trace peak selected by the user from the reference result trace. The reference peak is compared to the test peak and a relative length, height and area are then calculated by the system. These results are added to the Peak Ratio Results Table.
- **To select the reference peak**, right-click on an individual peak from the selected **Result Set Trace** located at the top of the **Fragment Result Set Traces** area of the window.
- From the drop-down menu displayed, select the **Reference Peak** command.
- Once a peak is selected as a reference peak the system will calculate the length, area and height of the test peaks by comparison and will enter this information into the Quantitation Result Table.
- **To de-select the reference peak from the Reference Result Peaks table**, **right-click** on the reference peak and de-select the Set Peak As Reference command to toggle the command to the "off" position (unchecked).
- **To de-select the reference peak from the Fragment Result Set Traces area**, **right-click** on the reference peak to open the drop-down menu.
- Open the **Peak** sub-menu and de-select the **Reference** command (unchecked).

Selecting a Test Peak

- A test peak is a trace peak selected by the user from the reference result trace. The test peak is compared to the reference peak and a relative height and area are then calculated by the system. These results are added to the Peak Ratio Results Table.
- **To select the test peak**, right-click on an individual peak from the selected **Result Set Trace** located at the top of the **Result Set Traces** area of the window.
- From the drop-down menu displayed, select the **Test Peak** command.
- Once a peak is selected as a test peak the system will calculate the area and height by comparison with the reference peak and will enter this information into the Peak Ratio Result Table.
- Each selected test peak is used 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 de-select a test peak from the Reference Result Peaks table**, **right-click** on the test peak and de-select the Set Peak As Test command to toggle the command to the "off" position (unchecked).
- **To de-select a test peak from the Fragment Result Set Traces area**, **right-click** on the test peak to open the drop-down menu.
- Open the **Peak** sub-menu and de-select the **Test** command (unchecked).

AFLP Analysis

AFLP (Amplified Fragment Length Polymorphism) 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 CEQ System includes AFLP Fingerprint analysis, which allows you to rapidly score AFLP fingerprints with output as a text format which is compatible with most statistical and pattern matching software. The AFLP analysis tool is a cluster analysis program that scores the presence or absence of a fragment of a given size per sample.

Using 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, a "1" is assigned if a fragment is found in that sample and "0" is assigned if the fragment is absent. A table of 1's and 0's is then created. The text can be exported to most other statistical analysis and spreadsheet software.

Setting the AFLP Analysis Parameters

Open the **Analysis** menu and select the **New AFLP Analysis** command.

- This can also be accessed from the **Analyses** tab of the **Study Explorer**.
- The **New AFLP** window will be displayed.

Figure 154: New AFLP window
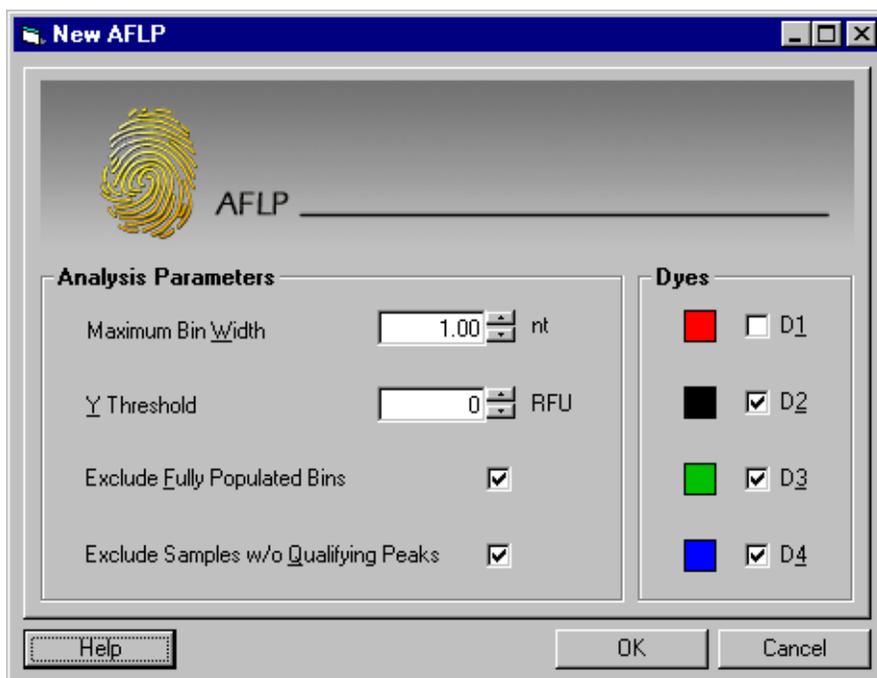
Analysis Parameters

The analysis parameters area allows you to set filtering parameters for including or excluding fragment results in your AFLP analysis.

The **Maximum Bin Width** field is used to set the limiting value given to the width of the individual bins.

- Set the width of the bin by entering a value in nucleotides.
- The default value is 1.0 nt.
- Within each dye label, the program will cluster the fragments with the sizes that fall within the set bin width and create a bin with size rounded to the next integer.

The **Y Threshold** parameter serves as a exclusion filter, defining the lowest acceptable value (peak height) for the y-axis.

- Set the y-threshold in relative fluorescence units (RFU).
- The default value is zero RFU.
- Fragments with heights less than this threshold value will be excluded from the clustering process.

Select the **Exclude Fully Populated Bins** check box if you would like to exclude bins which are represented in all samples (i.e., non-polymorphic fragments).

Select the **Exclude Samples without Qualifying Peaks** check box if you would like to exclude samples that have no qualifying peaks.

The **Dyes** parameters determine the dye colors to be used by the system during the cluster analysis.

- If the results list contains samples with fragments with 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.

Starting a New AFLP Analysis

When you have finished setting the analysis parameters, click **OK** to generate the
New AFLP Analysis.

Figure 155: New AFLP Analysis window

Viewing the Cluster Analysis

When the clustering operation is completed, a table will be displayed showing the
results of the AFLP analysis. The format of this table can be displayed as either rows
or columns of data for ease of export to a third party software platform for further
analysis (see sample axis below).

- The **Bin Column / Row** displays the bin number. Bins with A and B suffixes
  will be assigned if two adjacent bins fall in the same integer after rounding of the
  Bin X Mean.
- The **Dye Column / Row** displays the dye included.
- The **Samples Column / Row** displays the sample count within the bin.
- The **Fragments Column / Row** displays the fragment count within the bin.
- The **X-Min Column / Row** displays the minimum fragment size within the bin.
- The **X-Max Column / Row** displays the maximum fragment size within the bin.
- The **X-Mean Column / Row** displays the mean fragment size within the bin.
- The **X-Var Column / Row** displays the fragment size variation within the bin.
- The **Y-Mean Column / Row** displays the mean peak height within the bin.
- The **numbered columns / rows** to the right / bottom of the table show the
  number of sample components (peaks/fragments) within each bin for each sample.

Cluster Analysis Parameters and Display Settings

The selections available at the bottom of the AFLP Cluster Analysis window allow you to make additional settings that affect the values and output format of the fields displayed.

- Selecting the **Sample Bin: Fragment Count** option button will display the number of components within each sample bin as either zero components, one component or multiple components.

> **Note** *The likelihood of finding multiple components in a bin can be reduced by decreasing the bin width*

- Selecting the **Sample Bin: Binary Presence** option button will indicate the binary presence or absence within each sample bin.
- Selecting the **Sample Bin: Fragment(s) Max Y** option button will display the absolute peak height of the fragments within each sample bin.
- Select the **Exclude Fully Populated Bins** check box if you would like to exclude bins which are represented in all samples (i.e., non-polymorphic fragments).
- Select the **Exclude Samples without Qualifying Peaks** check box if you would like to exclude samples that have no qualifying peaks.
- Select the **Show Excluded Elements** check box to highlight all fragment data that was excluded from the analysis.
- The **Maximum Bin Width** and **Y Threshold** parameters are identical to those set in the analysis parameters area of the New AFLP window (see previous). The fields displayed here allow you to make additional adjustments to both the width and threshold.
- The sample names can be displayed in either rows or columns as listed in the **Sample Axis** drop-down list box. This can also be accomplished by selecting the **Transpose** command available in the right-click menu accessed from the column header area of the fragment list.

Customizing the AFLP Table

The components of the cluster analysis are user-defined and can be modified through commands available in the right-click menus. These commands allow you to select specific columns for display in the fragment list, customize the arrangement of fragment list information and modify several other parameters of the fragment list.

> **Note** *Information regarding use of the Column Selector window, the Column Sort window and other display parameters can be found in the "Customizing the Fragment List" section of this manual or in the Online Help.*

Exporting your Results

Exporting the AFLP Analysis
- Open the **File** menu and select the **Export AFLP** command.
- The **Export AFLP** window will be displayed.
- Select a file name and destination folder for the data and click **Save**.

Some 3rd party software may require that you export the sample information in a specific format. When choosing to display the sample names in rows, the alleles can be in separate columns or in one column. The "Bin Labels", etc., can be included in the file by checking the appropriate boxes in the "Export File Format" for file export.

The **Dyes** parameters determine the dye color to be used by the system during the cluster analysis. If the results list contains samples with fragments with 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.

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.

Beginning a LOH Analysis

- Open the **Analysis** menu and select the **Run LOH Analysis** command.
- **Microsoft Excel**$^{\text{TM}}$ will be launched and the **START** sheet will be displayed.

Figure 156: Run LOH Analysis
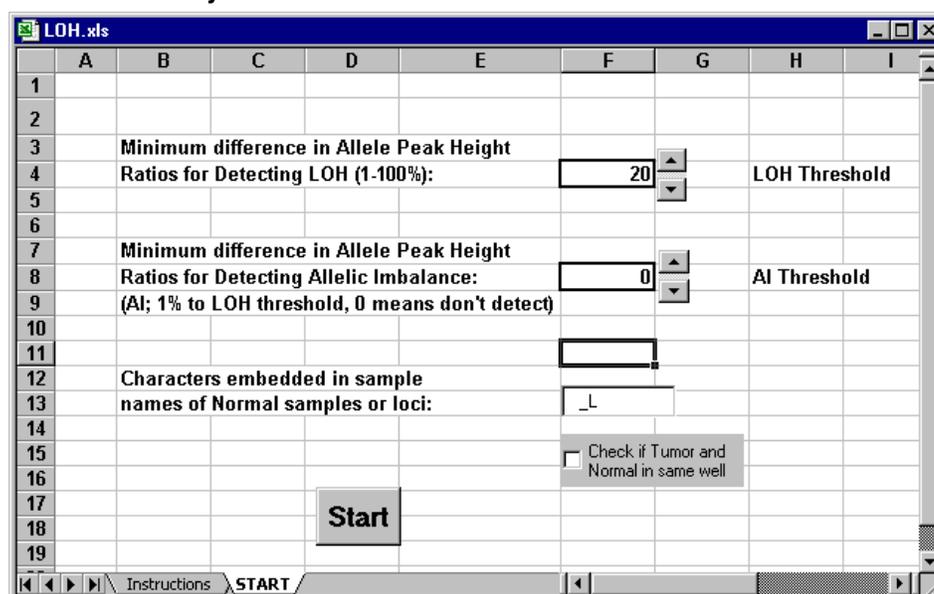
Pairs of samples to be compared must have the same "root" names followed by characters that allow the distinction between reference and test samples or loci. All data to be analyzed will reside in a temporary file created by the CEQ System Fragment Analysis application.

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).

> Note | *You must have "Enable Macros" selected to run the LOH Analysis. You will receive a prompt to enable this feature if it is not already selected.*

From the LOH.xls START sheet:

1. Set the LOH threshold (and the AI threshold if desired).
2. Specify the unique character combination used to identify Normal samples or loci (i.e. Norm).
3. If Normal and "Tumor" samples are being run in the same well, check the corresponding check-box. If Normal and "Tumor" samples are in different wells, make sure the check-box is unchecked.
4. Click the **START** button.
5. As the analysis is being processed, do not use the keyboard or the mouse.
6. When the processing is complete, you may save the results.

Note *The LOH Analysis results <u>must</u> be saved as a "Microsoft Excel Workbook (*.xls)" file type in order for the data to be saved correctly. Additionally, the file name <u>must</u> be changed from the default name displayed in the Save As... dialog; otherwise, the result will be saved as a temporary file and will be overwritten in the next LOH Analysis.*

Note *If more than 2 alleles are found for a locus, the last allele listed for the locus will be followed by an asterisk ("*").*

Customizing the Results List

The components of the results lists are user-defined and can be modified through commands available in the right-click menus. These commands allow you to select specific columns for display in the results list, customize the arrangement of information, add or remove columns and modify several other parameters of the results displayed. Customized lists are available in the Result Set View, the Fragment List, AFLP and Peak Ratio tables.

Selecting Columns for Display

The Column Selector window contains a list of all possible columns that can be displayed in the list. These columns can be selected individually or in groups, they can be arranged in any order and can be locked so that they always remain in view when scrolling through the fragment list.

Accessing the Column Selector window

- Right-click on the column header area of the list to display the pop-up menu.
- Select the **Select Columns** command.
- The **Column Selector** window will be displayed.

Figure 157: Column Selector window - Selecting columns for display in the table
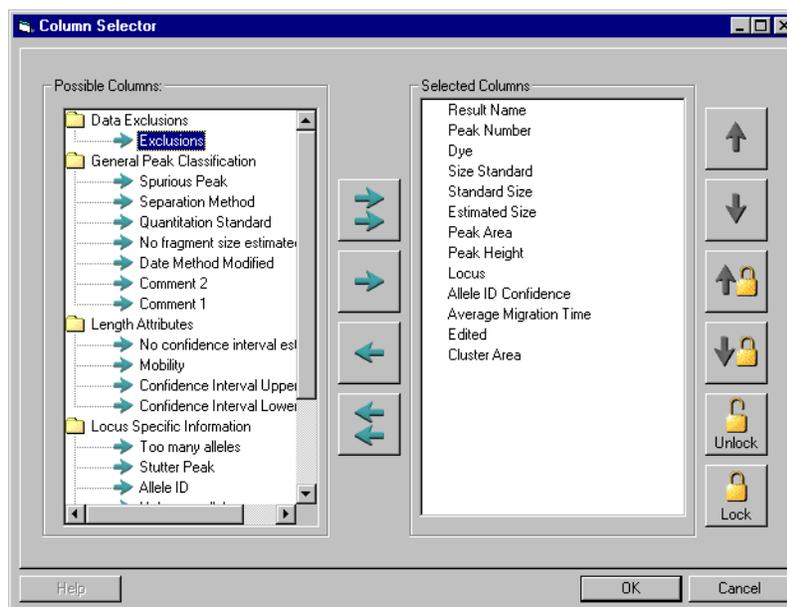
> *Note*
>
> *The columns available for display are determined by the application area in which the Column Selector window is accessed (i.e., Result Set View, Fragment List or AFLP Cluster Analysis).*

> *Note*
>
> *Refer to the Online Help for detailed descriptions of the available columns and their use.*

Selecting Columns for Display

- Highlight the column selection in the **Possible Columns** area of the window and click the right-arrow button to move the selection to the **Selected Columns** area of the window.
- To select all columns for display in the results list, click the right-double-arrow button.
- To remove columns from the Selected Columns area, highlight the selection and click the left-arrow button to move the selection back to the Possible Columns area.
- Columns can also be removed from the results list by right-clicking on the header and selecting the **Hide Column** command from the pop-up menu displayed.

Arranging the Column Display

- The left-to-right order of columns in the results list can be arranged by ordering the columns top-to-bottom 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 be displayed in the fragment result table.

Locking the Column Position

- Selected columns can be locked into position so that they remain in view as you scroll across the remaining columns in the results list.
- This is useful 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 the **Lock** button.
- A lock icon will be placed next to the selected column to indicate that the column position is locked.

> *Note* *All columns positioned above the lock location in the Selected Columns area, and thus to the left of the locked position in the results list will be locked. 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*

- The lock position can be moved to a different column location by clicking on the up-arrow/lock button or down-arrow/lock button in the **Column Selector** window.
- To remove the column lock, click the **Unlock** button in the **Column Selector** window.

- Columns can also be locked and unlocked directly from the fragment result table by right-clicking on the column header and selecting the **Lock Column** command or the **Unlock Column** command from the pop-up menu displayed.

Sorting the Results List

The components of the results list can be sorted in ascending or descending order for any of the column parameters displayed. For example, you can sort the results list by ascending bin number, descending bin fragment count, etc. Columns can be sorted in the Result Set View, the Fragment List, AFLP and Peak Ratios tables.

Sorting an Individual Column

- **Right-click** on the column header of the column you want to sort to display the pop-up menu.
- Select the **Sort Column** command.
- The column will be sorted in either ascending or descending order, depending upon the order in which it was previously arranged.
- To sort the same column in the reverse order, right-click on the header and select the **Sort Column** command again.

Accessing the Column Sort window

- **Right-click** on the column header area of the fragment list to display the pop-up menu.
- Select the **Sort Columns** command to display the **Column Sort** window.

Figure 158: Column Sort window - Sorting the results list data columns
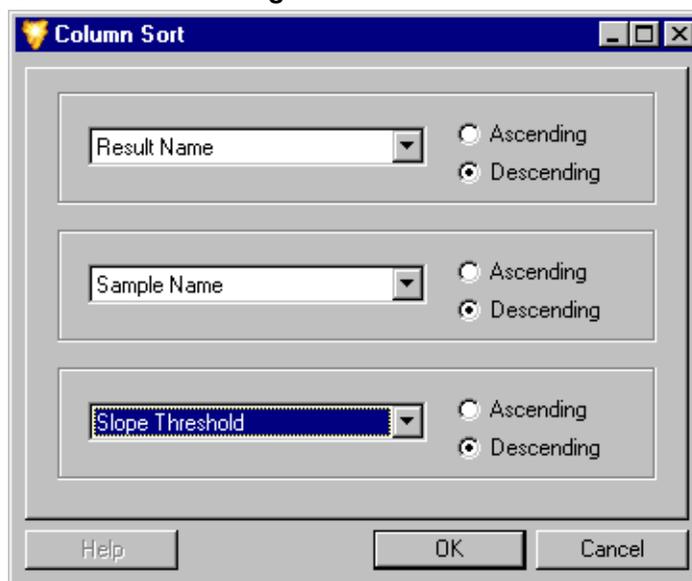
- Select the column parameter to be sorted and the order in which it is to be sorted (ascending or descending).
- Click **OK** to sort the fragment list.

More information on these and other display modifications can be found in the Online Help.

Reporting Results

The CEQ software includes a powerful suite of utilities for creating reports. It also allows you to export data and results to other 3rd party software packages.
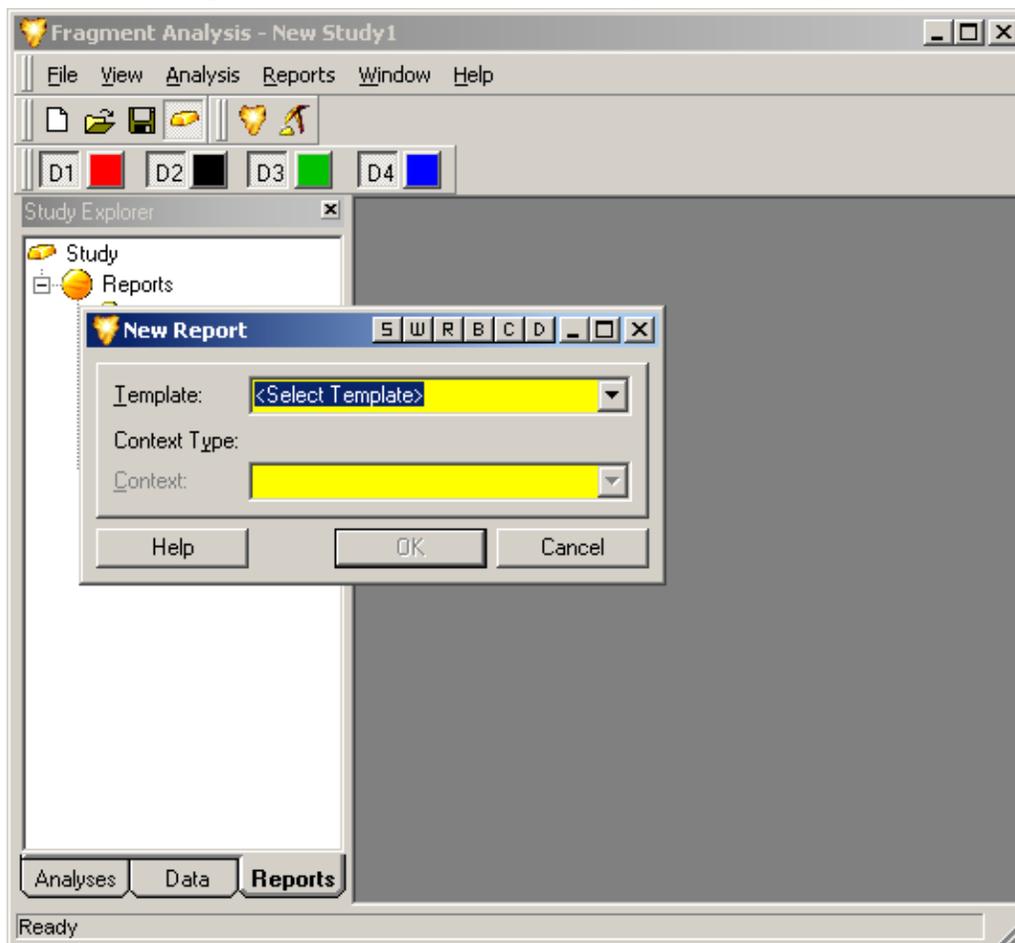
All of the reporting features available in the Fragment Analysis module are located under the **Reports** tab of the **Study Explorer**. Additionally, new report generation can also be accessed through the **Reports** menu located on the **Menu** bar.

The **New Report** window allows you to select the type of report you would like to generate from a list of available report templates (see *"Report Templates"* on page 291) and saved data. The **New Report** window can be accessed from either the **Report** menu or from the **Study Explorer**.

Creating a New Report from the Report menu:

- Open the Report menu and select the New Report command to display the New Report window.
- Select the type of report you would like to generate from the Template drop-down menu. The selections available depend upon the data contained in the open Study.
- Once the template has been selected, select the saved data that you would like to report from the Context drop-down menu.

- Click OK to display the new report containing the selected information.

Creating a New Report from the Study Explorer:

- 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 the New command from the menu displayed.
- The New Report window will be displayed with the Context Type reflecting the highlighted selection.
- Select the report template from the Template drop-down menu. The selections available depend on the Context Type.
- Click **OK** to display the new report containing the selected information.

Report Templates

There are several types of elements in each report template:

1. Header text for data, tables, or graphs
   E.g. **Bin Scatter plot**
2. Regular Data Objects
   E.g. **Context.Locus.Name (Locus)**
3. Loop Delimiters:
   BeginLoop: Allele = Context.Locus.Alleles

ID:	**Allele.ID**
Nominal Size:	FormatNumber(Allele.TrueSize, 0)
Apparent Size:	FormatNumber(Allele.ApparentSize, 2)
Standard Deviation:	FormatNumber(Sqr(Allele.FragmentSizeVariance))
Number of Data Points:	Allele.FragmentCount
Comment:	Allele.Comment

   EndLoop: Allele = Context.Locus.Alleles

4. Table Delimiters:

TableLoop: Allele = Context.Locus.Alleles

ID	Nominal Size (nt)	Apparent Size (nt)	Standard Deviation (nt)	Num. Points	Comments
Allele.ID	FormatNumber (Allele.TrueSize, 0)	FormatNumber (Allele.Apparent-Size, 2)	FormatNumber (Sqr(Allele.Frag-mentSizeVari-ance))	Allele.Fragment-Count	Allele.Comment

5. Graph Objects

E.g. **Graph: Service.BinGraph (Context, Site, DisplayOptions)**

6. Graph Placeholder pictures:
   E.g.:
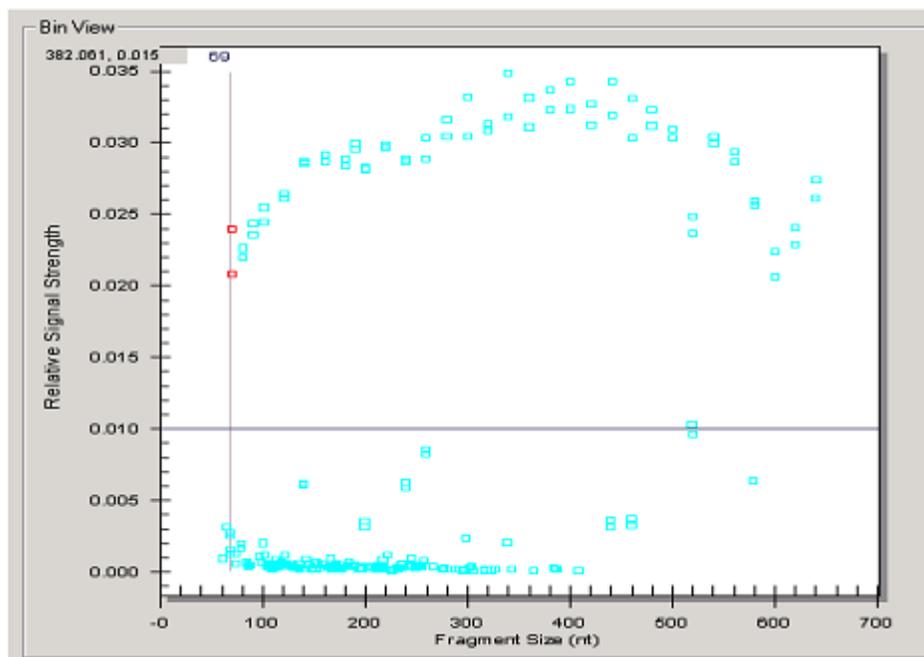
Figure 159:Placeholder pictures

Header text can be edited or removed at will. The header text in the Report Templates provided can be used as references to identify the data objects that follow.

Data object descriptions, highlighted in gray, can be removed, but should not be edited, because they are very 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.

Iterations of similar data can be achieved using Loop delimiters or Tables. 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 for proper graph display. Changing the graph display is discussed below.

Editing Graph Displays

Five features of the graph displays can be edited: 1) X scale type. 2) X-axis scale, 3) Y-axis scale, 4) Pane Count or Pane Width and 5) Dyes displayed.

To Edit the graph:

1. Right click on the graph placeholder.
2. Select Format Object.
3. Go to the "Web" tab and create the following text:

    ```
    <DisplayOptions
    (Your commands will go here)
    />
    ```

Editing 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 display by size or by time. If the XScaleType attribute is not specified, 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"
/>
```

Editing the 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"
/>
```

Editing the 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 either or both MinY and MaxY are not specified, they take on the minimum and maximum values from Unzoom all graph.

Editing the Pane Count or the Pane Width

Only one of PaneCount or PaneWidth should be specified. If PaneCount is specified, it represents the number of panes that the graph will be broken into. If neither PaneCount nor PaneWidth is specified, the default is to have a PaneCount of "1". PaneCount is an integer.

If PaneWidth is specified, it represents either the size or time per pane, depending on the XScaleType. The graph will be 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"
/>
```

Editing the number of dyes displayed on the Graph

SelectedDyes is dye selection options. It allows Egram graph to select which dye to report. The FA view dye visibility will not affect this option. The possible value for selectedDyes are "all", "1", "2", "3", "4" or combination of "1", "2", "3", "4". "All" means display all four dyes.

By default, the display for the four dyes is: 1 = D1, 2 = D2 etc.

In the DisplayOptions section of the Alternative text: box, enter the desired dyes as follows:

```
<DisplayOptions
XScaleType="BySize"
MinX="100"
MaxX="300"
MinY="0"
MaxY="10000"
PaneCount="3"
SelectedDyes="1,4"
/>
```

Exporting Results

You are able to include all of the property information when exporting a fragment result in text format. You may open the exported file in any text editor and/or in MS Excel and Preview the file prior to printing.

Text File Format

Follow these steps to export the Properties information with a fragment result:

- Launch the Fragment Analysis application and open a fragment result.
- Select the **Export** command from the **File** menu.
- The **Export Results** window will be displayed.

Figure 160: Export Results
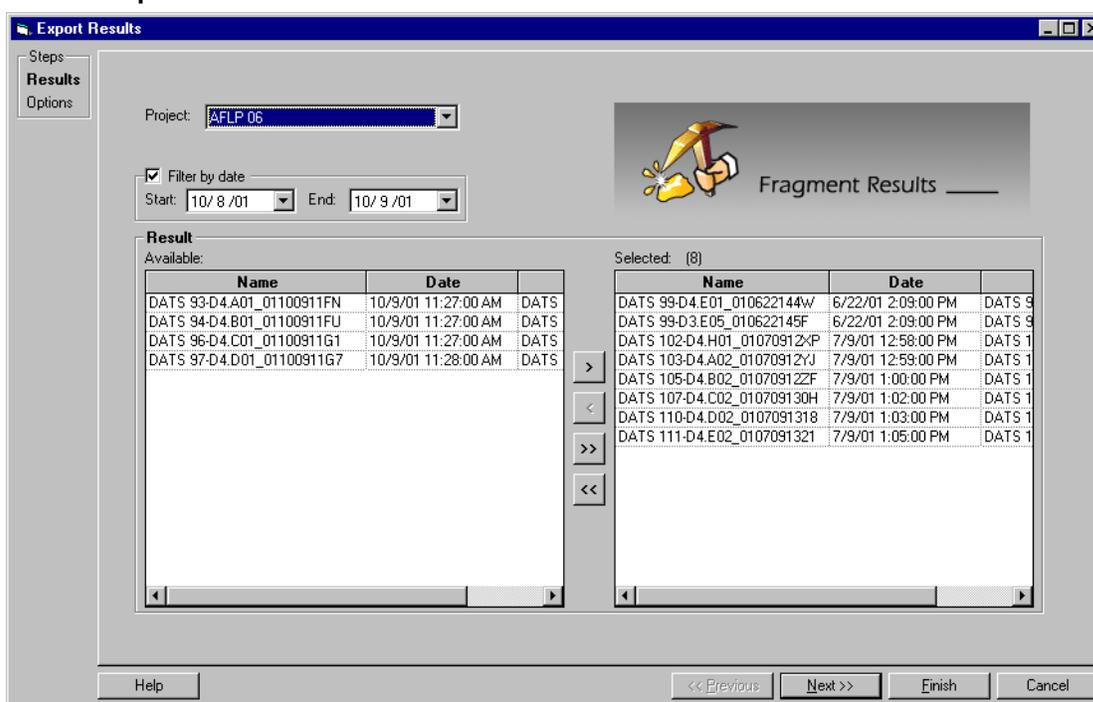
The first step involves selecting the results to be exported from a list of available results located in various projects. Select the Project folder that contains the sample data that you would like to export from the **Project** drop-down list box. To select the samples to be exported, click to highlight the sample, then click the right-arrow button to move the selection to the **Result / Selected** area.

> **Note**
>
> *The functionality of this window is very similar to that of the Select Raw Data window described in the "Creating a Study" section of this manual.*

When you have finished making your selections, click **Next** to proceed to the **Export Options** window.

The Export Options window allows you to define the export parameters and options, including the file name and location.

Figure 161: Export Options
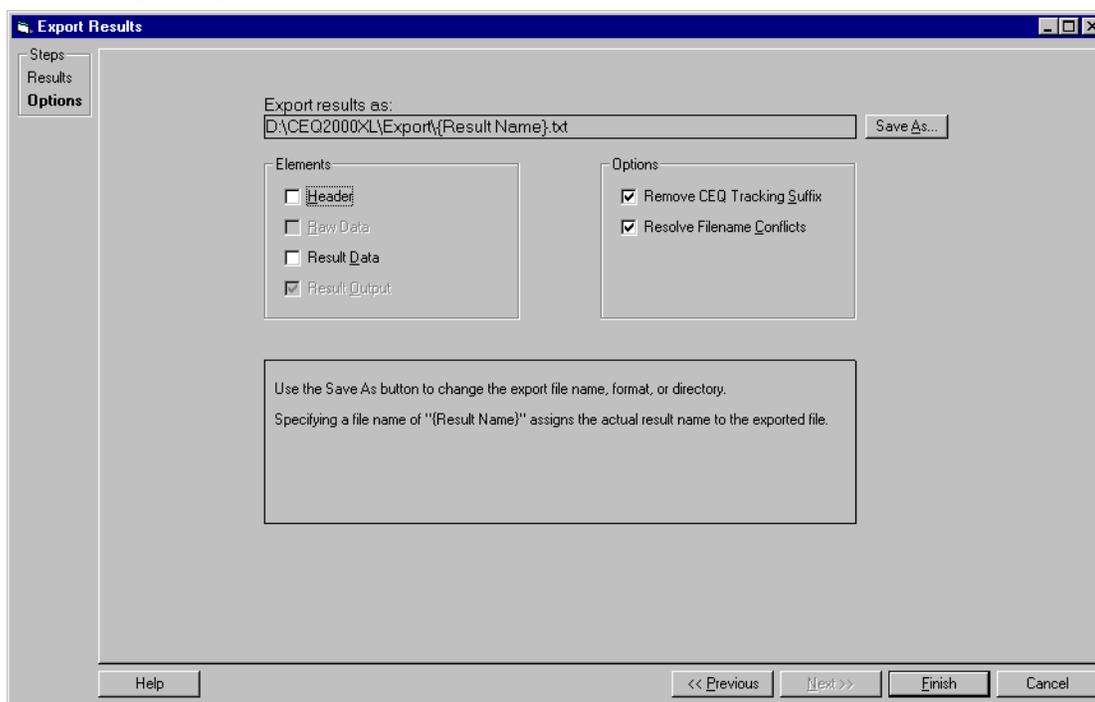
Features of the Export Options window:

- The **Export result as...** field displays the selected export format (file name extension), the file name and the directory path.
- This is a *read only* field. To change these selections, click the **Save As...** button and input the required information.
- Select the **Header option** button to include general information regarding the sample and run conditions with the exported results.
- Select the **Raw Data** option button to include raw data with the exported results.
- Select the **Result Data** option button to include dye traces and data points with the exported results.
- Select the **Result Output** option button to include a fragment list with the exported results.
- Select the **Remove CEQ tracking suffix** option button to remove the sample plate and date from the exported file name.
- Select the **Resolve filename conflicts** option button to automatically append a unique sequence number to exported files of the same name.

When you have finished making your selections, click **Finish** to complete the export application.

Text File Format

The text file format is an export of the results file. The data in this file are tab delimited and the export format has the extension *.txt.

Figure 162:Fragment Analysis Export Dialog
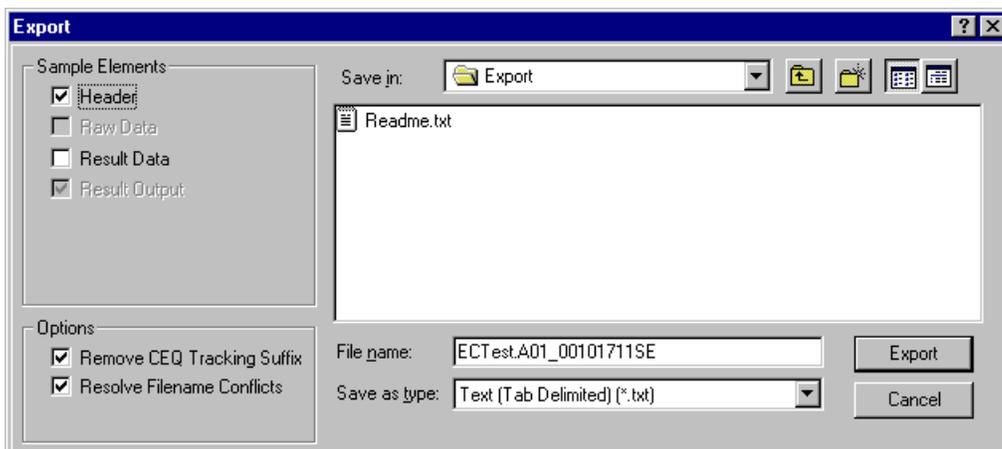
- Select a target folder and file name.
- Choose "Text (Tab Delimited) (*.txt) in the "Save as type:" text box.
- Make sure **Header** is selected in the Sample Elements section.
- Click **Export**.
- Now navigate to the target folder and double click on the exported file to open it in Excel, Notepad or any other text editor.

You can view the "Properties" information at the beginning of the file.

CEQ File Format

The CEQ System offers the CEQ 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 163: Fragment Result Export Dialog
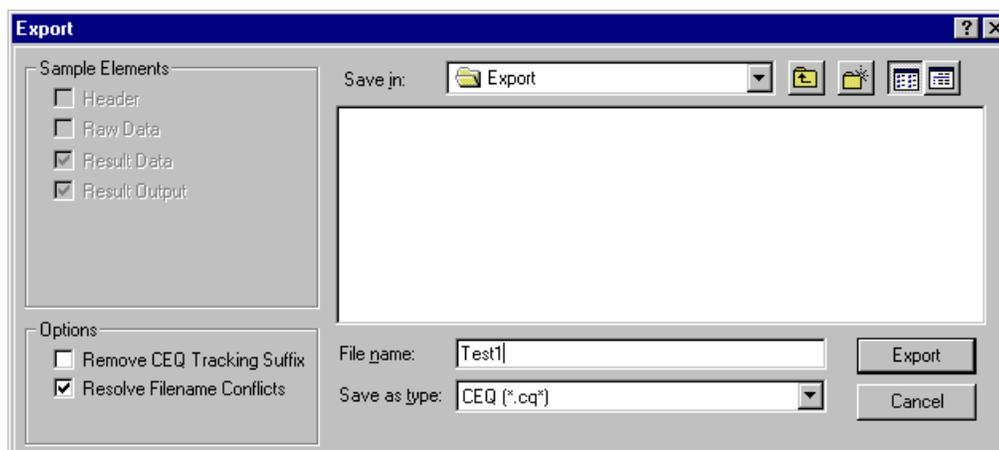
To export fragment results in CEQ (*.cq*) format, perform the following steps.

- Launch Fragment Analysis and open a fragment result.
- Select **File | Export** to invoke the Export dialog box.
- Navigate to a folder to save the data file and enter a file name.
- Select CEQ (*.cq*) from the Save as type text box.
- The Sample Elements section of the dialog box will change to reflect this selection.
- Check **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 CEQ System Software.
- Check **Resolve Filename Conflicts** to enable the system to increment the sample name(s) on export if it encounters a sample(s) with the same name(s).
- Now, click **Export** to export the Result Data and the Result Output.

Exporting Fragment Lists or Genotypes from a Study

This feature allows the export of the opened study's fragment list or genotype data. The pedigree and genotype data included in the export comes from the results included in the opened study.

Follow these steps to export the Properties information with a fragment result:

- Launch the Fragment Analysis application and open a Study.
- Select the **Export Fragment List** command from the **File** menu.
- The **Export Fragments / Genotypes** window will be displayed.

Figure 164: Exporting Fragment Lists and Genotypes
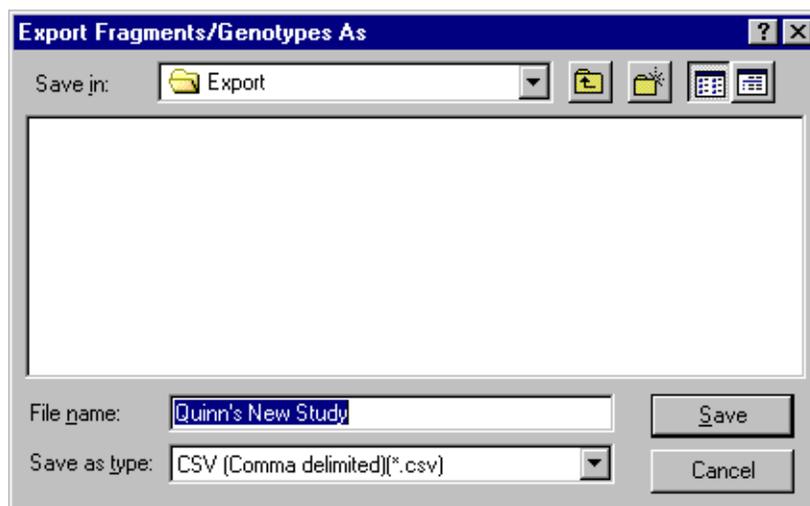
- Input the desired export format, the file name and the directory path.
- There are four export formats in the Save as type: drop-down list:
    - CSV (Comma) *.csv
    - Pedin (Linkage) *.pre
    - DMPopulation (Discovery Manager) *.txt
  - Genotype Summary Report *.gsr
- The status of the exporting, along with a processing log, is displayed in a status window. This window indicates when the export procedure is complete and any export errors that may occur.

Format Descriptions and Exporting Requirements

CSV (Comma) *.csv

This is an export of the 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 3rd party spreadsheet applications. The export format has the *.csv extension.

Pedin (Linkage) *.pre

This is an export of the Study's pedigrees and genotypes. The Pedin file is designated to be imported into a Linkage application's MakePed utility program. The pedigree and genotype information from each result in the Study is included in the Pedin file. Up to two alleles per locus is supported.

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**". This file must be stored in the CEQ application'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 will determine the order of the loci in the Pedin file.

The Pedin 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 (i.e., Pedigree ID, Individual ID, Father ID, Mother ID & Gender).

Pedigree information is associated with samples through the sample plate editor, using the sample properties set "Pedigree" template. Alternatively, the pedigree information can be associated with results, using the single result view's property set tab.

DMPopulation (Discovery Manager) *.txt

This is an export of the 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.

This 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 CEQ application'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 will determine 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 properties set "Pedigree" template. Alternatively, the pedigree information can be associated with results, using the single result view's property set tab.

Genotype Summary Report *.gsr

The Genotype Summary Report has been designed to accommodate Subject IDs, the occurrence of more than two alleles and the absence of alleles (Figure 165). Use the **"Locus List Editor"** on page 302 to customize the LocusList.txt file before exporting a Genotype Summary Report.

The report layout is described below:
- 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 *None found*.

	Subject 33	Subject 63	Subject 65
GATA193A07 A1			
GATA193A07 A2			
D11S1984 A1	182	Too many (3)	None found
D11S1984 A2	190	Too many (3)	None found
D14S599 A1			
D14S599 A2			
D1S1679 A1			
D1S1679 A2			

Figure 165:Genotype Summary Report

Locus List Editor

The locus list (LocusList.txt) is required to create the Genotype Summary Report (GSV) and to perform some exports. The locus list editor is used to customize the LocusList.txt file. To open the Editor, press the **F7** key or select **View | Locus List Editor**. Figure 166 shows the Locus List Editor and Table 73 details its function.
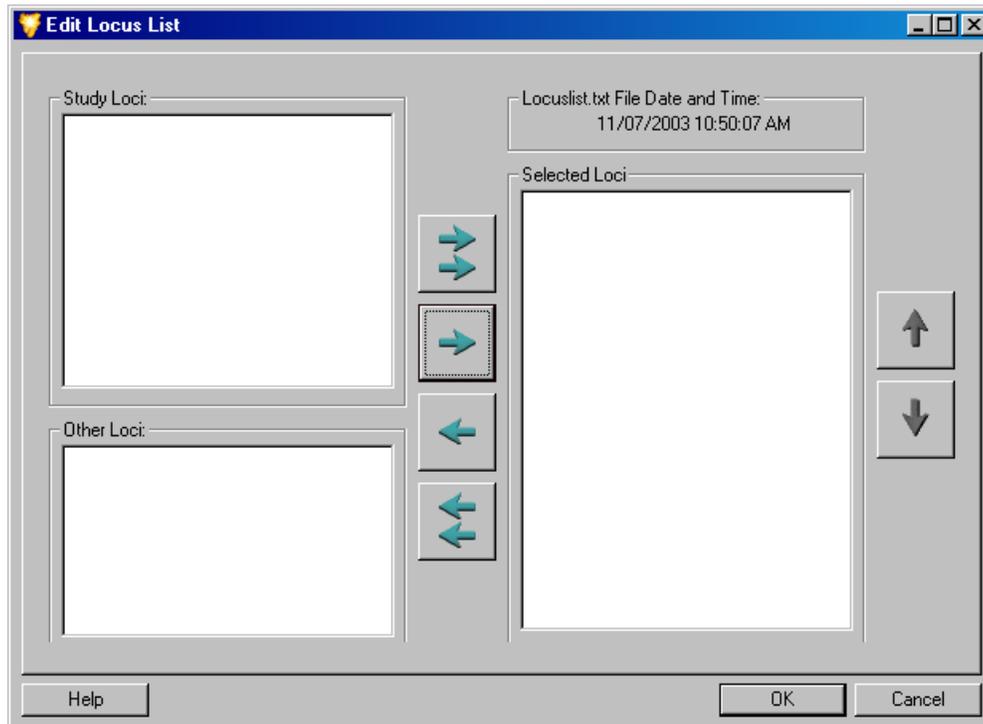
Figure 166:Locus List Editor

Table 73: Locus List Editor - Legend

Study Loci	The loci from the database that have not yet been moved to the Selected Loci list box.
Other Loci	The loci that will be removed from the LocusList.txt file.
Selected Loci	The loci that will be added to the LocusList.txt file.
	Moves all loci from the Study Loci and Other Loci list to the Selected Loci list.
	Moves only the selected loci from the Study Loci and Other Loci list to the Selected Loci list.
	Moves all loci from the Selected Loci list to the Study Loci or Other Loci list. If the loci exist in the database, they will be moved to the Study Loci list box.
	Moves the selected loci from the Selected Loci list to the Study Loci or Other Loci list. If the loci exist in the database, they will be moved to the Study Loci list box.
	Used to rearrange the list order. Select an item and click the appropriate arrow to move it up or down the list.

Data Manager Procedures

User's Database Template

A database template called USERTEMPLATE is added upon installation of the CEQ software. The USERTEMPLATE database is used to store newly created methods and parameters. 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.

The USERTEMPLATE database cannot be set as the *Working Database*, but it can be deleted. If the USERTEMPLATE database is deleted, no template will be used for subsequent databases. If a new template is required, create a new one named `USERTEMPLATE`.

Creating a Database

To create and name a database, perform the following steps.

1. In the Data Manager window, select **File | New Database**.
2. In the **New Database** dialog box, enter the name for this new database, select **Set as Working Database** (if desired) and select **OK**.

Creating and Naming a Project Folder

To create and name a project folder, perform the following steps.

1. In the Data Manager window, highlight the desired 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. Close the Data Manager module (**File | Exit**).

Deleting a Project Folder or Database

To delete a project folder or database, perform the following steps.

1. In the Data Manager window, highlight the folder or database to be deleted.
2. Select **File | Delete** from the menu.
3. In the **CEQ Data Manager** dialog box, click **Yes** to delete the folder or database.

> *You cannot delete the working database.*

> **CAUTION** **When a folder is deleted, all of the data contained within that folder will be deleted. You will be warned by the system.**

Renaming a Project Folder or Database

To rename an existing folder, perform the following steps.

1. In the Data Manager window, highlight the project folder or database.
2. Select **File | Rename** from the menu bar.
3. Enter the new name for the database and press the **Enter** key.

Database Size

The database size is displayed in the Database Manager. To view the database size, mouse-click the CEQ DBASE item at the top of the left pane (see Figure 170). The size for each database is shown in the right pane.

> **Note** *If data has recently been removed from a database, the size shown may not be accurate. Refer to "Shrink Database" below for more information.*
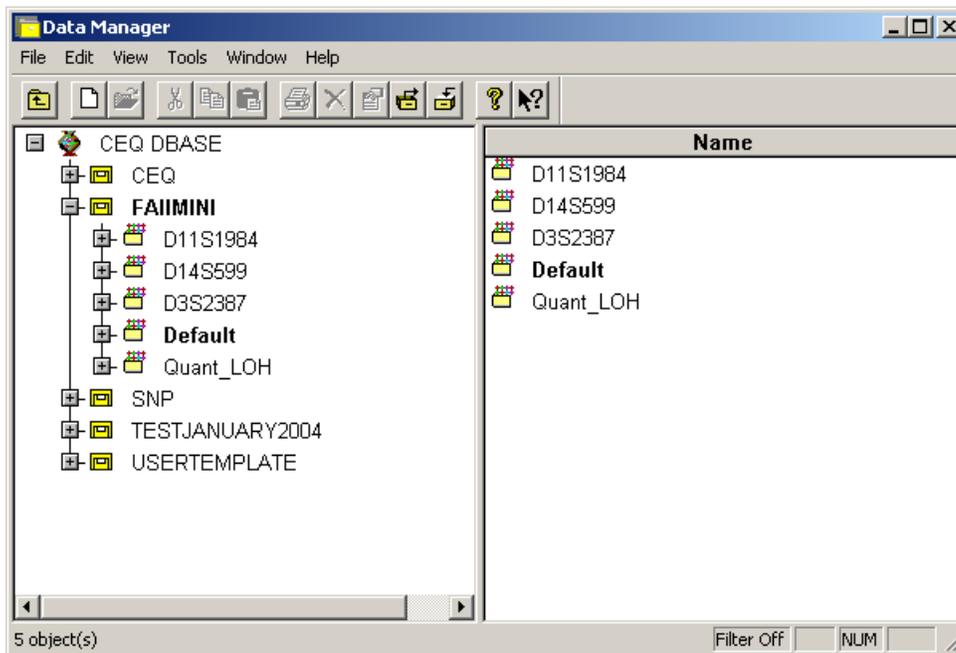
Figure 167: Data Manager

Shrink Database

Use this menu option to reduce the size of the currently selected database. Often, when studies or results are removed from the database, the size of the database does not immediately reduce in size. Use this option to manually shrink the database to its new (smaller) size. This is useful before a database is backed-up.

Note

You cannot shrink a database while simultaneously performing a backup of the same database. It is advised to first shrink the database before backing it up.

Shrink the database using the following steps.

1. Open the *Data Manager* module.
2. Select the database to shrink.
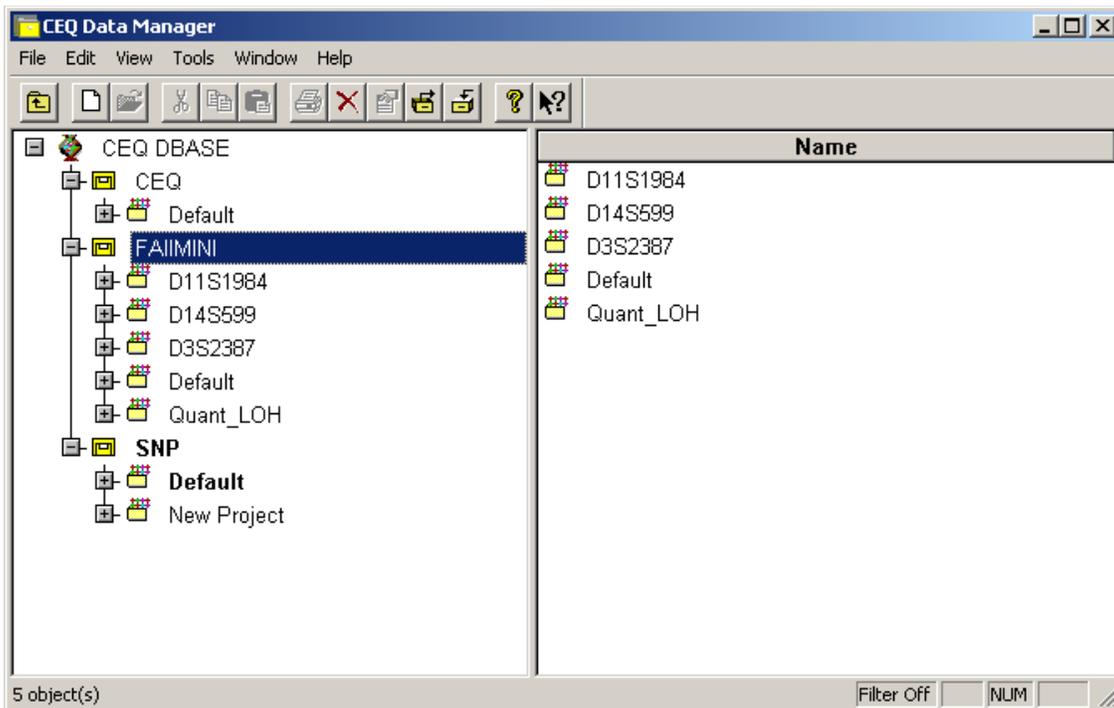
Figure 168: Data Manager

3. Select the **Tools | Shrink Database** menu item.
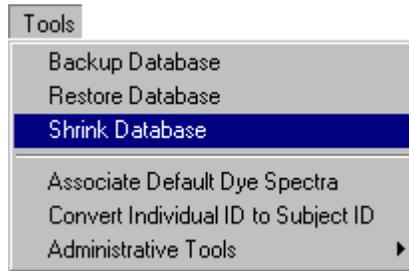
Figure 169: Shrink Database menu item

Backup Database

Periodic backups are essential in reducing the risk of losing data. If a database is deleted or damaged, only the data created since the last backup will be lost. Backups can be saved to any folder on a local drive.

> *Follow the Shrink Database procedure before backing-up any database.*

Backup a database using the following steps.

1. Open the *Data Manager* module.

2. Mouse-click the database to be backed up as shown in Figure 170.
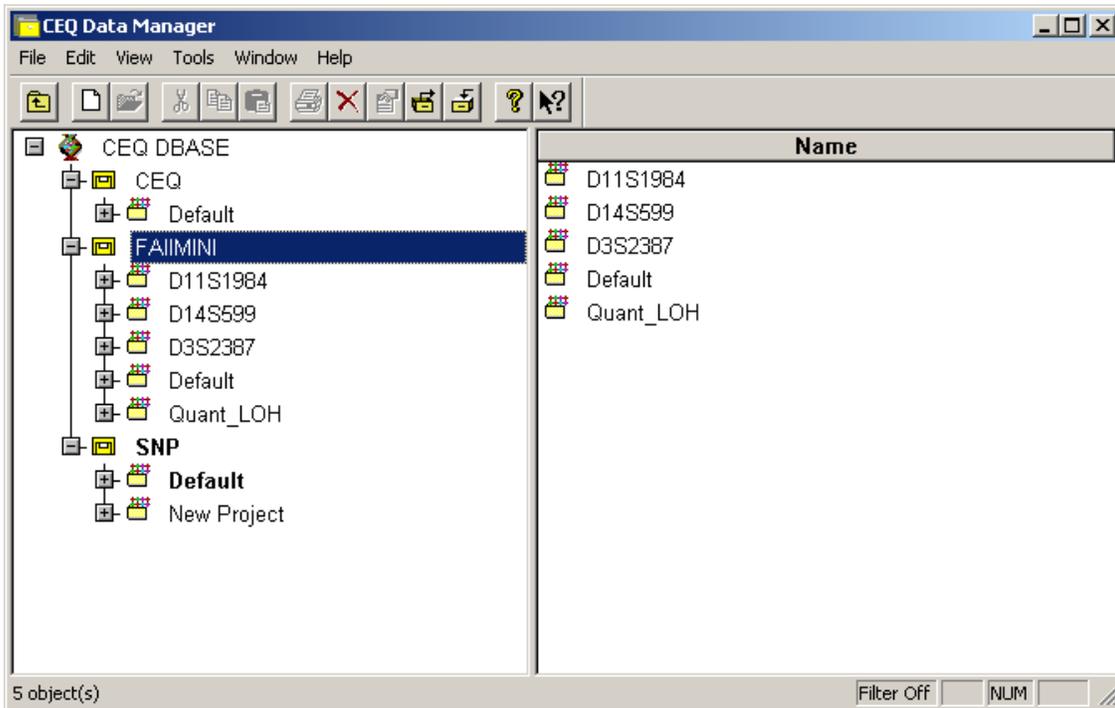
Figure 170: Database selection

3. Select the **Tools | Backup Database** menu item.
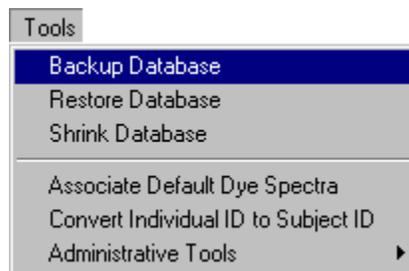
Figure 171: Backup Database menu item

4. Select the location, on the local disk, to save the backup file.
5. Accept the file name or rename it as necessary.

> *Note* *Typically, databases are frequently backed-up. It may be prudent to add the date as part on the name to prevent overwriting valuable backups as well ensuring accurate database Restore.*

6. Click **Save**.
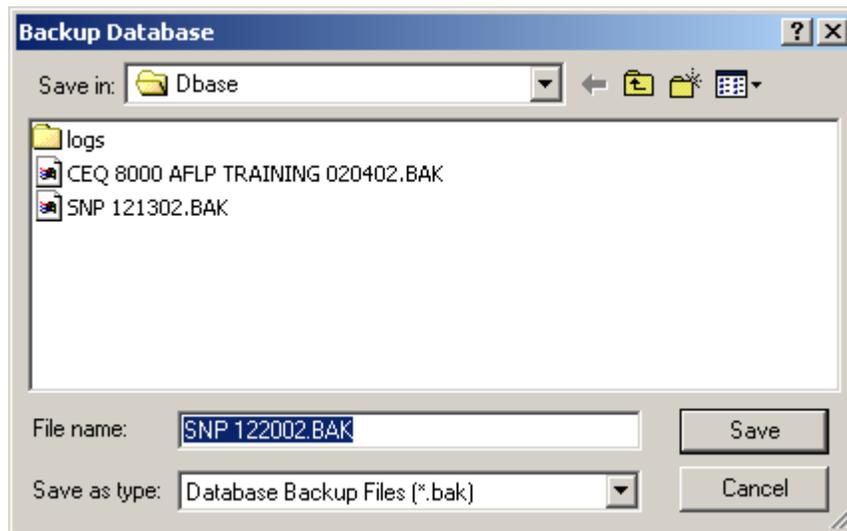
Figure 172: Backup Database dialog

Restore Database

Restore Database allows for the recovery of a database that was previously saved using the backup procedure. If you are restoring a database that has the same name as one already residing in the Database Manager, you must either restore it with a new name or delete the existing database before restoring it (**NOT Recommended**).

Restore a Database using the following steps.

1. Open the *Data Manager* module.

2. Mouse-click in any window.
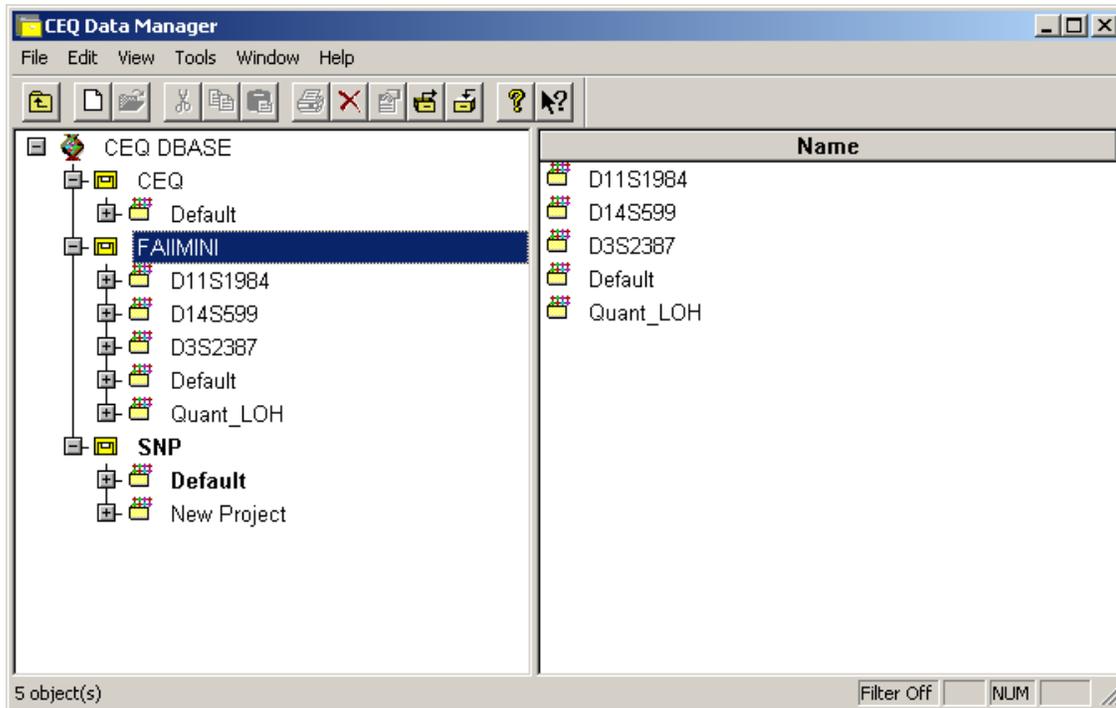
Figure 173: Data Manager

3. Select the **Tools | Restore Database** menu item
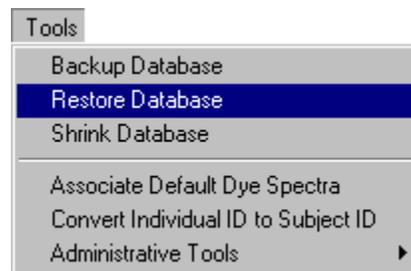
Figure 174: Restore Database menu item

4.   Select the location, on the local disk, where the backup file was saved.
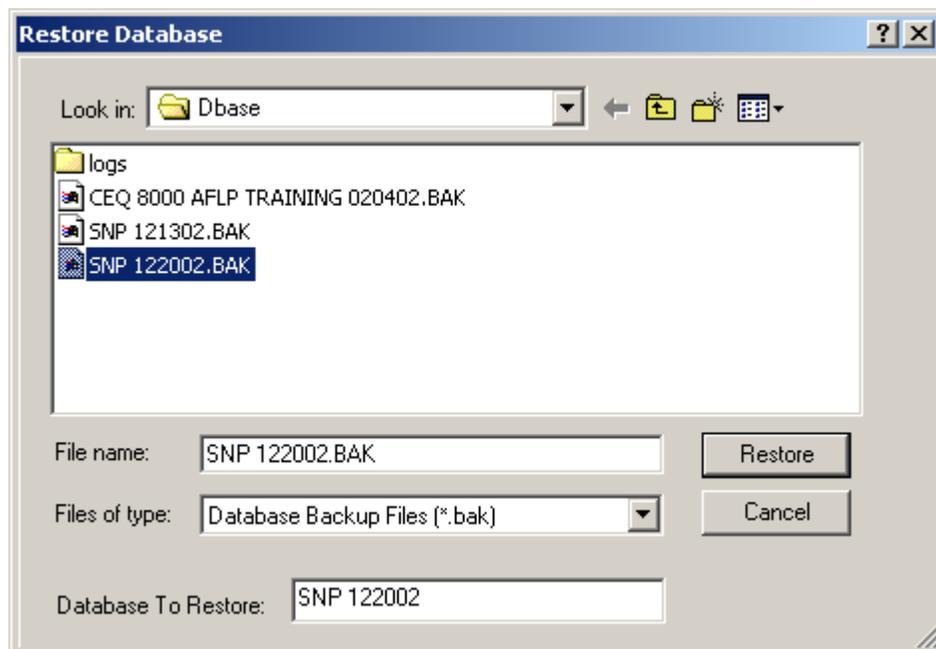
Figure 175: Restore Database dialog

5.   Select the file to restore and click **Restore**.

Note — *If a database with the same name exists, a warning will appear (Figure 176).*
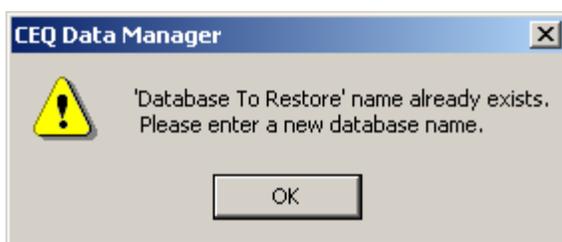
Figure 176:

If this occurs, click **OK** *and either:*

•*Delete the database in the Data Manager (NOT recommended), or*

•*Rename the database being restored. This is done by entering a new name in the* **Database To Restore** *field.*

Convert Individual IDs to Subject IDs

This menu option (Figure 177) is used to convert all sample data records that use Individual IDs to records that use Subject IDs. This applies only to the working database *only* and is active only when the working database is selected. *All other modules must be closed prior to the start of conversion.*

To convert individual IDs to subject IDs, follow these steps:

1. Select the database to convert and ensure that it is the **Working Database**.
2. Select To**ols | Convert Individual ID to Subject ID** (see Figure 177).
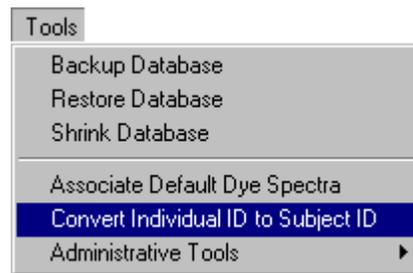
Figure 177:Convert Individual ID to Subject ID menu item

The conversion takes place in the background and the message *Conversion Completed Successfully* message will appear, in the message bar, when completed (see Figure 177).
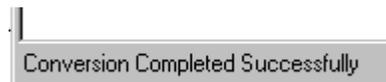
Figure 178:Conversion Completed Successfully message

The Subject ID attribute is carried to the sequence and fragment records that were generated from the converted sample data record. A new *Subject ID* column is added to all existing studies and the *Individual ID* column is removed.

When a non-working database is selected for conversion, the menu item is not available.

Exporting Database Items

Sample Plate Result Export

When exporting a sample plate from the Data Manager the dialog in Figure 179 will appear if any of the sample results are missing. This dialog box provides a list of the sample results that were not exported.

Figure 179:Sample Results Missing Dialog
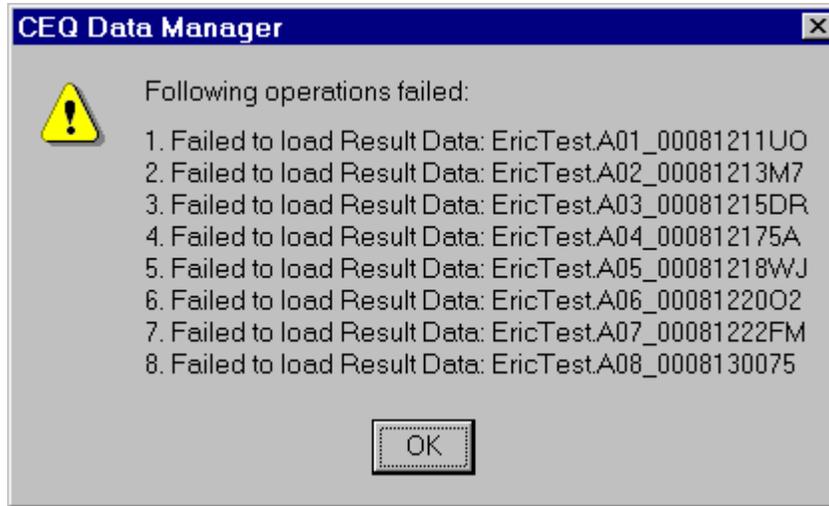
A maximum of twenty missing results will be listed. If there are more than twenty missing results, a message will be displayed at the bottom of the dialog box as shown in Figure 180.

Figure 180:
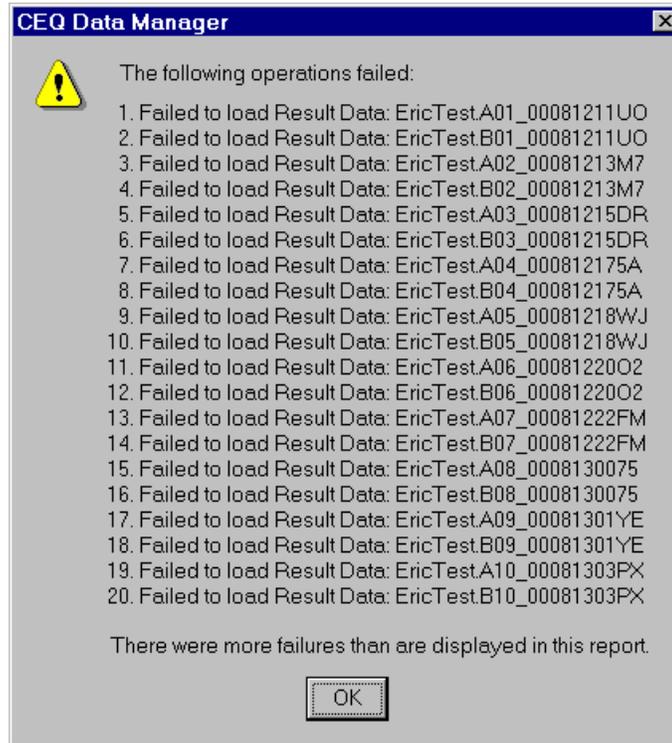
Exporting from the Data Manager

To export from the Data Manager Module, follow the steps below:

1. Launch the Data Manager and open a sequence result.
2. Select **File | Export** to open the Export dialog box.
3. Select a target folder and file name.
4. Select the desired format in the Save as type text box as shown in Figure 181.

Figure 181:Sequence Analysis Export Dialog
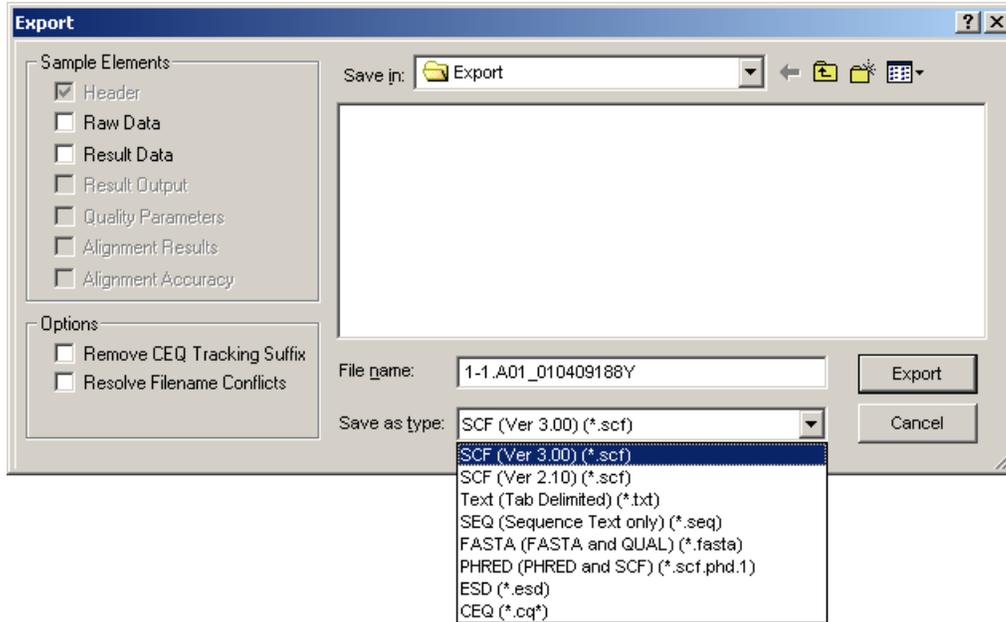
5. Click **Export**.

Data Manager Export File Types

Listed in Table 74 are the available export file types.

Table 74: Data Manager Export Options

Format	Description
SCF (.scf)	The SCF (Standard Chromatogram Format) format is used to store analyzed DNA sequence or fragment data for a single sample. The format describes both "public" and "private" data sections. The well-defined public data section contains trace data points, the called sequence or identified fragments and the positions of each called base or fragment within the trace data. A public comment section is also defined. The "private" section can be used to store other information that will generally be ignored by most software packages. The CEQ System uses the private data section to store sample data (including raw data along with current and voltage data). Sample data from either sequencing or fragment analysis samples can be stored. The notes and property sets are stored in the comment section. The CEQ 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 Inc.). 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 CEQ Sequence Analysis module. If you use SCF to exchange data between instruments you will lose informational data such as sample plate name, capillary array serial number, etc. If you wish to maintain exact copies of data including header information, use the CEQ format and import and export these data via the Data Manager.
Tab-Delimited Text (.txt)	The Tab-delimited format is valid for raw data, sequence results and fragment results. Check the Raw Data check box to include raw data, as well as current and voltage data. Check the Results Data and Results Output check boxes to include the sequence results. The data points will be expressed as text values and can be viewed in any text editor or in Excel.
SEQ (.seq)	The SEQ format only applies to the sequence results portion (e.g., text bases) of analyzed data. You would use this format, for instance, to export data into Lasergene's SeqMan from DNASTAR Inc.

Table 74: Data Manager Export Options (Continued)

Format	Description
FASTA (.fasta)	The FASTA format only applies to the sequence results portion (e.g., 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 will have a value of 99. You would use this format, for instance, to export data into PHRAP. Note: *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 CEQ System, we bypass PHRED and export directly into PHRAP.*
PHRED (.phd.1)	The PHRED format will produce 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 CEQ System. The TIME stamp is the current time at the time of export. The DNA section is composed of the base-call (always lower-case), PHRED Quality Value, and the Peak Index (data point) of the analyzed data contained within the associated SCF file. Values are separated by spaces.
ESD (.esd)	The ESD, electropherogram sample data, format is used to export raw data from the CEQ System to a third-party software package for analysis. When you select this option, on the raw data will be exported, and no other information is included.

Table 74: Data Manager Export Options (Continued)

Format	Description
CEQ (.cq*)	If you select the CEQ format, the format native to our database, you will export 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 box, such that all of the associated information listed above will be exported. This format essentially takes a "snapshot" of the file and is able to retain all of the information belonging to that file. This format can only be used when importing data into the Data Manager. This format can also be used when exporting data from the Sequence and the Fragment Analysis modules and the Data Manager. 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 Data 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 74: Data Manager 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 batches are exported, the resulting four files will 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* *Default dye colors. The dye colors may be reassigned 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 will result in five files; one for each dye and one to assist in determining the trace order. See the table below: {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

Importing Database Items

To import items into the database, perform the following steps.

1. In the Data Manager window, select the project where the imported file will reside.
2. Select **File | Import** from the menu.
3. From the **Import** dialog box, locate the folder where the file resides, select the import format from the **Files of type** drop-down menu, highlight the filename, and then click **Import**.

Generating a Sample Run History

To generate a sample run history, perform the following steps.

1. Select **View | Sample Run History** from the menu.
2. In the **Sample Run History** dialog box:
   a. Select a **Project** name from the drop-down menu.
   b. Select **Enable** and enter a filtering start and end date.
   c. Click the **Compute** button.
   d. View the total number of runs during the specified dates in the **Total Sum of Samples Run** field.
   e. Print a report of all samples run during the specified dates by selecting the **Print** button.

Administrative Tools

This version of CEQ Software allows users to access the database without logging onto the computer as an Administrator. This operation must be done by the System Administrator. Before using the Administrative Tools, the Administrator must first setup a CEQUSERS group and the users that will be using the databases in the CEQ software.

Add Users and Groups

Adding Users and Groups is done outside of CEQ. Once the CEQUSERS group is created and users added, the group must be activated within the *Data Manager* module.

Adding Users

Add Users by selecting, from the computer desktop, **Start | Programs | Administrative Tools | Computer Management**. The *Computer Management* console shown in Figure 182 will be displayed. Add New Users following the steps below:
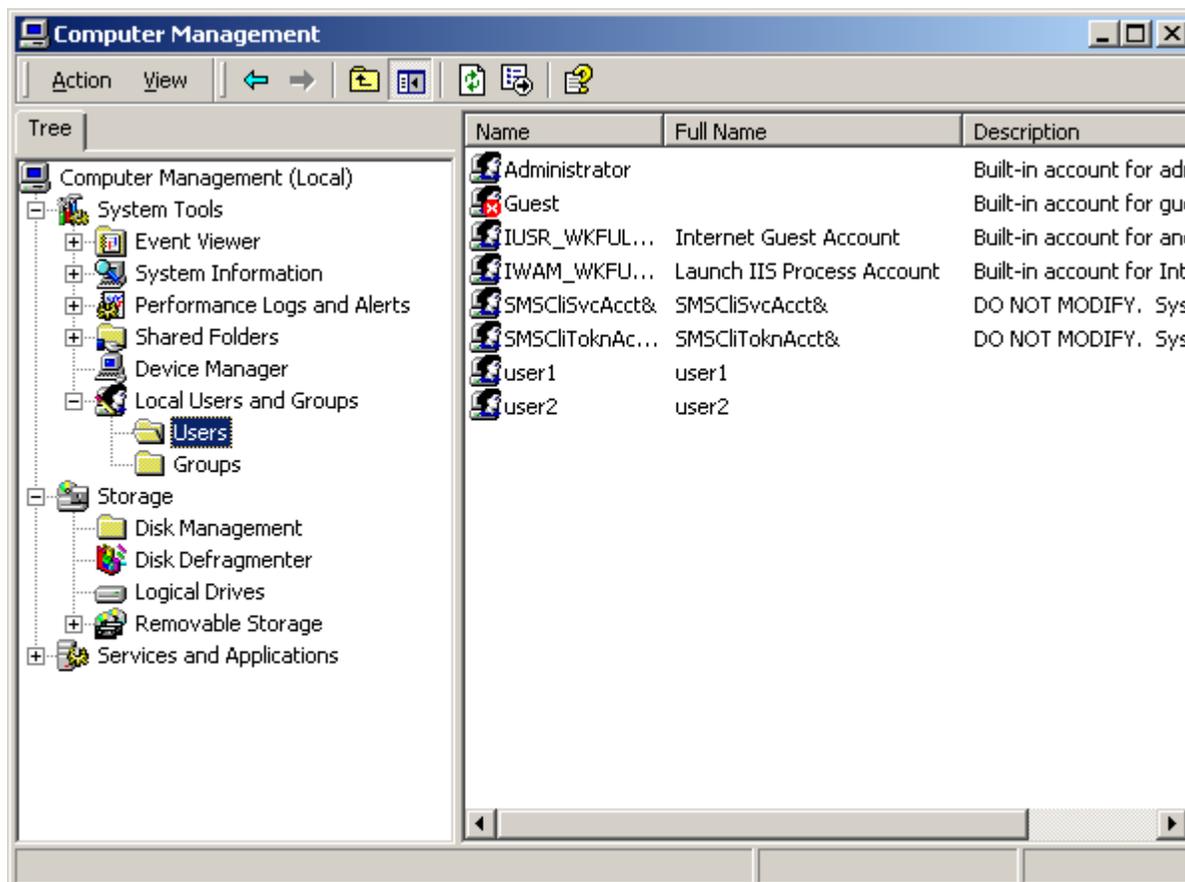
Figure 182: Windows 2000 Computer Management console

1. Mouse-click on the "+" symbol to the left of the *System Tools* item, if needed.
2. Mouse-click on the "+" symbol to the left of the *Local Users and Groups* item, if needed.

3. Right-click on *Users* and select **New User** (Figure 182).
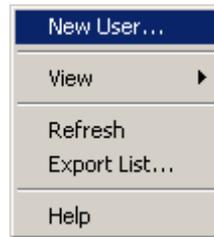
Figure 183:Add New User menu

4. In the New User dialog (Figure 184), enter the new User name (required) and edit the other fields as necessary.
5. Click **Create**.
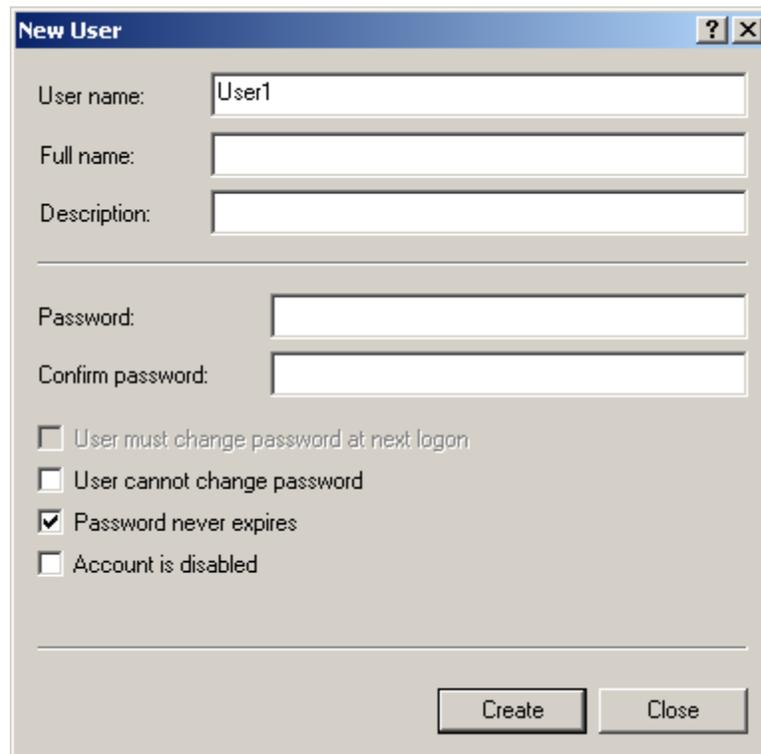
Figure 184:New User dialog

Adding Groups

1. Mouse-click on the "+" symbol to the left of the *System Tools* item, if needed.
2. Mouse-click on the "+" symbol to the left of the *Local Users and Groups* item, if needed.

3. Right-click on Groups and select **New Group** (Figure 185).

Figure 185:Add New Group menu

4. In the New Group dialog (Figure 186), enter the new Group name CEQUSERS and a description if necessary.
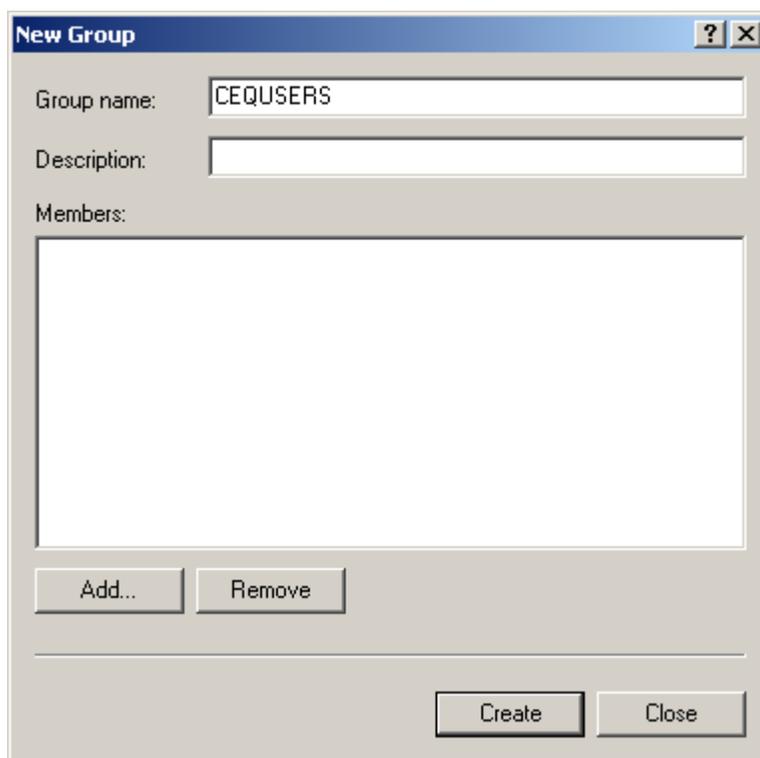
Figure 186:New Group dialog

5. Click the **Add** button to add the users created in the *Adding Users* section above.
6. The *Select Users or Groups* dialog will open. The local computer will be the default location in the *Look in* field. If it isn't, use the drop-down list and select the local computer.

7. Scroll down the **Name** list and select the user names created in the *Adding Users* section above. If more than one User was created, mouse-click on the first name, press and hold the **Ctrl** key on the keyboard and select any additional names to add to the group.

8. Click **Add** and then **OK** to add the selected users to the new group.
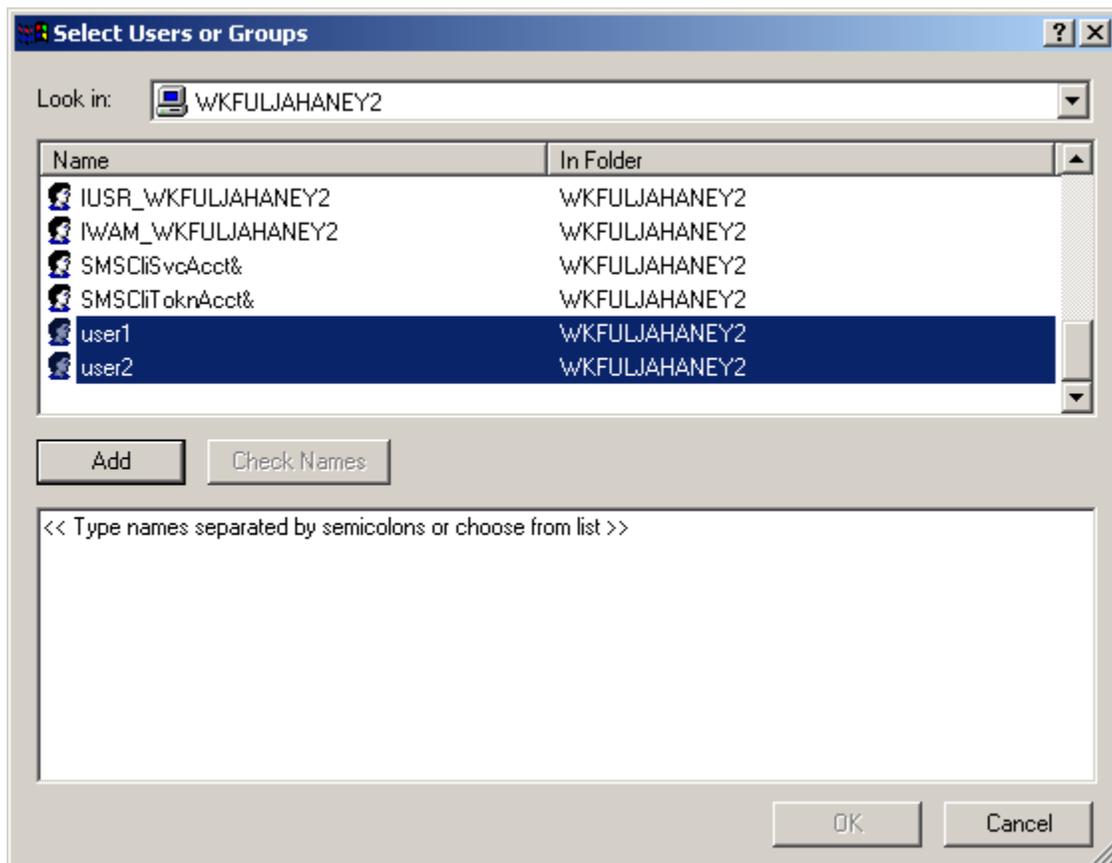
Figure 187: Adding users to the new group

9. Click **Create** to save the new group.
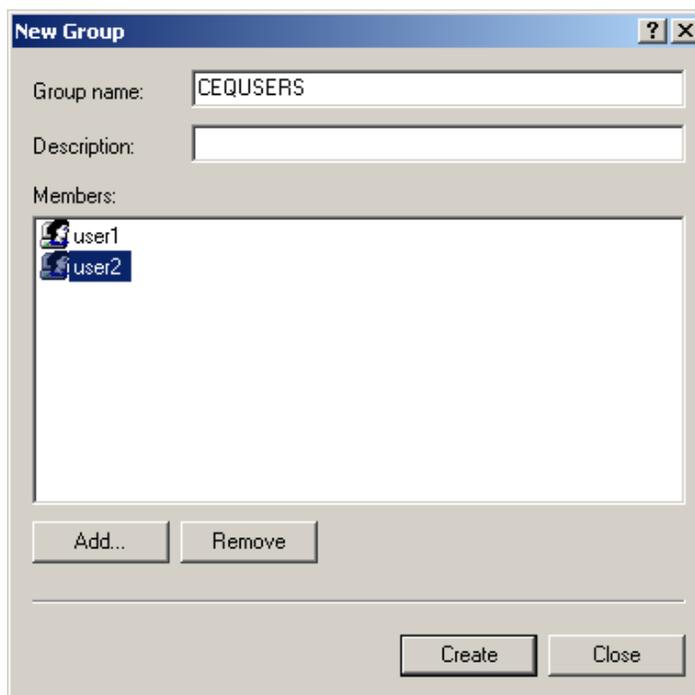
Figure 188: New Group dialog

Activating the CEQUSERS Group

Once the CEQUSERS group has been created and the users added, it must be activated within the *Data Manager* module. This is done only once and users can be added/removed at any time thereafter.

The steps below describe the activation process. Be sure the steps outlined in *"Adding Users'* on page 322 and *"Adding Groups'* on page 323 are completed before continuing.

1. Open the *Data Manager* module.
2. From the Menu bar select **Tools | Administrative Tools | Add CeqUsers Group to SQL Login** as shown in Figure 189.

Figure 189: Activating the CEQUSERS Group

All users added to the group CEQUSERS now have access to the database.

Direct Control and Replenishment

The following procedures apply to the Direct Control functions of the Run module. Direct Control functions can be accessed via the **Direct Control** menu, the **Replenish** menu and the **Hot Areas** of the Direct Control window.

> *Note* — *Use Figure 190, User Accessible Hardware Components, to locate hardware components referenced in this section.*

Figure 190: User Accessible Hardware Components
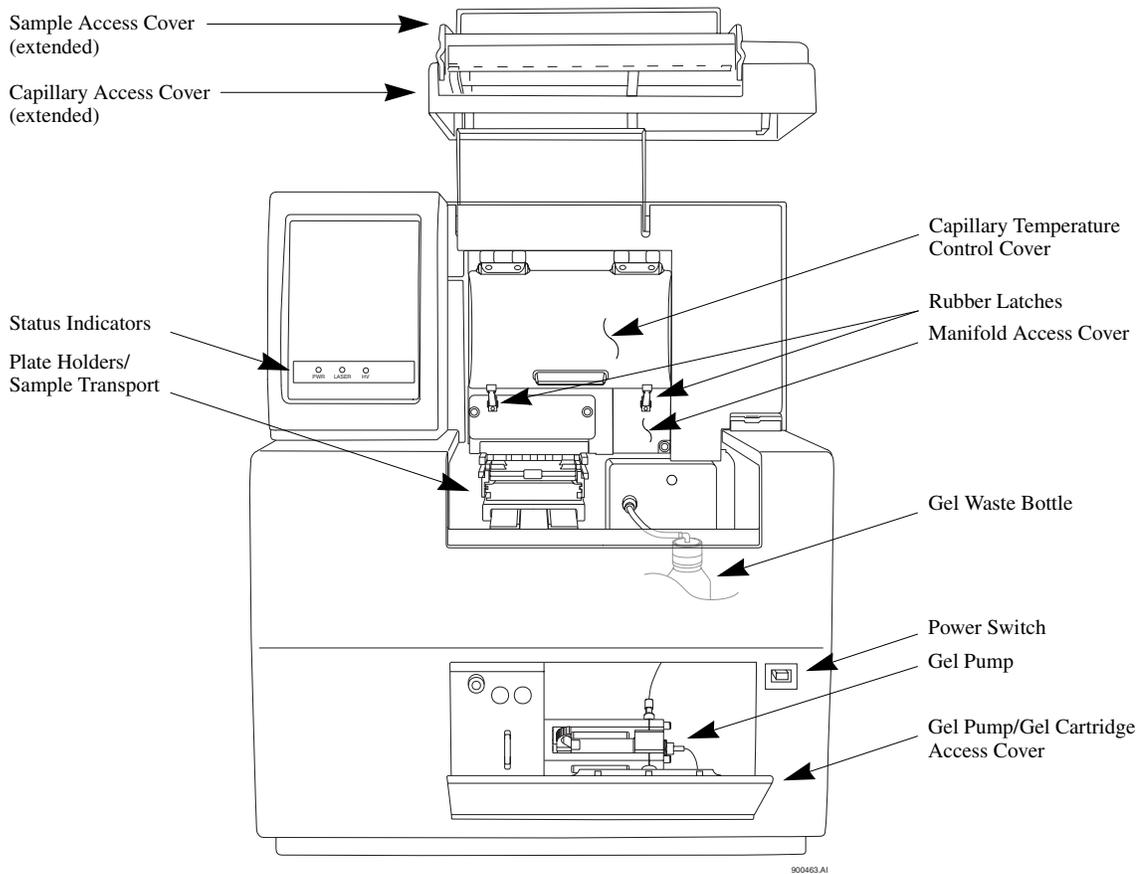
Accessing the Direct Control Window

From the CEQ Toolbar, click on the **RUN** icon. Verify that the Run window is displayed and then click on the **Direct Control** tab.

Direct Control Procedures

Replacing the Wetting Tray

Removing the Wetting Tray

1. Select **Replenish | Replace Wetting Tray** from the Run Module main menu.
2. Press **OK** when prompted to verify this action.
3. Open the Sample Access Cover (Figure 190) and lift to the vertical locking position.
4. Remove the sample plate and set aside.
5. Rotate the Wetting Tray Retainers outwards to release the Wetting Tray.
6. Lift the Wetting Tray vertically.

Installing the Wetting Tray

1. Select **Replenish | Replace Wetting Tray** from the Run Module main menu.
2. Press **OK** when prompted to verify this action.
3. Open the Sample Access Cover (Figure 190) 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 (Figure 191) 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, it must be removed before installing the wetting tray to allow movement of the wetting tray retainers.*

Figure 191: Replacing the Wetting Tray
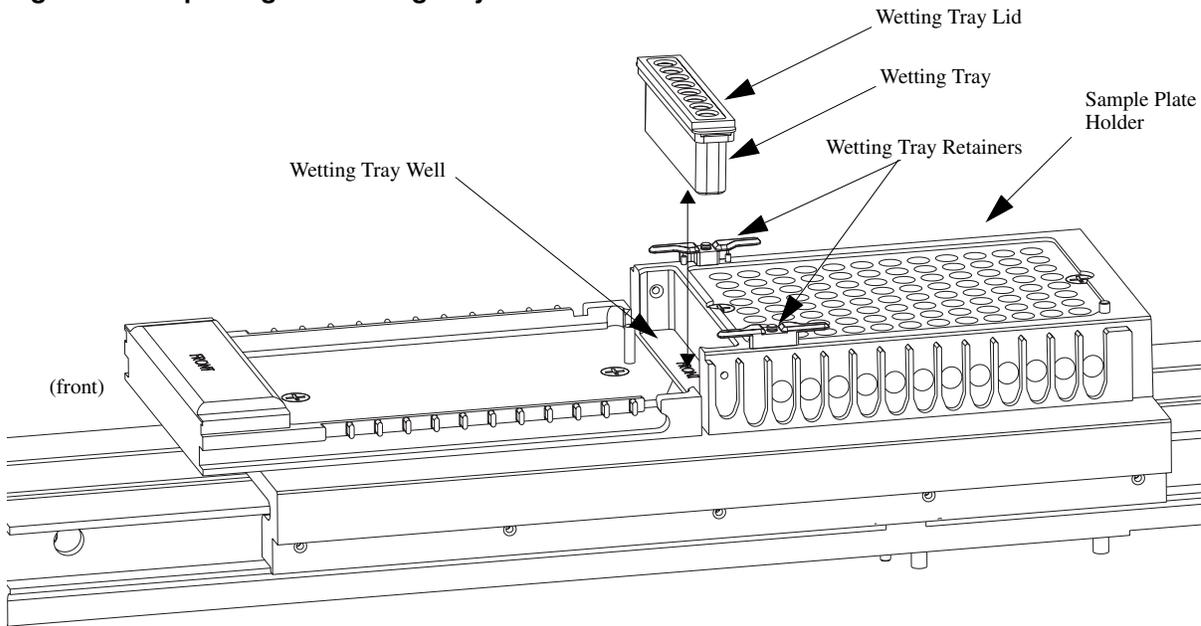
6. Close the Sample Access Cover and then click the **Done** button in the **Replace Wetting Tray** dialog box (Figure 192).

Figure 192: Replace Wetting Tray Dialog
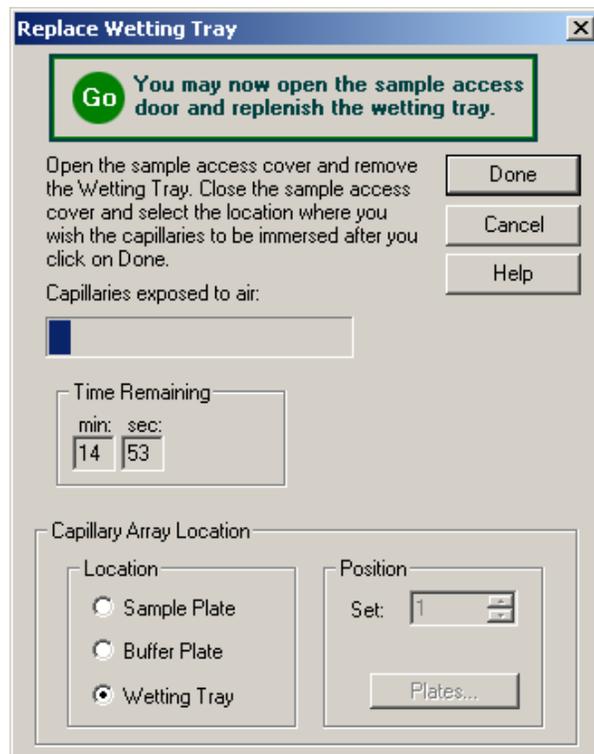
Loading the Sample Plate or Buffer Plate

1. Select **Direct Control | Access Plates** from the menu.

Figure 193: Access Plates Dialog
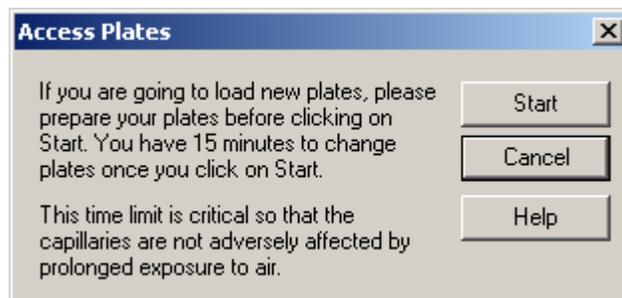
2. Click **Start**. The Capillaries Exposed dialog (Figure 194) will appear.

Figure 194: Capillaries Exposed Dialog
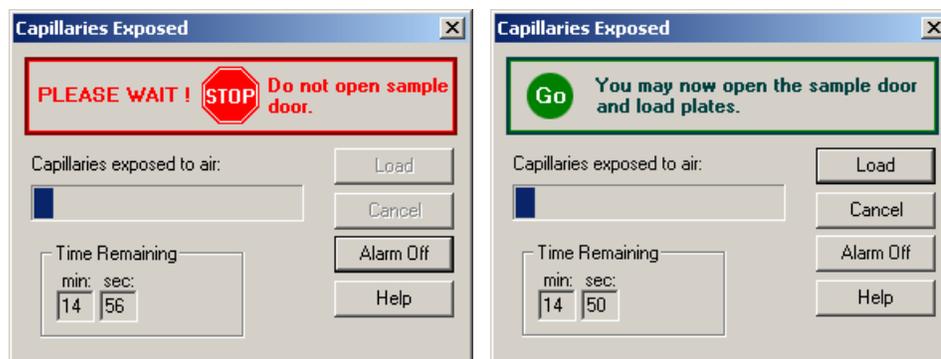
3. Wait until the dialog warning turns green.
4. Open the Sample Access Cover (Figure 190) and lift to the vertical locking position.

   - If **Sample Plate** was selected, skip to "*Loading the Sample Plate*."
   - If **Buffer Plate** was selected, skip to "*Loading the Buffer Plate and Evaporation Cover*."

Loading the Sample Plate

1. Make sure the Wetting Station is installed or perform the procedure "*Installing the Wetting Tray*" on page 328, 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 (Figure 195).

Figure 195:Loading the Sample Plate
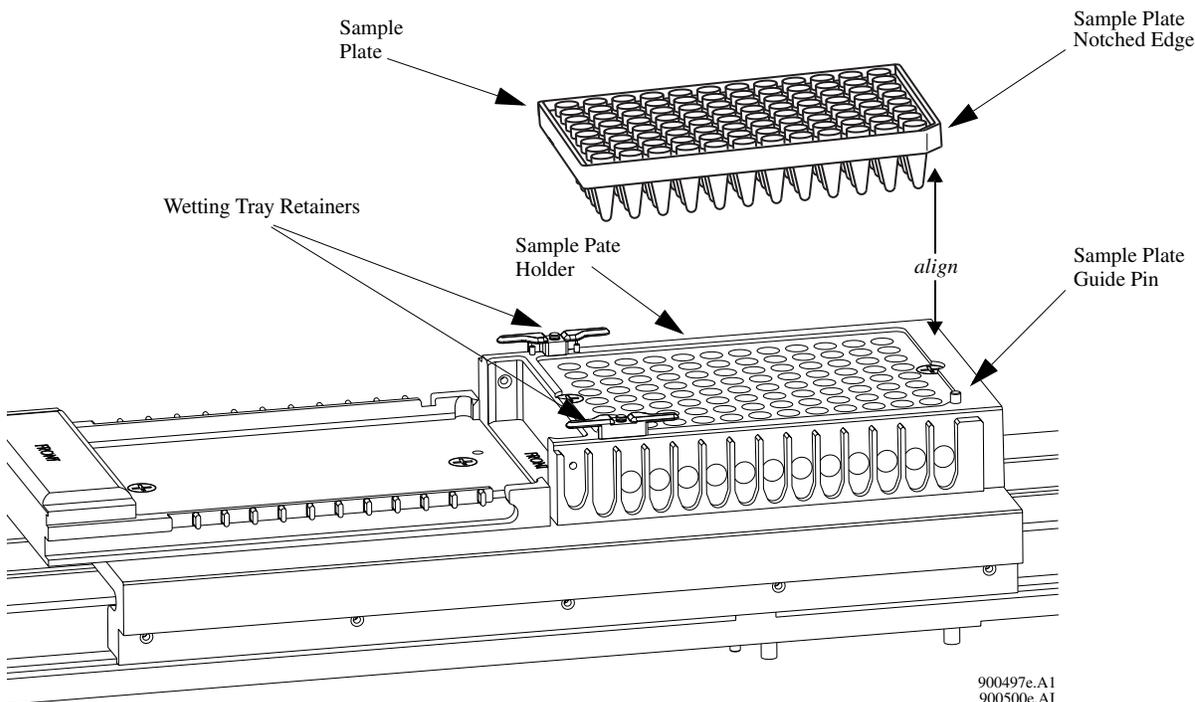
3. When finished positioning the plate, close the Sample Access Cover and then click the **Load** button of the **Capillaries Exposed** dialog box (Figure 196).

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

Figure 196:Capillaries Exposed Dialog
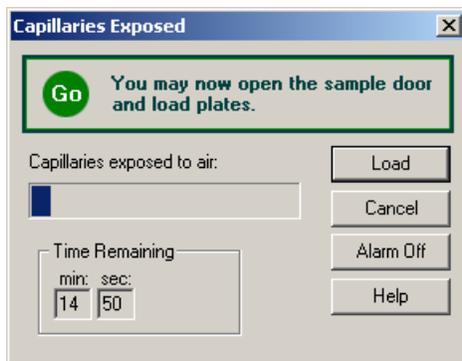
Loading the Buffer Plate and Evaporation Cover

1. Align the notched corner of the Buffer Plate with the alignment line on the Buffer Plate Holder (Figure 197).
2. Gently push the Buffer Plate towards the front of the instrument 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 197: Loading the Buffer Plate and Buffer Evaporation Cover
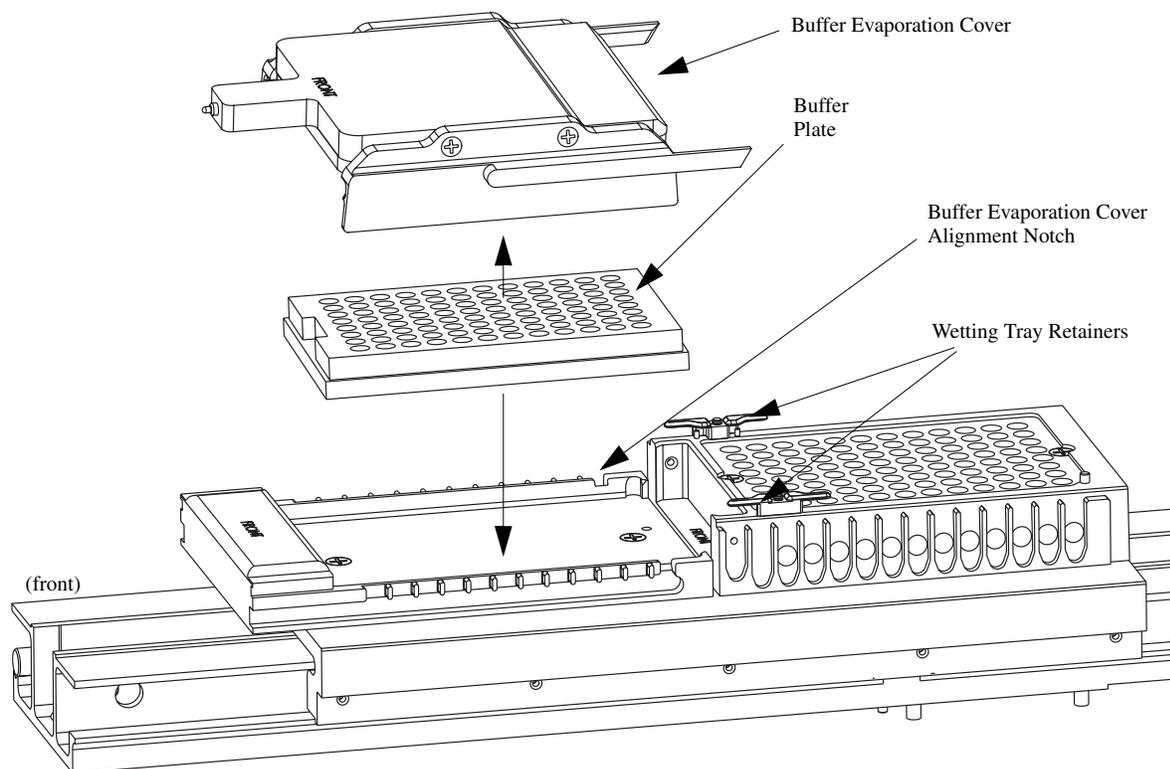
4. When finished positioning the plate, close the Sample Access Cover and then click the **Load** button of the **Capillaries Exposed** dialog box (Figure 196).

Specifying Capillary Temperature

1. Select **Direct Control | Capillary Temperature** from the menu.
2. In the **Capillary Temperature** dialog box:

    a. Enter a capillary holding temperature value in degrees centigrade.
    b. Select **Wait for temperature to be reached** and then click **Start**.

Denaturing a Sample

1. Select **Direct Control | Denature** from the menu.
2. In the **Denature Samples** dialog box:

    a. Enter a time duration in seconds.
    b. Identify the position of the **Sample Set** and click **Denature**.

Injecting a Sample

1. Select **Direct Control | Inject** from the menu.
2. In the **Inject** dialog box:

   a. Enter a value for the voltage in kV.
   b. Enter a time duration in seconds.
   c. Identify the position of the sample using the **Sample Set** spin controls and then select **Inject**.

Performing a Separation

1. Select **Direct Control | Separate** from the menu.
2. In the **Separate** dialog box:

   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.*

Replenishing the Capillaries with Gel

1. Select **Direct Control | Gel Capillary Fill** from the menu.
2. From the **Gel Capillary Fill** dialog box, select the **Buffer Plate** or **Wetting Tray** radio button to identify the position where waste will be expelled from the capillaries.

   a. If **Buffer Plate** was selected, use the **Buffer Plate** spin controls to identify the waste position in the buffer plate and then select **Fill**.
   b. If **Wetting Tray** was selected, click the **Fill** button.

Purging the Manifold

1. Select **Direct Control | Manifold Purge** from the menu.
2. In the **Manifold Purge** dialog box:

   a. Enter a volume in milliliters (mL) (0.4mL default).
   b. Enter the number of cycles and then select **Purge**.

Performing an Optical Alignment

To align the lasers with the detection windows of the eight capillaries, perform the following steps.

1. Select **Direct Control | Optical Alignment** from the Run menu.
2. To save the alignment data:

   a. Select the **Autosave** check box.

b. Enter a name in the **Name** field.
c. Select a **Project** from the drop-down menu.
d. Select **Align**.

> **Note**
>
> *Prior to performing an Optical Alignment, it is advisable to purge the Array Manifold and fill the capillaries with fresh gel (in that order). See "Purging the Manifold" and "Replenishing the Capillaries with Gel".*

Viewing or Changing Capillary Information

1. Select **Replenish | Capillary Information** from the Run module menu bar.
2. Use the **Capillary Information** dialog box (Figure 198) to view or change the following information:

a. View the part number of the installed capillary array.
b. View the serial number used to track the capillary array along with the samples that used the array.
c. The system automatically updates the date and time installed.
d. Change the number of runs imparted on the capillary array since it was installed using the spin control, if you wish to change the system's alert mechanism.
e. Change the number of days the capillary array has been on the instrument using the spin control, if you wish to change the system's alert mechanism.
f. Click **Advanced** to view additional information specific to the installed array.
g. Click **OK** to enter the information and close the dialog box.

8. When finished, select **OK** to close the window(s).

Figure 198: Capillary Information Dialog
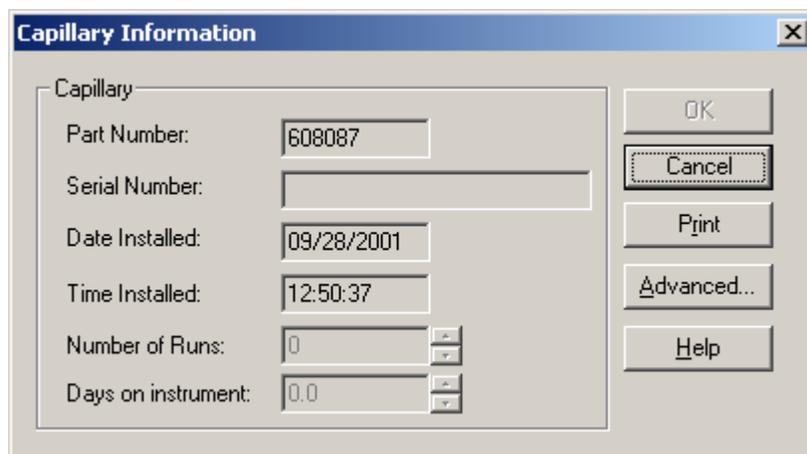
Viewing or Changing Gel/Buffer Information

1. Select **Replenish | Gel Cartridge/Buffer Information** from the Run menu.
2. Use the **Gel Cartridge/Buffer Information** dialog box (Figure 199) to view and/or change the following information:

    a. The system automatically updates the new buffer part number.
    b. The system automatically updates the date and time installed.
    c. Use the spin control to change the **Hours on Instrument** if you wish to change the system's alert mechanism.

4. When finished, select **OK** to close the window.

Figure 199:Gel Cartridge/Buffer Information Dialog

Removing and Replacing the Capillary Array

CAUTION **Care must be exercised when installing/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.*

1. Select **Replenish | Release Capillary Array** from the Run menu. After the system prepares for release of the capillary array, the **Remove Capillary Array** dialog box (Figure 200) is displayed.

Figure 200:Remove Capillary Array Dialog
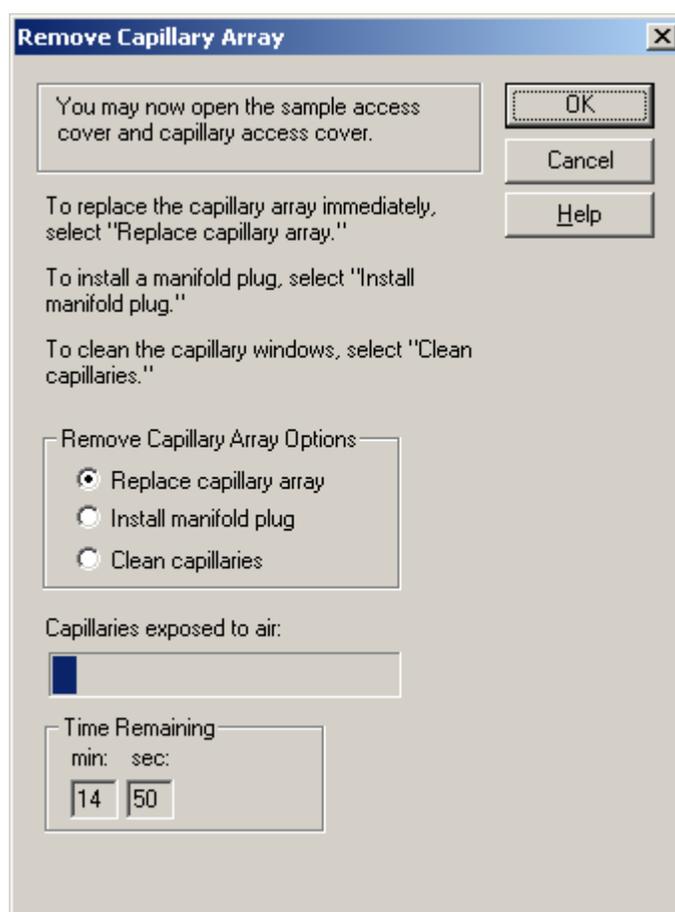
2. Open the Sample Access Cover (Figure 190) and lift to the vertical locking position.
3. Open the Capillary Access Cover and lift to the vertical locking position.
4. Unlatch the two rubber latches holding the Capillary Temperature Control Cover and lift to the vertical locking position.
5. Loosen the Manifold Access Cover captive screw (Figure 201), remove the cover and set it aside.

Figure 201: Manifold Access Cover

Capillary Temperature
Control Cover (open)

Short Plenum
Assembly

Manifold
Access
Cover

captive screw

Manifold
Access
Cover (removed)

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900488e.AI
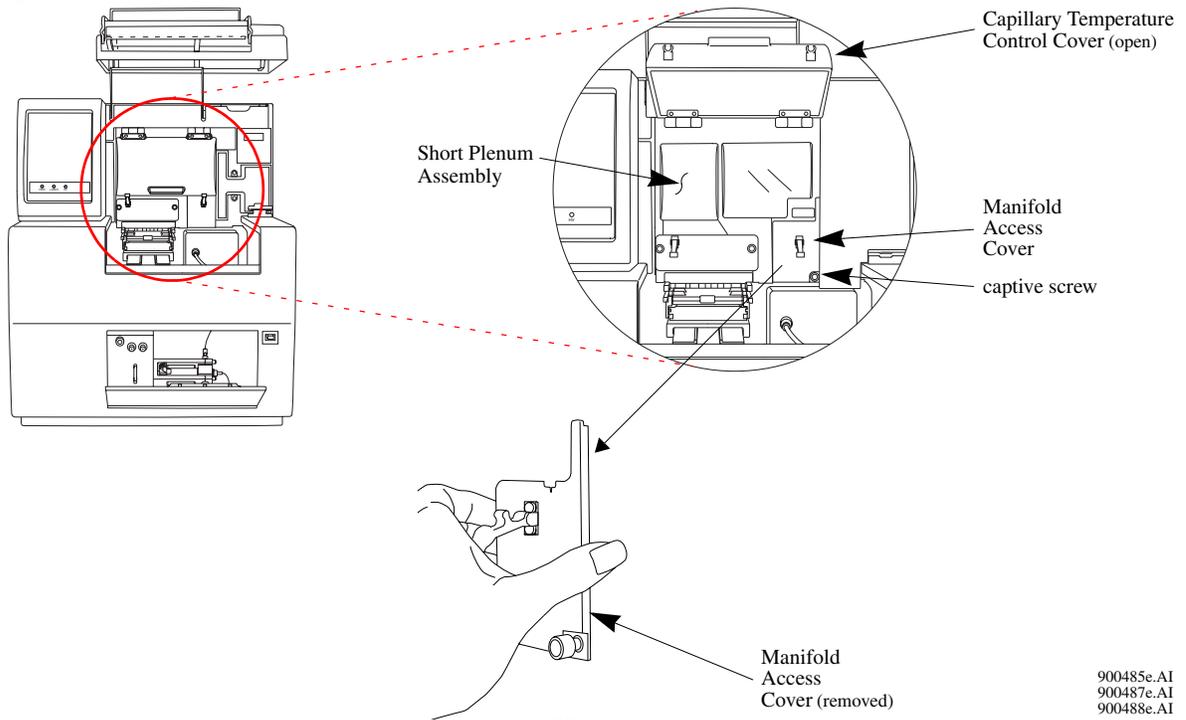
6. Loosen the two Plenum Assembly captive screws (Figure 202), pull the Plenum Assembly straight back and away from the instrument and set it aside.

> **⚠ CAUTION** **Slowly remove the Plenum Assembly as the electrode block may disengage from its mounting posts and become damaged.**

Figure 202: Plenum Assembly Removal

Plenum Assembly

captive screw

captive screw
rubber latch
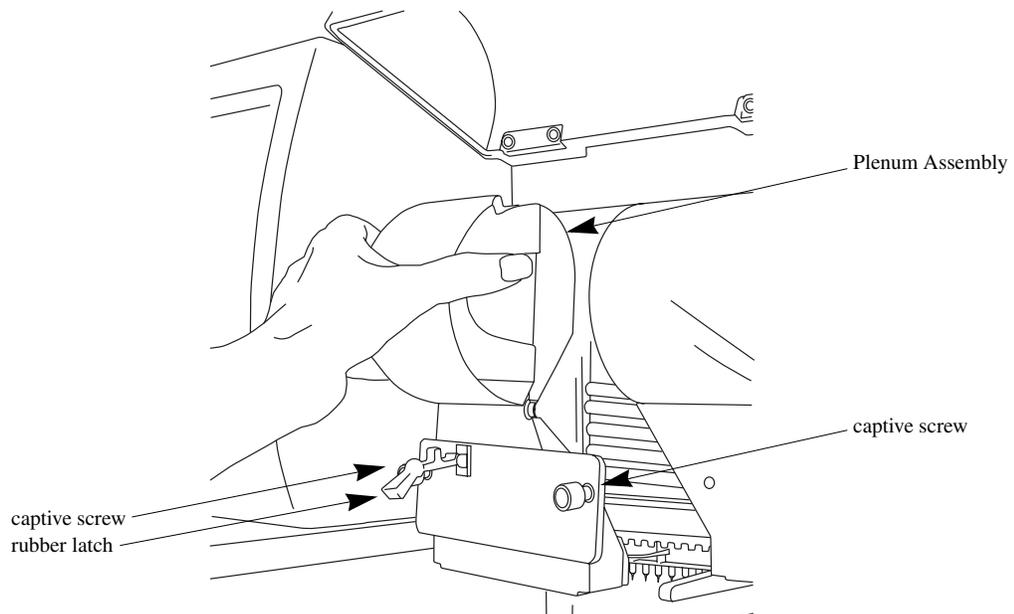
7. Lift the Eject Lever (Figure 203) to release the Array Fitting.

8. Grasp the Array Fitting tab (Figure 203) and then:

    a. Pull the fitting approximately one inch out of the manifold.

    b. Touch the tip of the fitting to the bottom of the Optics Base Plate.

    c. Hold and wait five seconds for the gel strand to dry.

    d. Pull the fitting away from the instrument.

    e. Wipe gel strands off of the instrument using a damp tissue.

> **Note** *Note the number of runs and days on the instrument 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.*

Figure 203: Removing/Replacing the Array Fitting
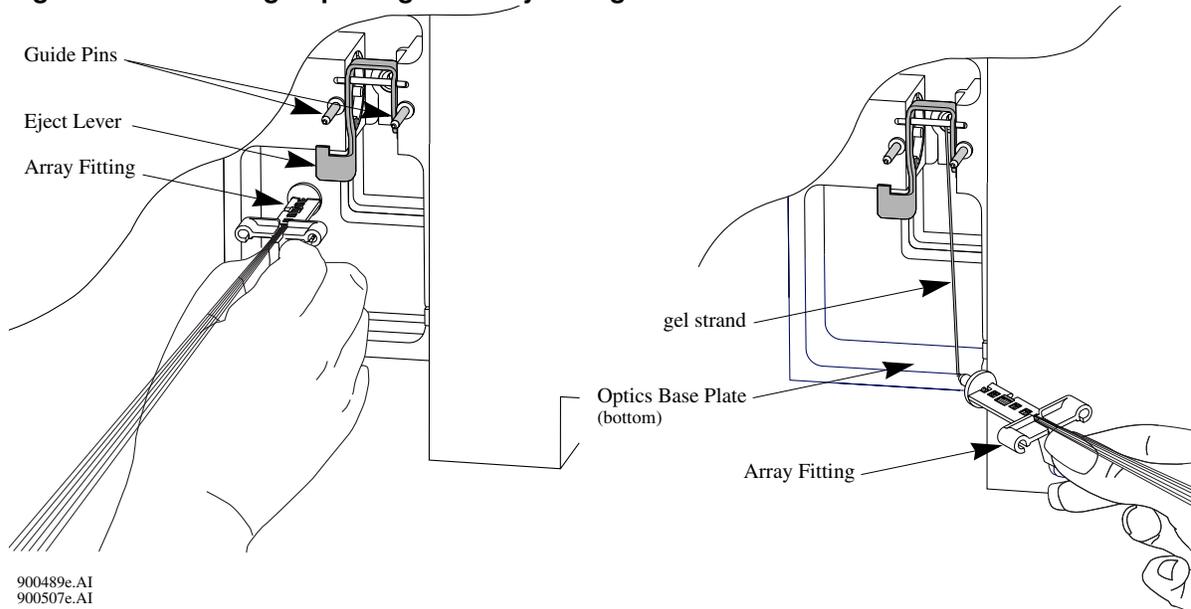
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900507e.AI

6.   Grasp the Electrode Block tab (Figure 204) with your left hand and pull the block out and away from the instrument.

Figure 204: Removing/Replacing the Electrode Block
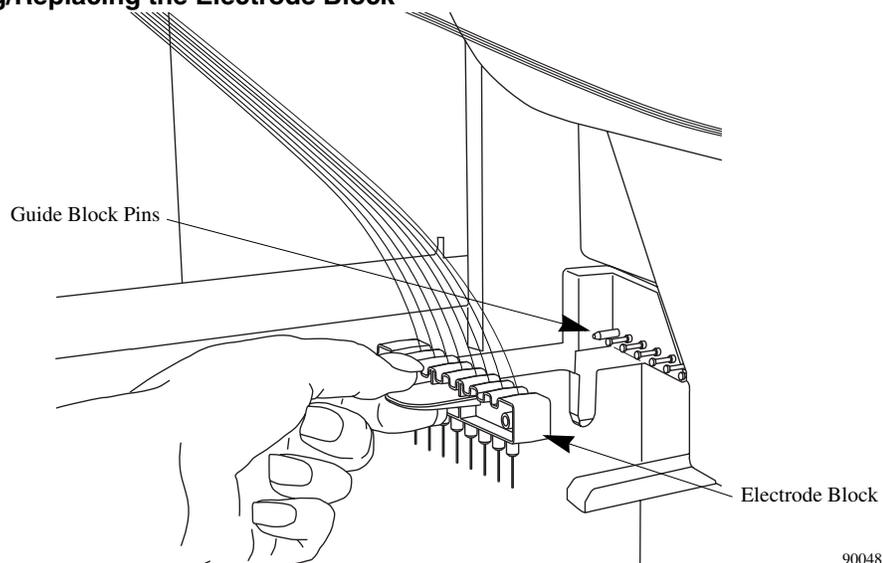
900481e.AI

7.   From the **Remove Capillary Array** dialog box (Figure 200), select the **Replace Capillary Array** radio button and click **OK**.

! **CAUTION** **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.**
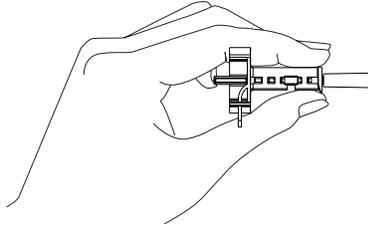
8.   While holding the Array Fitting tab (of the new capillary array), align the Array Fitting (Figure 203) with the manifold opening and guide pins. Push the fitting into the manifold until it is completely seated against the bases of the Guide Pins. Make sure that the fitting tab is placed downward when inserting the capillary array into its slot.

9.   While holding the Electrode Block tab, align the Electrode Block (Figure 204) with the guide block pins and gently push it in until resistance is met. (The resistance is from the spring-loaded contacts.)

10.   When installing the capillary array, carefully route the capillaries through the hole in the Plenum Assembly (Figure 205) and straight across into the guide block pins (Figure 206).

Figure 205:Plenum Assembly - Front View

900697L.AI
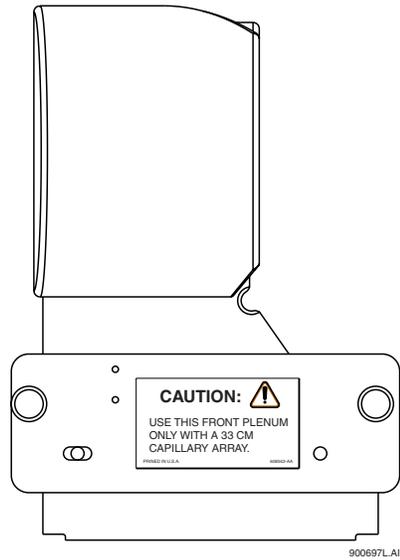
Figure 206:Capillary Array Routing

900694L.AI
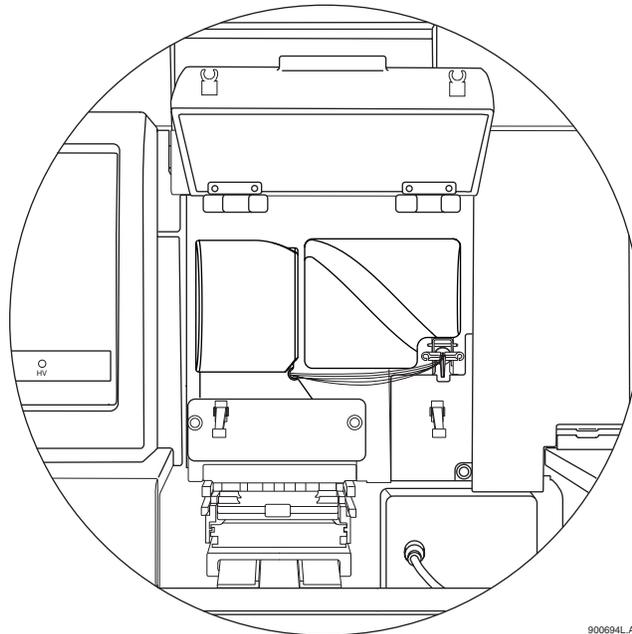
> **Note** *When reinstalling the Plenum Assembly, gently gather the capillaries between your thumb and forefinger to make sure they pass through the hole in the Plenum Assembly without any constriction.*

11. Replace the Plenum Assembly and tighten the two captive screws (Figure 202).
12. Replace the Manifold Access Cover and tighten the captive screw (Figure 201).
13. Lower the Capillary Temperature Control Cover and secure the two rubber latches.
14. Lower the Capillary Access Cover and Sample Access Cover to their locking positions

15. If you are installing a new capillary array, in the **Install Capillary Array** dialog box (Figure 207)**,** select the correct part number, enter the serial number, click the **Set to New** button then **Done**. The number of runs and days on instrument will revert to "0."

If you are installing the previous capillary array, do not change the serial number, number of runs, or the number of days on the instrument as they will be correct.

If you are installing a capillary array that was previously used, but not the last capillary array on the instrument, enter its part number (if applicable) serial number, and adjust the number of runs and the previous cumulative days on the instrument. Then click **Done**.

> **Note** *Note the number of runs and days on the instrument for this capillary array for reference.*

Figure 207: Install Capillary Array Dialog

16. The Confirm Capillary Array Selection dialog will appear. Read it and make the selection **Yes** to continue, or **No** to abort the capillary installation.

Removing and Replacing a Gel Cartridge/Gel Pump Plug

⚠ CAUTION **Care must be exercised when installing/removing a gel cartridge due to the viscosity of the gel mixture. Do not allow gel to remain on the instrument.**

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. Select **Replenish | Release Gel Cartridge** from the Run menu. When the system is ready to release the gel cartridge, the **Release Gel Cartridge** dialog box will be displayed. Wait until the lead screw is completely disengaged.

2. Open the Gel Pump/Gel Cartridge Access Cover by gently pushing in on the top of the cover. (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° angle from its locked position.)

Figure 208: Removing/Replacing the Gel Cartridge

Cartridge Locking Lever (closed)

Cartridge Barrel

Cartridge Locking Lever (open)

Cartridge Barrel

Gel Pump/
Gel Cartridge
Access Cover

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900491e.AI
900492e.AI
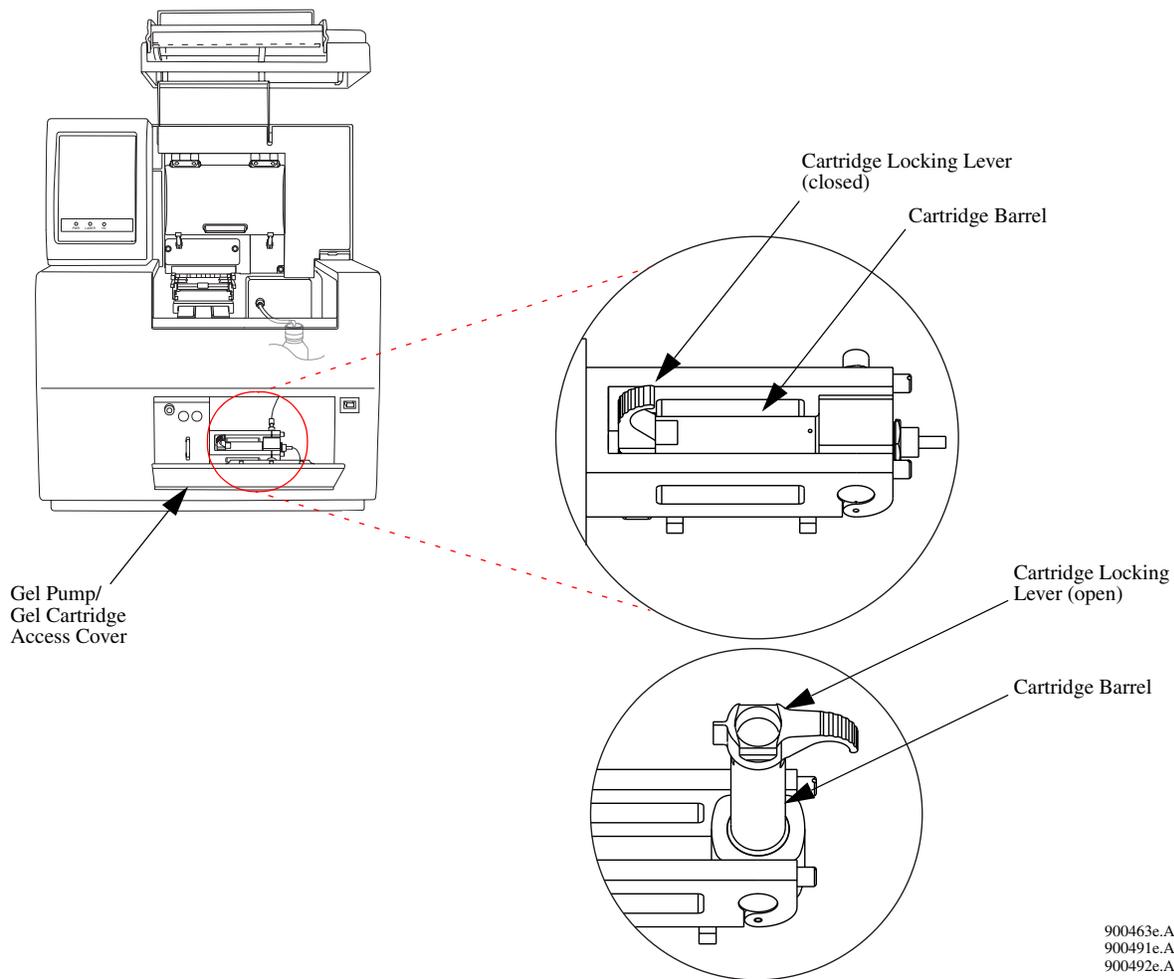
4. Grasp the wings of the gel cartridge/gel pump plug and pull it out of the barrel.

> **Note** *Always note the lot number and the hours on the instrument for a gel cartridge if you are planning to use it for more than one session.*

5. If necessary, use a tissue to wipe gel strands off of the instrument.

Figure 209: Removing/Replacing the Gel Cartridge - Continued

Cartridge Wings

Gel Cartridge OR Gel Pump Plug

Cartridge Locking Lever (open)

Cartridge Barrel
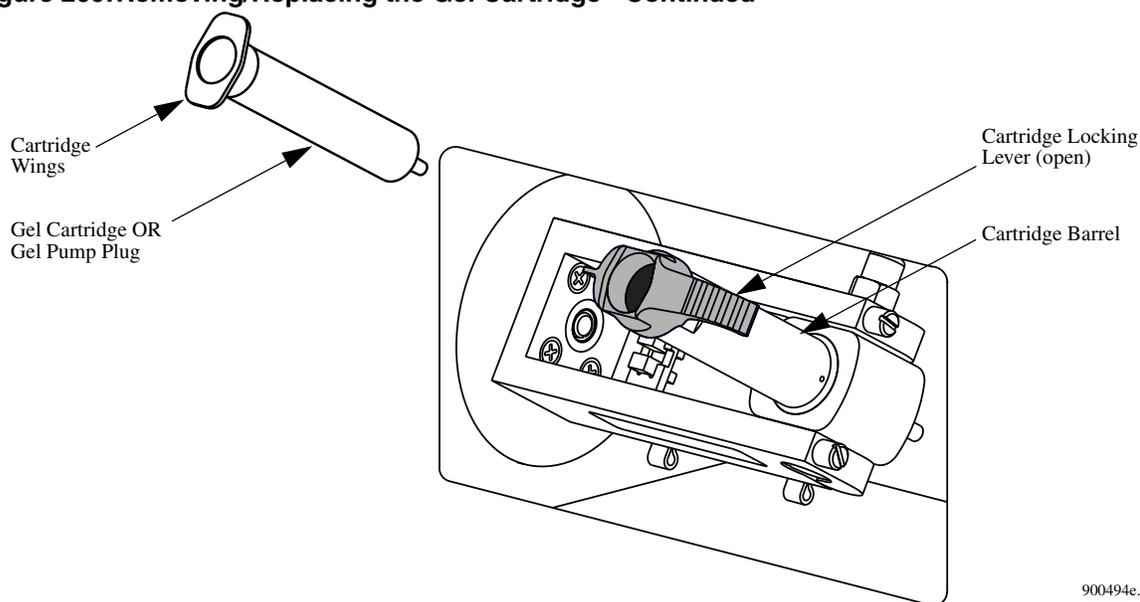
900494e.AI
900493e.AI

6. Remove any air pockets from the gel cartridge by grasping the cartridge wings between your first two fingers, and depressing the plunger with your thumb until a small amount of gel is pushed out of the cartridge tip.

> **Note** *If there are bubbles present in the gel, do not install the cartridge. Repeat step 6 above before installing this cartridge.*

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 instrument (approximately a 90° angle) into its locked position.

9. Close the Gel Pump/Gel Cartridge Access Cover.

10. In the **Remove Gel Cartridge** dialog box (Figure 210):

   • Click **Install Cartridge** to indicate that a gel cartridge was installed.

   OR

   • Click **Install Plug** to indicate that the gel pump plug (or empty cartridge) was installed.

Figure 210:Remove Gel Cartridge Dialog
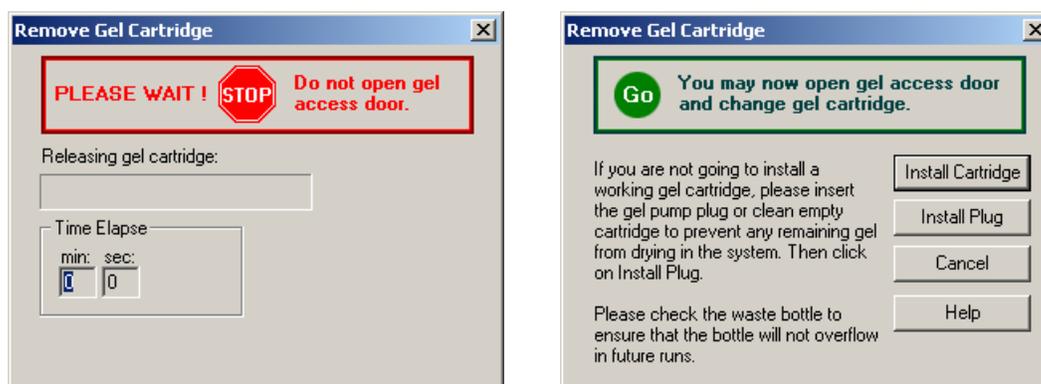
11. If **Install Plug** was selected in step 10, proceed to step 5.

   If **Install Cartridge** was selected in step 10, use the **Install Gel Cartridge** dialog box (Figure 211) to enter the following information:

   a. If you are installing a new gel cartridge, click the **Set to New** button, select the part number from the drop-down 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."

   b. If you are installing the previous gel cartridge, do not change the lot number, or the hours on the instrument, as they will be correct.

   c. If you are installing a previously used gel cartridge, but it was not the last one on the instrument, enter its part number and/or lot number, and adjust the hours to properly reflect the hours this cartridge has been on the instrument.

   d. Click **Done** in the **Install Gel Cartridge** dialog.

   > **Note** *Always note the lot number and the hours on the instrument for a gel cartridge if you are planning to use it for more than one session.*

   > **Note** *The lot number is an alphanumeric text box for your own identification purposes.*

   CAUTION *If the gel cartridge has been on the instrument for more than 72 hours, it is likely that the gel will produce undesirable results.*

Figure 211: Install Gel Cartridge Dialog

5. Dispose of the used tissue and spent gel cartridge in accordance with the procedure, *"Disposal of the Gel Cartridge'* on page 360.

Removing the Manifold Plug

The Manifold Plug is installed in the CEQ instrument during shipping and when the instrument is not is use to prevent drying of gel.

> *This procedure assumes that the Manifold Plug is being removed in preparation for Capillary Array installation.*

1. Select **Replenish | Release Capillary Array** from the Run Module main menu (wait for the **Remove Manifold Plug** dialog box to appear).
2. Open the Sample Access Cover (Figure 190) and lift to the vertical locking position.
3. Open the Capillary Access Cover and lift to the vertical locking position.
4. Unlatch the two rubber latches holding the Capillary Temperature Control Cover and lift to the vertical locking position (Figure 212).
5. Loosen the Manifold Access Cover captive screw, remove the cover and set it aside.

Figure 212: Manifold Access Cover
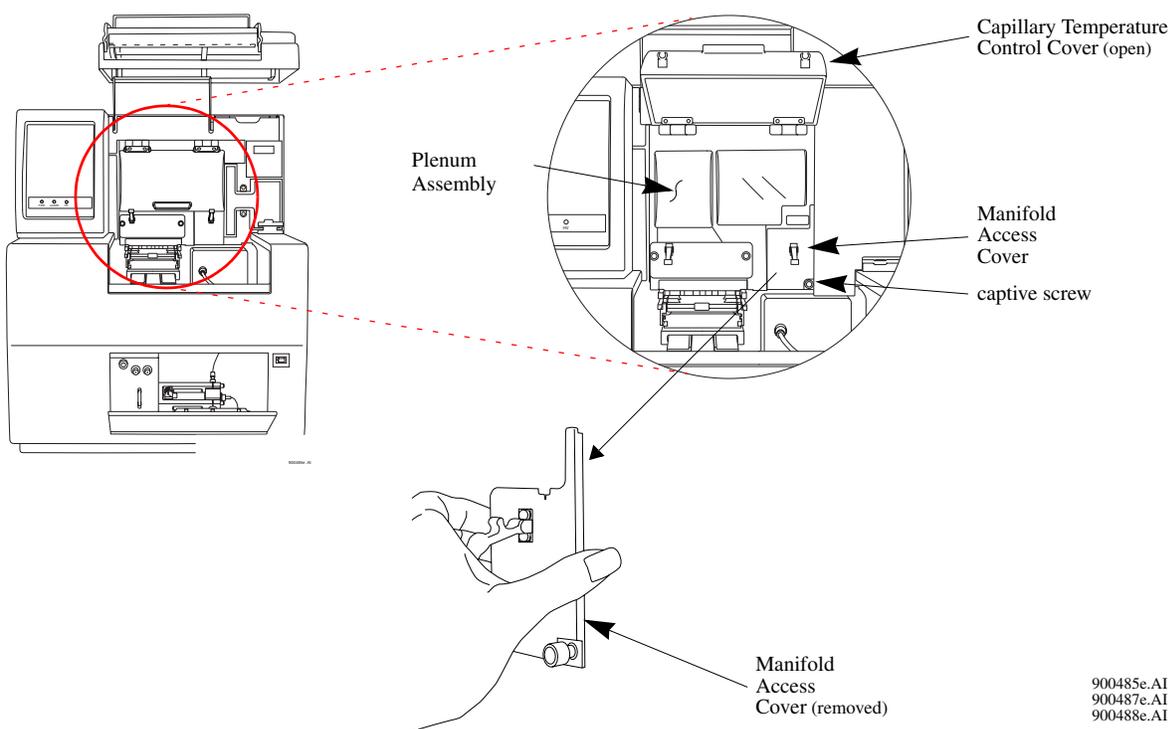
6. Lift the Eject Lever to release the Manifold Plug.
7. Grasp the Manifold Plug tab (Figure 213) and then:

   a. Pull the plug approximately one inch out of the manifold.
   b. Touch the tip of the plug to the bottom of the Optics Base Plate.
   c. Hold and wait five seconds 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 instrument using a damp tissue.

Figure 213: Removing the Manifold Plug

Guide Pins
Eject Lever
Array Fitting
gel strand
Optics Base Plate
(bottom)
Array Fitting

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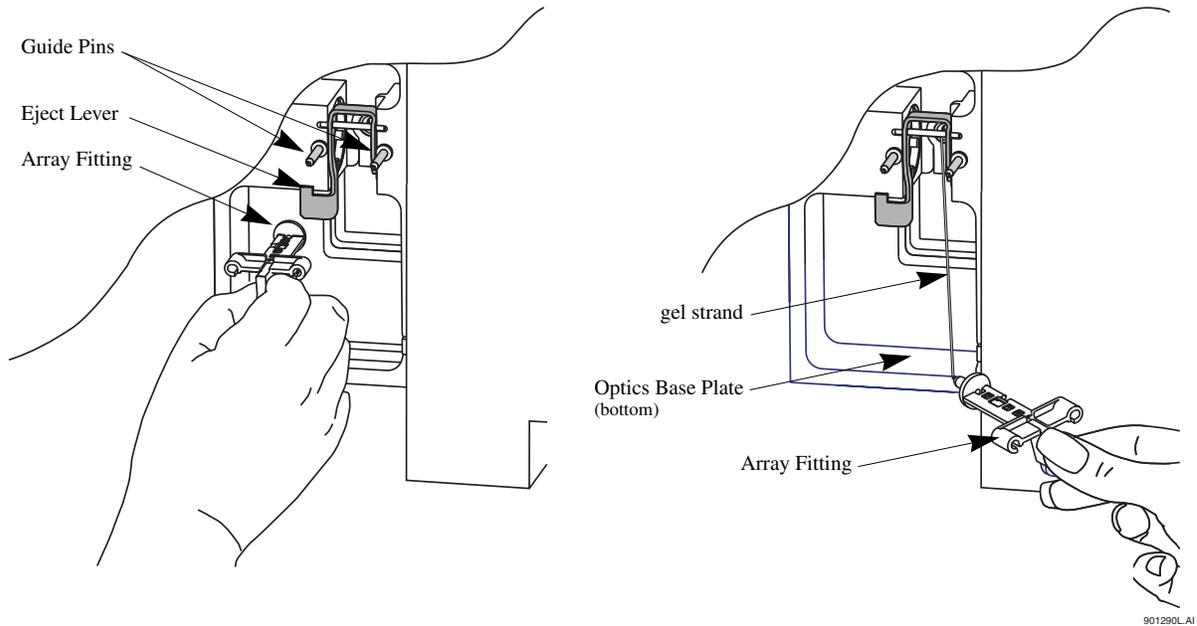
6. Click **OK** from the **Remove Manifold Plug** dialog box (Figure 214).

Figure 214: Remove Manifold Plug Dialog

Remove Manifold Plug

The system is performing functions to allow for the removal of the manifold plug. Please wait.

OK
Cancel
Help

Remove Manifold Plug

You may now open the sample access cover and capillary access cover.

Please remove the manifold plug before installing the capillary array.

OK
Cancel
Help
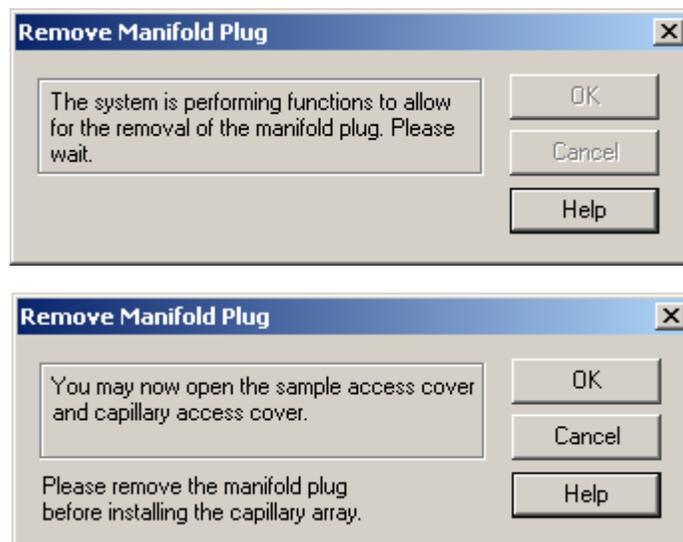
7. To install the capillary array, see *"Removing and Replacing the Capillary Array"* on page 336.

Routine Maintenance

Overview

This section provides routine maintenance and biological waste disposal procedures. It also provides a list of the consumable materials used in the system.

Routine Maintenance

> **Note** *Use Figure 215 to locate hardware components referenced in this section.*

Figure 215: User Accessible Hardware Components
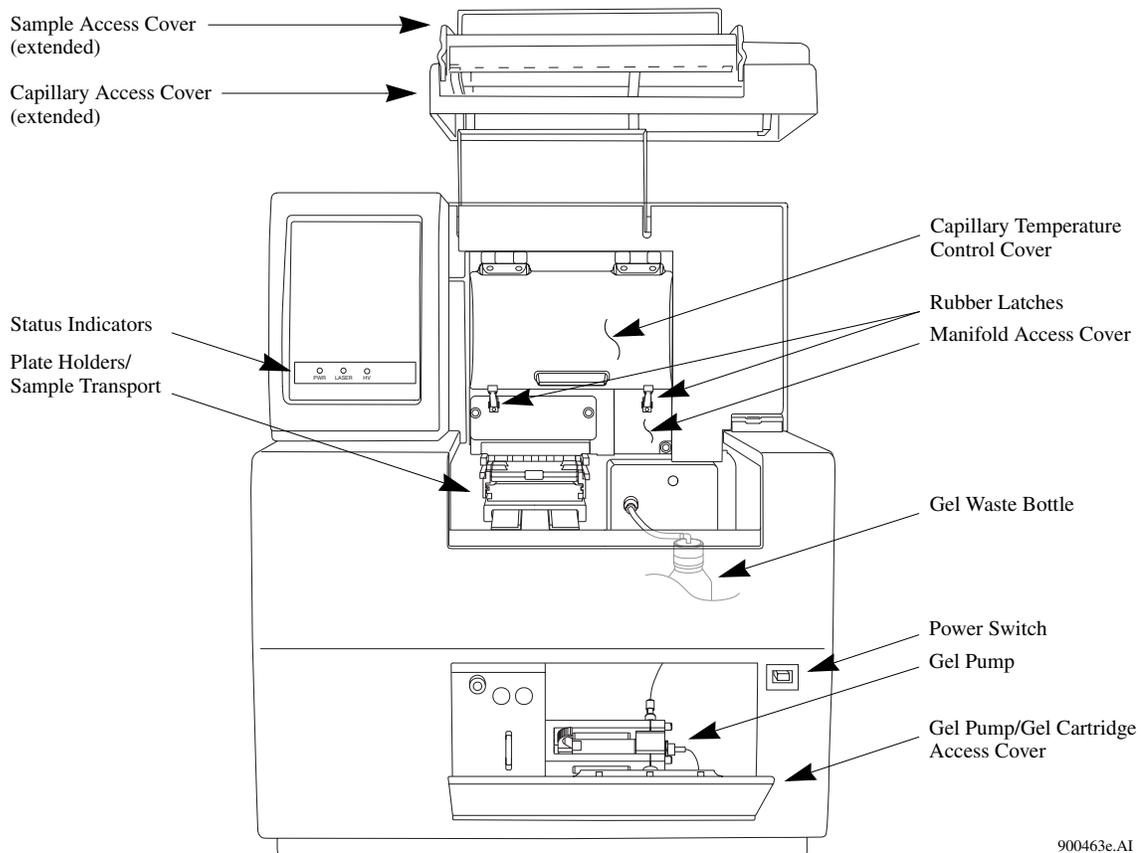
900463e.AI

Cleaning the Capillary Array

⚠️ **CAUTION** **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 6000 counts.**

Water used during this procedure must be fresh, distilled, deionized water (18 Mohm/cm water).

1. Select **Replenish | Release Capillary Array** from the Run Module Main window. Wait for the Remove Capillary Array dialog to appear.

2. Open the Sample Access Cover (Figure 215) and lift to the vertical locking position.

3. Open the Capillary Access Cover and lift to the vertical locking position.

4. Unlatch the two rubber latches holding the Capillary Temperature Control Cover and lift to the vertical locking position.

5. Loosen the Manifold Access Cover captive screw (Figure 216), remove the cover and set it aside.

Figure 216: Manifold Access Cover

Capillary Temperature Control Cover (open)

Plenum Assembly

Manifold Access Cover

captive screw

Manifold Access Cover (removed)

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900487e.AI
900488e.AI
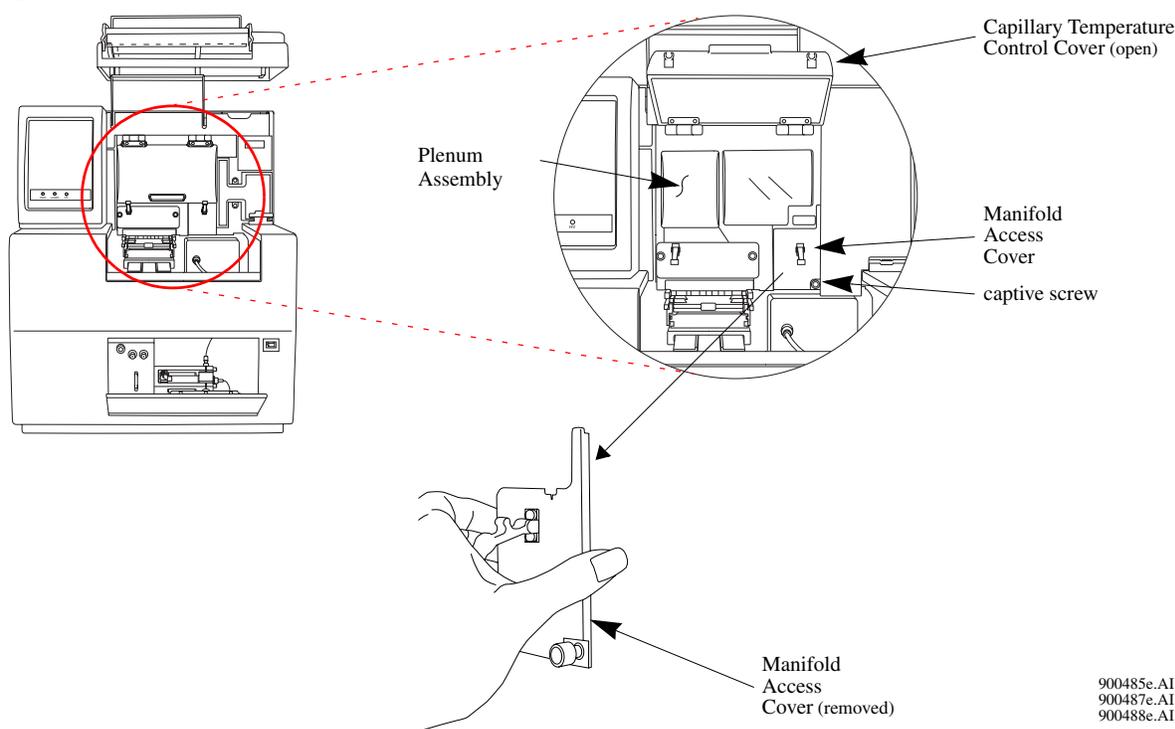
6. Lift the Eject Lever (Figure 217) to release the Array Fitting.

7. Grasp the Array Fitting tab (Figure 217) and then:

   a. Pull the fitting approximately one inch out of the manifold.
   b. Touch the tip of the fitting to the bottom of the Optics Base Plate.
   c. Hold and wait five 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 217: Removing/Replacing the Array Fitting
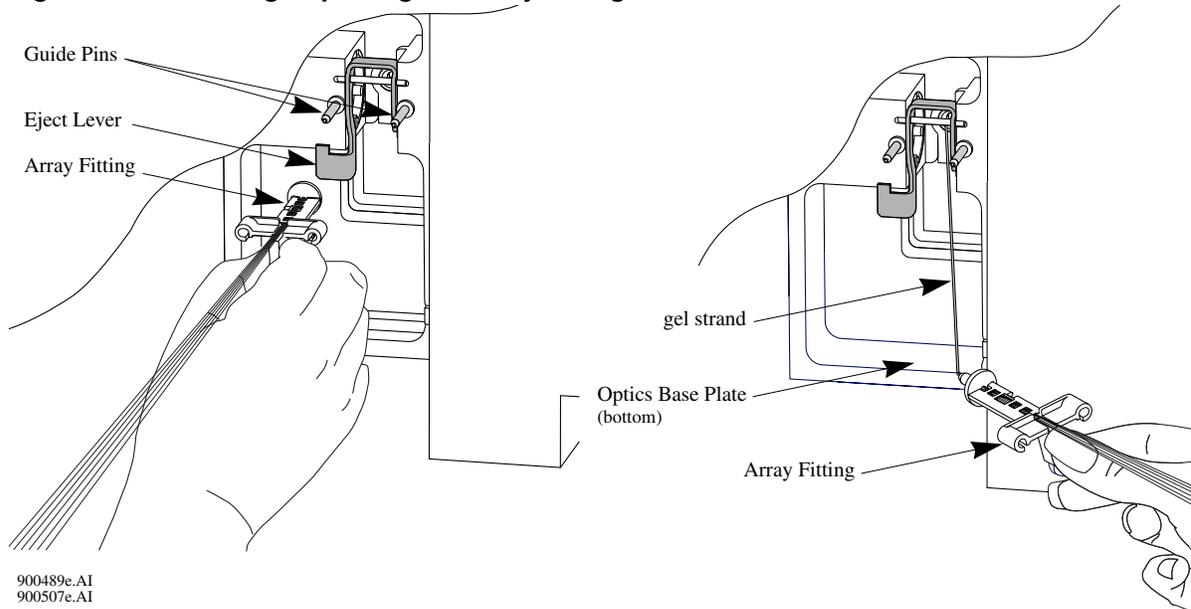
8. With the array fitting in hand, blow dust and debris off of the windows with compressed gas (Texwipe Microduster III, P/N: TX2511).

9. Using a water-moistened swab (Texwipe Swab, P/N: TX754B), gently wipe the Detection Window by stroking in one direction *only* as shown in Figure 218.

Figure 218: Cleaning the Detection Window
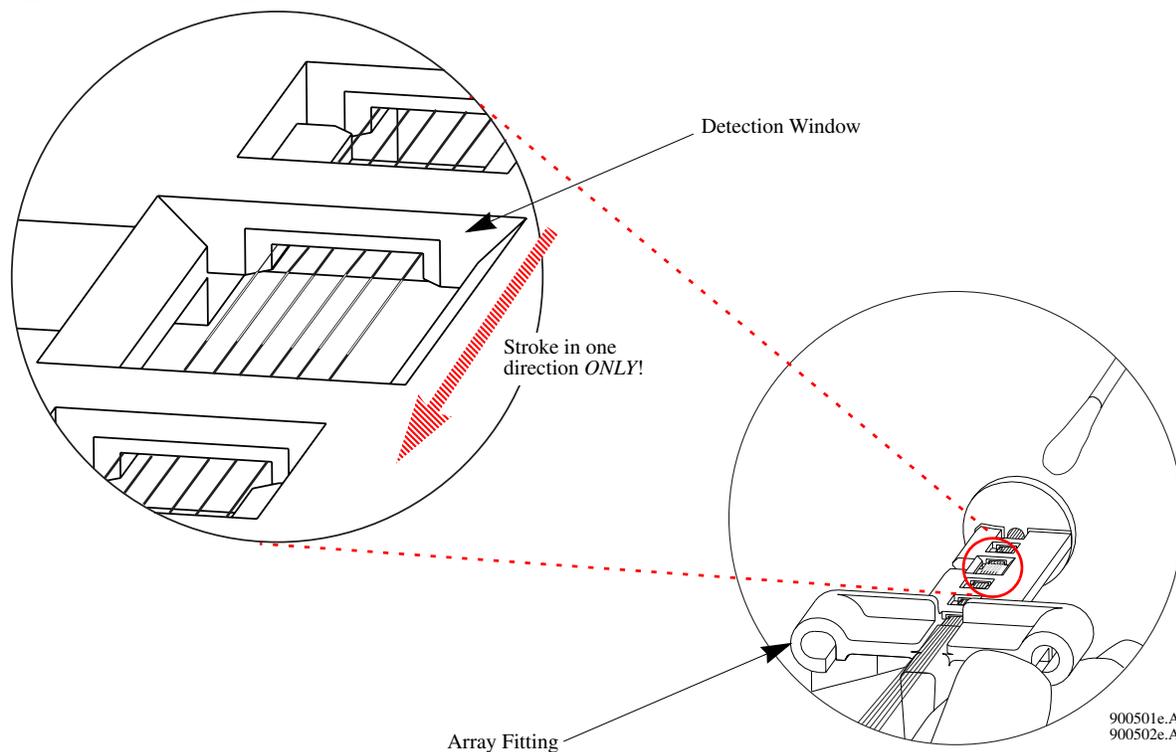
10. With a new water-moistened swab, repeat wiping on the other side of the window.

11. With a dry swab, gently wipe the windows to remove excess water, again repeating on the backside with a new, dry swab.

12. Blow compressed gas on the windows to remove all excess water.

> ⚠ **CAUTION** **Care must be taken not to invert the compressed gas bottle, otherwise propellant will contaminate the capillary windows.**

13. If dried gel or other debris remains on the window, repeat this procedure.

14. From the **Remove Capillary** dialog box (Figure 219), select the **Clean Capillaries** radio button and click **OK**.

Figure 219: Remove Capillary Dialog
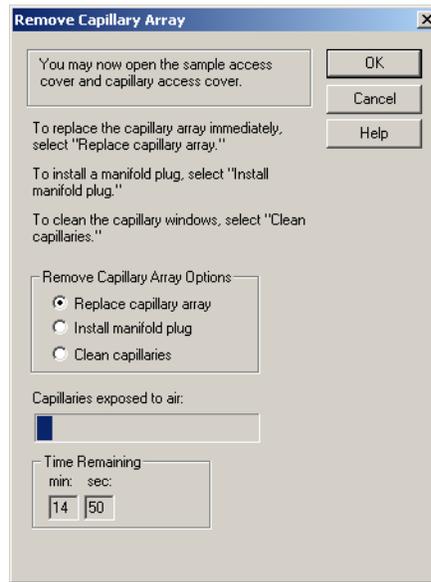
15. While holding the Array Fitting tab (of the clean capillary array), align the Array Fitting with the manifold opening and guide pins. Push the fitting into the manifold until it is completely seated against the bases of the Guide Pins. See Figure 217.
16. Replace the Manifold Access Cover and tighten the captive screw.
17. Lower the Capillary Temperature Control Cover and secure the two rubber latches.
18. Lower the Capillary Access Cover and Sample Access Cover to their closed/locked positions.
19. Click **Done** in the Install Capillary Array dialog shown in Figure 220.

Figure 220: Install Capillary Array Dialog
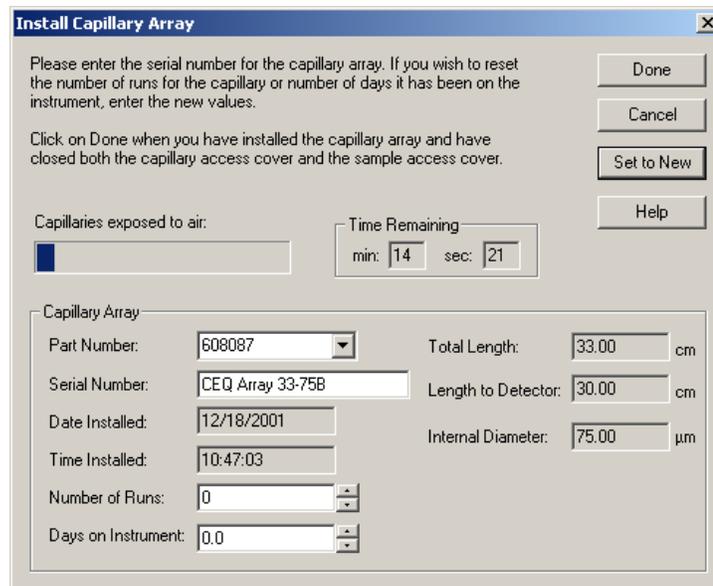
> ! CAUTION **After cleaning the Detection Windows, perform the Optical Alignment procedure and monitor the baseline. If background levels are above 6000 counts, repeat the cleaning process.**

Replacing the Gel Waste Bottle

> Note *This procedure assumes that a used (full) waste bottle is being replaced with an empty waste bottle.*

1. Remove the cap from the new (empty) waste bottle.
2. Open the Sample Access Cover (Figure 215) and lift to the vertical locking position.
3. With the Gel Waste Bottle 80-90% full, remove the cap and pull the bottle out of the instrument.
4. Place the cap from the new bottle over the full waste bottle and secure.
5. Thread the new bottle onto the cap attached to the instrument and set the bottle into position.
6. Close the Sample Access Cover.
7. Dispose of the full waste bottle according to procedures found in *"Disposal of the Gel Waste Bottle'* on page 361.

Replacing the Wetting Tray

Remove the Wetting Tray

1. Select **Replenish | Replace Wetting Tray** from the Run Module menu.
2. Open the Sample Access Cover (Figure 215) and lift to the vertical locking position.
3. Remove the Sample Plate and set aside.
4. Rotate the Wetting Tray Retainers outwards to release the Wetting Tray. (Figure 221)
5. Lift the Wetting Tray vertically.

Cleaning 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.

Fill with D.I. Water

1. Remove the lid of the Wetting Tray and fill with D.I. water to the indicator line.
2. Replace the wetting tray lid.

> **!** **CAUTION** **No more than one 96-well plate should be processed, for each wetting tray, without replenishing the Wetting Tray.**
>
> **Periodically check the liquid level in the wetting tray. 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.**

Installing the Wetting Tray

1. Rotate the wetting tray retainers outward.
2. Insert the Wetting Tray into the receptacle between the Sample and Buffer plates (Figure 221) and then gently press it down into the well.
3. Rotate the Wetting Tray Retainers inwards to lock the Wetting Tray in place.

Figure 221: Replacing the Wetting Tray
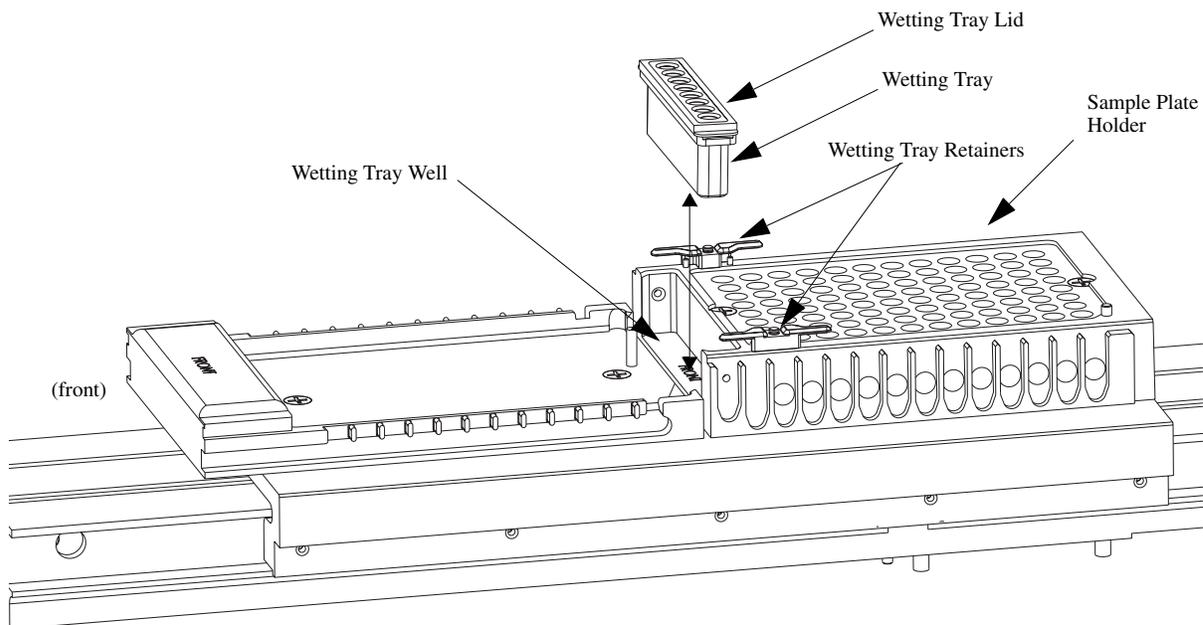
4. Close the Sample Access Cover and then click the **Done** button of the **Replace Wetting Tray** dialog box (Figure 222).

> **Note** *If a sample plate is installed in the system, it must be removed before installing the wetting tray to allow movement of the wetting tray retainers.*

Figure 222: Replace Wetting Tray Dialog
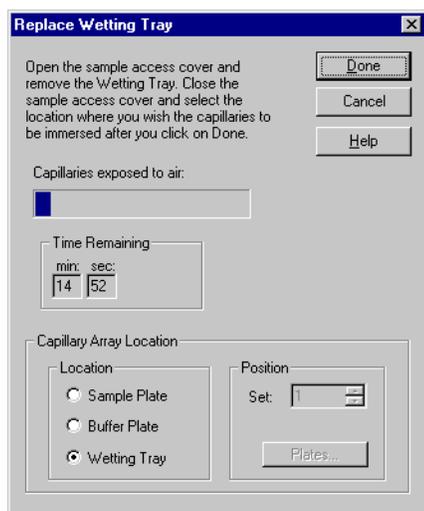
Biological Waste Disposal

WARNING **The CEQ System has been designed to minimize exposure to hazardous chemicals and biological waste. However, care must still be exercised when removing used chemicals and biological samples from the instrument. The information below provides the minimum protocols to use when handling hazardous chemicals and biological waste.**

Disposal of Formamide from the Sample Plate

Multi-Channel Pipettor

WARNING **When performing this procedure, use an exhaust ventilation unit that meets TLV requirements.**

1. Aspirate 40 μL of formamide from the thermo-cycling plate.

2. Dispense the formamide 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.

WARNING **Dispose of formamide in accordance with all applicable federal, state and local environmental regulations concerning hazardous liquid waste.**

Bulk Disposal

WARNING **When performing this procedure, use an exhaust ventilation unit that meets TLV requirements.**

1. Using a 1L side arm flask as a trap, connect the trap to a vacuum.
2. Attached a pipette to the trap using chemical resistant tubing.
3. Aspirate the formamide from each well and dispose of it in a hazardous liquid organic waste container.
4. After complete removal of all formamide 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.

WARNING **Dispose of formamide in accordance with all applicable federal, state and local environmental regulations concerning hazardous liquid waste.**

Disposal of Buffer/Gel Mixture from the Buffer Plate

Multi-Channel Pipettor

WARNING **When performing this procedure, use an exhaust ventilation unit that meets TLV requirements.**

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.

WARNING **Dispose of buffer/gel mixture in accordance with all applicable federal, state and local environmental regulations concerning hazardous liquid waste.**

Bulk Disposal

WARNING When performing this procedure, use an exhaust ventilation unit that meets TLV requirements.

1. Using a 1L side arm flask as a trap, connect the trap to a vacuum.
2. Attached 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.

WARNING Dispose of buffer/gel mixture in accordance with all applicable federal, state and local environmental regulations concerning hazardous liquid waste.

Disposal of the Capillary Array

WARNING After removing the expended capillary array from the instrument, dispose of it in a solid hazardous waste container.

Disposal of the Gel Cartridge

WARNING 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.

Disposal of D.I. Water/Gel Mixture from the Wetting Tray

WARNING When performing this procedure, use an exhaust ventilation unit that meets TLV requirements.

1. Pour the D.I. 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.

WARNING **Dispose of D.I. water/gel mixture in accordance with all applicable federal, state and local environmental regulations concerning hazardous liquid waste.**

Disposal of the Gel Waste Bottle

WARNING **When performing this procedure, use an exhaust ventilation unit that meets TLV requirements.**

Dispose of buffer/gel mixture in accordance with all applicable federal, state and local environmental regulations concerning hazardous liquid waste.

Consumable Items List

Table 75 provides a list of the required consumable items for the Sequence Analysis system.

Table 75: Consumable Items Required for Sequence Analysis

Item	P/N	QTY[a]	Description
DTCS Kit (DNA Sequencing RXN Kit) *or*	608000	1	Dye Terminator Cycle Sequencing Kit for 96 reactions. Includes: • DNA polymerase • CEQ Dye Terminators (ddUTP, ddGTP, ddCTP, ddATP) • dNTP Mix Solution • Sequencing Reaction Buffer • pUC18 Control Template • -47 Sequencing Primer • Glycogen • Mineral Oil • Sample Loading Solution (SLS)
DTCS Quick Start Kit	608120	1	Dye Terminator Cycle Sequencing Kit for 96 reactions in a single tube containing: • DNA polymerase • CEQ Dye Terminators (ddUTP, ddGTP, ddCTP, ddATP) • dNTP Mix Solution • Sequencing Reaction Buffer • pUC18 Control Template • -47 Sequencing Primer • Glycogen • Mineral Oil • Sample Loading Solution (SLS)
Sequencing Test Samples	608070	1	
CEQ Separation Gel LPA 1	608010	1	11.5 mL of gel in CEQ 8000 compatible container. Sufficient for 12 fills of the Capillary Array (96 sequencing runs).
CEQ Separation Buffer	608012	1	Each container has a screw top and pour tip. The container has enough buffer (30 mL) to fill a CEQ System 96-well, flat bottom Buffer Plate. (Each well being ¾ full.) 4/Pack
DNA Separation Capillary Array 33-75B	608087	1	Package of 100 flat-bottom polystyrene plates, nonsterile, without lids. Required for use as the CEQ 8000 Separation Buffer Plate.

Table 75: Consumable Items Required for Sequence Analysis (Continued)

Item	P/N	QTY[a]	Description
Buffer Plates	609844	Box	Package of 100 flat-bottom polystyrene plates, nonsterile, without lids. Required for use as the CEQ Separation Buffer Plate.
Sample Plate	609801	Box	Package of 25 V-bottom thermal cycler-compatible polypropylene plates with 200 µL volume capacity. Required for use as the CEQ 8000 sample plate. 25 Plates/Box

a. Contact your local Beckman Coulter representative for specific consumable material requirements based on your application and frequency of use.

Table 76 provides a list of the required consumable items for the Fragment Analysis system.

Table 76: Required Consumable Materials for Fragment Analysis

Item	P/N	QTY[a]	Description
DNA Separation Capillary Array 33-75B	608087	1	Eight capillaries, 75 µm i.d., 33 cm long, 200 o.d. complete with electrode block and detector array fitting. Ready for installation into CEQ 8000.
CEQ Separation Gel LPA 1	608010	1	11.5 mL of gel in CEQ 8000 compatible container. Sufficient for 12 fills of the Capillary Array (96 sequencing runs).
CEQ Separation Buffer	608012	1	Each container has a screw top and pour tip. The container has enough buffer (30 mL) to fill a CEQ System 96-well, flat bottom Buffer Plate. (Each well being ¾ full.) 4/Pack
CEQ DNA Size Standard Kit - 80	608395	1	Contains fragments of 13 and 80 nucleotides designed to accomodate a wide range of sizes for multiplexed and poolplexed SNP fragments.
CEQ DNA Size Standard Kit - 400	608098	1	DNA size standard for analysis of fragments up to 400 nucleotides. Includes: • Mineral Oil • DNA fragments of the following sizes labeled with CEQ WellRED fluorescent dye: 60, 70, 80, 90, 100, 120, 140, 160, 180, 190, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, and 420 nucleotides Sufficient for 96 fragment analysis separations.

Table 76: Required Consumable Materials for Fragment Analysis

Item	P/N	QTY[a]	Description
CEQ DNA Size Standard Kit - 600	608095	1	DNA size standard for analysis of fragments up to 600 nucleotides. Includes: • Mineral Oil • DNA fragments of the following sizes labeled with CEQ WellRED fluorescent dye: 60, 70, 80, 90, 100, 120, 140, 160, 180, 190, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 420, 440, 460, 480, 500, 520, 540, 560, 580, 600, 620, and 640 nucleotides Sufficient for 96 fragment analysis separations.
CEQ SNP-Primer Extension Kit.	390280	1	Includes: • DNA Polymerase • CEQ dye-labeled terminators (ddUTP, ddGTP, ddCTP, ddATP) • Reaction buffer • Mineral oil Sufficient for 96 reactions.
CEQ Sample Loading Solution (SLS)	608082	1	Sample Loading Solution (SLS), 6.0 mL
Buffer Plates	609844	Box	Package of 100 flat-bottom polystyrene plates, nonsterile, without lids. Required for use as the CEQ Separation Buffer Plate.
Sample Plate	609801	Box	Package of 25 V-bottom thermal cycler-compatible polypropylene plates with 200 μL volume capacity. Required for use as the CEQ 8000 sample plate. 25 Plates/Box.

a. Contact your local Beckman Coulter representative for specific consumable material requirements based on your application and frequency of use.

Materials Required but not Supplied

	Sequence Analysis	Fragment Analysis
Refrigerated microfuge	✔	✔
Molecular Biology Grade: sterile dH_2O, 95% (v/v) ethanol/dH_2O, 70% (v/v) ethanol/ dH_2O	✔	✔
Sterile tubes - 0.5 mL microfuge, 0.2 mL thin wall thermal cycling tubes or plates with caps	✔	✔
Thermal cycler with heated lid	✔	✔
CEQ Sample Loading Solution (SLS)	—	✔
Mini Alpha Swab from Texwipe, P/N: TX754B (VWR)	✔	✔
Microduster III AccTech, P/N: 58019-538 (VWR)	✔	✔
20 mg/mL Glycogen - Boehringer Mannheim Cat. # 901 393	—	✔
3M Sodium Acetate pH5.2 - Sigma, Cat. # S7899	✔	✔
100mM Na_2-EDTA pH8.0 (500 mM Sigma Cat. # 7889)	✔	—
PCR enzyme and buffer	—	✔
Labeled primers (contact your local Beckman Coulter representative for information on vendors carrying the appropriate primers)	—	✔
Thermal cycling plates and caps	—	✔

Diagnostics

Re-Initializing the System

To re-initialize the system, select **Run | Reset** from the Run menu.

Homing the Plates and/or Gel Pump

To re-establish the position of the plates and/or gel pump, perform the following steps.

1. Select **Run | Diagnostics** from the Run menu.
2. From the **Diagnostics** dialog box, select the **Home Plate** and/or **Gel Pump** check box and click **OK**.
3. Monitor the **Tests** dialog box and verify that the final result(s) is **Test Passed** and then click the **Close** button.

Viewing PC Settings

To view the computer settings, perform the following steps.

1. Select **Run | Diagnostics** from the Run menu.
2. From the **Diagnostics** dialog box, select the **PC Settings** button.
3. View the settings in the **PC Communication Settings** dialog box and then exit the dialog box.

Viewing Instrument Status

To view the instrument status, perform the following steps.

1. Select **Run | Diagnostics** from the Run menu.
2. From the **Diagnostics** dialog box, select the **Status** button.
3. View and/or change the settings in the **CEQ Monitor** dialog box and then select **OK**.

Viewing Optical Scan Data

To view the optical scan data in either the Sequence or Fragment Analysis module, perform the following steps.

1. Select **File | Open** from the desired Analysis module menu.
2. In the **Open** dialog box, select the **Optical Scan Data** tab, highlight the desired sample name and then select **OK**.
3. Verify that the **Optical Scan** window is displayed.

Monitoring the Baseline

Note	*You must perform the optical alignment procedures prior to monitoring the baseline ("Performing an Optical Alignment' on page 333).*

To monitor the baseline, perform the following steps.

1. Select **Run | Monitor Baseline** from the Run Module main menu.
2. From the **Monitor Baseline** dialog box:

    a. Select the **Enable Monitor Baseline** check box.
    b. Select the **Autosave** check box (to save monitor baseline data).
    c. Enter a name in the **Name** field.
    d. Select a **Project** from the drop-down menu and then select **OK**.

5. To view the baseline trace:

    a. Access the desired Analysis module.
    b. Select **File | Open** from the menu.
    c. Click on the **Sample Data** tab and select the desired baseline data (the baseline data is displayed).