



GenomeLab GeXP Genetic Analysis System

Troubleshooting Guide

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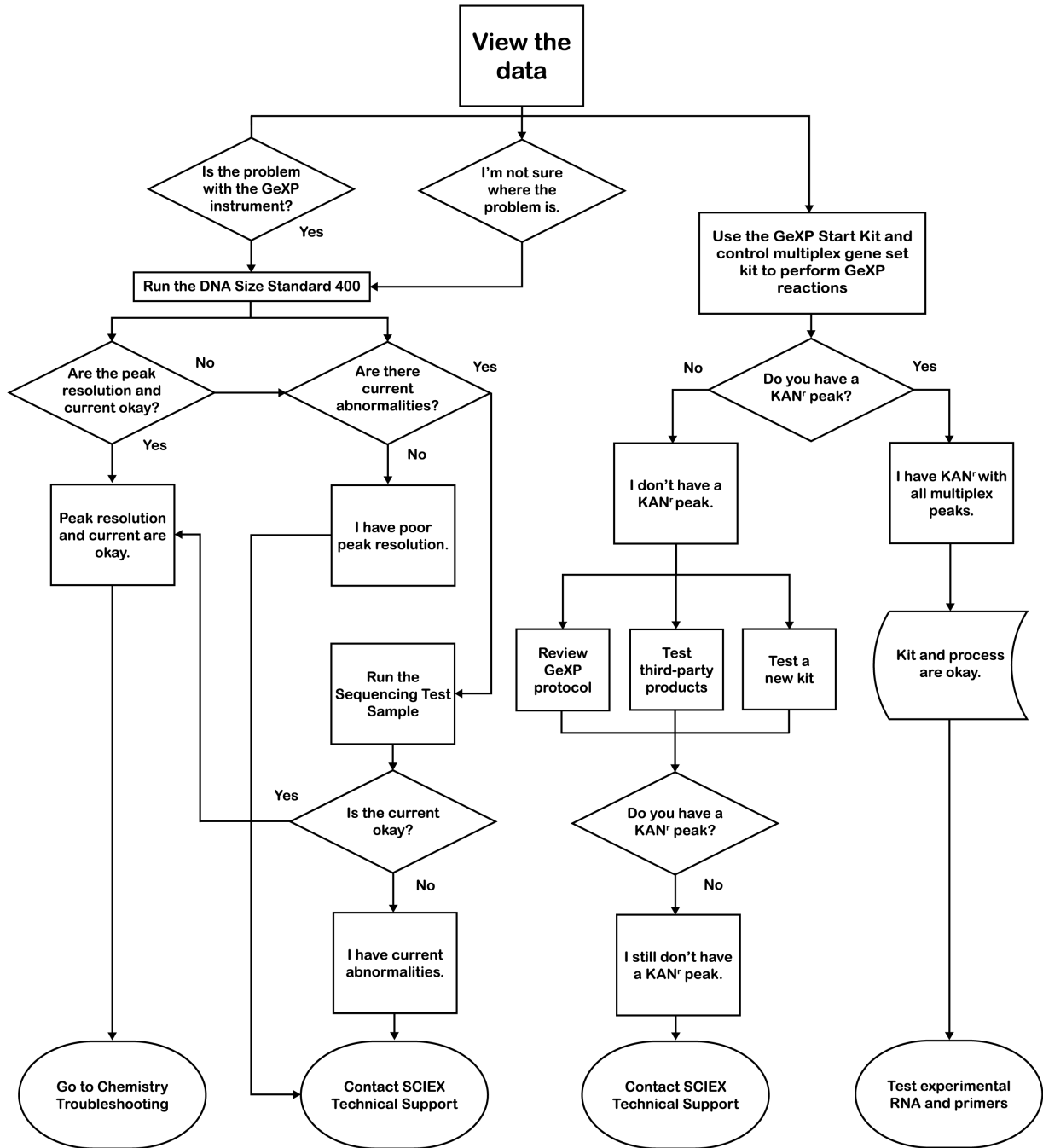
Overview

The *GenomeLab GeXP Genetic Analysis System Troubleshooting Guide* has been designed to help you identify and correct the issues most commonly encountered with the gene expression application of the GenomeLab GeXP Genetic Analysis System. Read through the entire document first, before performing troubleshooting experiments.

Begin with the [General Diagnostics Guidelines](#) section, which will inform you of the required data and where it can be found. Next, the [Instrumentation & Chemistry](#) section will provide a systematic approach to enable you to effectively diagnose the problem. [Figure 1-1](#) can serve as a visual aid for the troubleshooting process.

Finally, the [Reference Tables](#) and [Questions & Answers](#) sections are particularly helpful for resolving specific problems. These sections can also aid in rapid identification of the most likely suspects for poor results.

Figure 1-1 Gene Expression Troubleshooting Workflow



Overview

The GenomeLab System software provides functions that can help identify and resolve problems. Two key features in this regard are the raw data and current profile.

Note: The analyzed data can also be used for troubleshooting, but only after the raw data and current profile have been shown to be acceptable.

Use the raw data, current profile, and analyzed data to determine whether the issue is caused by instrumentation, chemistry, or possibly both. Be sure to note the following aspects of each type of data for all of the affected samples and appropriate controls:

Raw and Analyzed Data

- Baseline for D4 (blue) and D1 (red)
- Signal strength
- Signal profile (level or drop-off)
- Landmarks
 - Unincorporated primers (raw only)
 - Multiplex peaks
 - KAN^r peak
 - Size standard peaks

Refer to [Evaluating the Raw Data](#).

Current Profile

- Ramping profile
- Maximum separation current (μA)
- Level of current throughout separation

Refer to [Evaluating the Current Profiles](#).

Evaluating the Raw Data

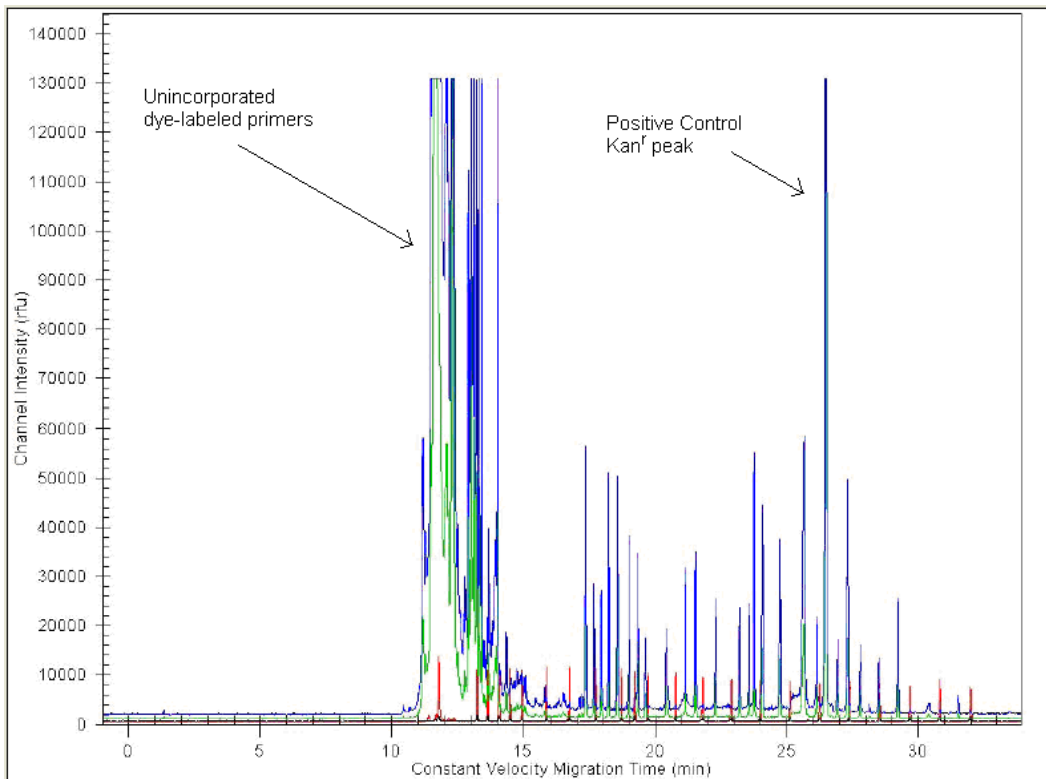
The raw data generated by the GenomeLab GeXP System shows in the Data Monitor toolbar of the Run module during the actual separation. The data is also accessible by using the Fragment Analysis and Sequence Analysis modules.

Examining Signal Strength and Multiplex Profile

Refer to the signal strength and multiplex profile as the first step in diagnosing problems when looking at raw data.

- Although peak heights vary for different multiplex fragments, the signal strength of D4 (blue) labeled multiplex peaks should be fairly even across the separation after the initial unincorporated primer dye front.
 - One exception is the positive control, the 325-nucleotide kanamycin resistance (KAN^r) peak, which is usually over-range.
- All of the D1 (red) labeled DNA Size Standard peaks should be appropriately spaced and approximately the same height.

Figure 2-1 Raw Data



Checking the Raw Data

The electropherogram in [Figure 2-1](#) shows a typical separation with a start time at approximately 11 min for the Frag-3 separation method. The signal strength at the beginning of the separation is over-range due to excess primers remaining in the PCR Reaction Mix. This is common for GenomeLab GeXP System reactions. The multiplex fragments show at approximately 17.5 min for this particular sample. The positive control KAN^r fragment shows at approximately 26.5 min. The D1 (red) labeled DNA Size Standard peaks are appropriately spaced and even in height.

The data shows as signal intensity, measured in relative fluorescence units (RFU) versus time. [Figure 2-1](#) shows excellent signal strength. However, it is not necessary for each sample to have such high signal in order to obtain accurate peak area measurements. More important is that all the peaks fall within the limits of detection (less than 130,000 RFU in the raw data) and the electropherogram shows a balanced profile with no signal drop-off.

Signal Drop-Off

The example of analyzed data in [Figure 2-2](#) shows a balanced profile, whereas the analyzed data in [Figure 2-3](#) shows signal drop-off. Signal drop-off is characterized by relatively high signal for shorter fragments and significantly lower signal for the longer fragments. The result is a multiplex profile with a downward slope toward the larger fragments, while the DNA Size Standard peaks are even and appear normal. The KAN^r peak may or may not be affected by signal drop-off.

Irregularities in PCR thermal-cycling conditions, particularly the extension temperature, are the most likely cause of signal drop-off. This is often seen in the edge wells of a thermal cycler. Make sure that the thermal cycler is calibrated and consider using only the non-edge wells to avoid signal drop-off. Refer to [Gene Expression Chemistry](#).

Note: Signal drop-off can be caused by using the incorrect DNA polymerase. Thermo-Start *Taq* DNA Polymerase has been validated for use with the GenomeLab GeXP System.

Figure 2-2 Analyzed Data: Normal Peak Profile

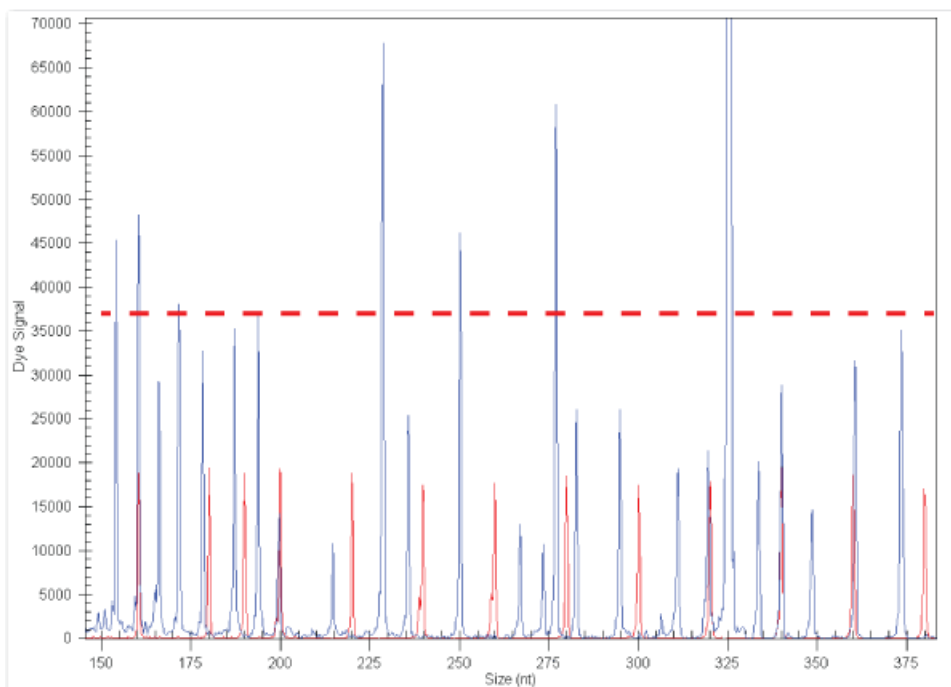
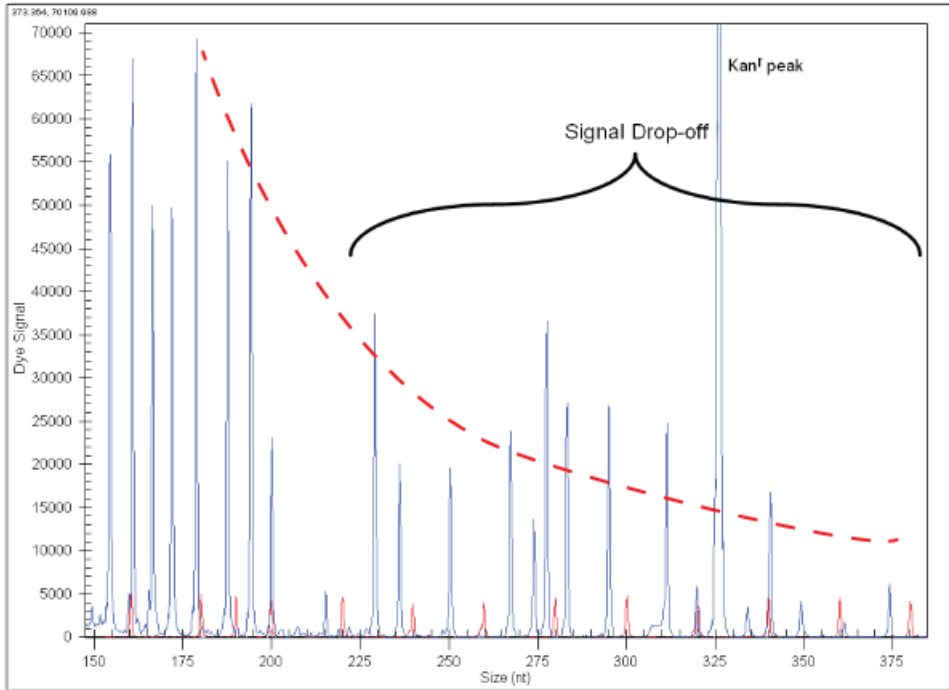


Figure 2-3 Analyzed Data: Peak Profile with Signal Drop-off



Note: Signal drop-off can lead to high %CV in analyzed data. Do not use the results of these wells for data analysis.

Evaluating the Current Profiles

The GenomeLab GeXP System continuously monitors the current in each of the eight capillaries while the system is running. The current profile can be extremely useful in diagnosing certain issues. The current profile shows in the Data Monitor toolbar of the Run module during the actual separation, and is also accessible by using the Fragment Analysis module.

Confirming the Separation Current

The figures below illustrate the raw data from the same sample separated with normal current and with abnormal current.

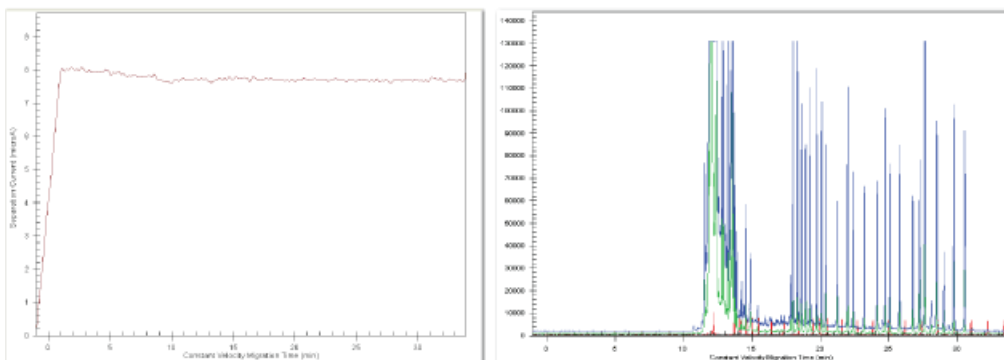
Normal Current

The current should ramp up to the final level in a single stage and then plateau. The level of the plateau is determined by the separation voltage, which is set in the separation method (Frag-3)

General Diagnostic Guidelines

and is maintained throughout the separation. The final level should be approximately 7 μA to 11 μA . The current profile should look similar to the following figure. Refer to [Figure 2-4](#).

Figure 2-4 Normal Capillary Current Profile and the Corresponding Raw Data



Abnormal Current

A number of characteristics can be used to determine a current failure. The most commonly observed characteristics for a failed current are:

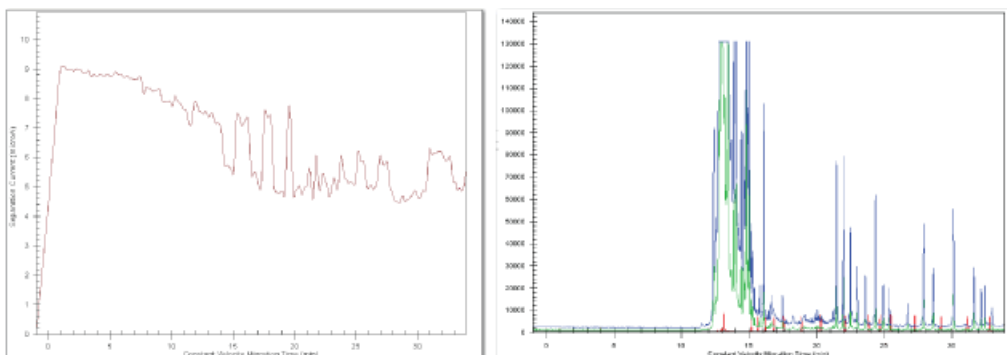
- Ramps in two or more stages
- Does not maintain the separation current at a steady level
- A change in current level of more than 10%

A current failure will cause the fragments to:

- Separate abnormally with delayed peaks (Refer to [Figure 2-5](#))
- Have reduced peak resolution
- Have low signal

IMPORTANT:

If a capillary has a current failure, do not use data from these wells because the peak area calculation is affected by abnormal current.

Figure 2-5 Abnormal Capillary Current Profile and the Corresponding Raw Data

Causes of Current Abnormalities

Current profile abnormalities can be caused by the following:

- Air bubble in the manifold
- No separation buffer
- Impurities or debris in the sample

For instructions on how to verify the cause of the problem, refer to [Performing Instrument Diagnostics](#).

Overview

Issues encountered with the GenomeLab GeXP System process can be divided into two areas: instrumentation and chemistry.

A systematic approach to troubleshooting is described below, to enable the user to effectively diagnose a problem with the GenomeLab GeXP System process.

First, use the raw data, the current profile, and analyzed data to determine whether the issue is caused by one of the following:

- The GenomeLab GeXP System
- The GenomeLab GeXP System chemistry

Initial Indications

- If the DNA Size Standard peaks and/or current are abnormal, then begin by troubleshooting the GenomeLab GeXP System. Refer to [Performing Instrument Diagnostics](#).
- If the DNA Size Standard peaks and current are normal, then begin by troubleshooting the chemistry. Refer to [Testing the Chemistry](#).
- If you are unsure where to start, begin by troubleshooting the GenomeLab GeXP System.

Performing Instrument Diagnostics

A separation of DNA Size Standard in the absence of gene expression reaction products is performed to test the integrity of the GenomeLab GeXP System and the various reagents used by the system. These reagents include:

- Separation Buffer
- Separation gel, 10 mL, for single-rail systems
- Separation gel, 20 mL, for dual-rail systems
- Sample Loading Solution
- DNA separation capillary array

- DNA Size Standard

Running DNA Size Standard

1. Thaw the DNA Size Standard and the Sample Loading Solution at room temperature.

Note: It is recommended that the lot numbers of all consumables be recorded during troubleshooting.

2. Combine 13 μL of DNA Size Standard with 1,027 μL of Sample Loading Solution in a microcentrifuge tube and mix.

Use non-barrier pipette tips when pipetting Sample Loading Solution.

Note: Use only SCIEX Sample Loading Solution. Do not substitute the solution with formamide from a third party.

3. Pipette 40 μL of the DNA Size Standard 400 + Sample Loading Solution mixture to each of 24 wells of a sample plate (three rows total).
4. Put a drop of mineral oil over the DNA Size Standard 400 + Sample Loading Solution mixture in each well.
5. Add the Separation Buffer to each corresponding well of a buffer tray, filling each well about $\frac{3}{4}$ full (250 μL).
6. Set up the sample plate.
7. Select the **Frag-3** separation method for all three rows.
8. Select the **Default Fragment Analysis Parameters** for automatic analysis of each well.
9. Run the samples according to the standard procedures.
10. Review the data as described in [General Diagnostic Guidelines](#).

Interpreting the DNA Size Standard Results

The DNA Size Standard provides acceptable results by meeting the following criteria:

- All DNA Size Standard peaks are present and called
- The peaks are well resolved
- The current is normal

If all of the above criteria are met, then the GenomeLab GeXP System and reagents are fine and further diagnostics of the chemistry are needed.

If any of the above criteria are not met, then try testing the system consumables such as the separation gel, Sample Loading Solution, DNA separation capillary array, and another lot of the DNA Size Standard 400. The individual components should be tested in separate experiments to identify the faulty component.

If a current failure is the problem, proceed with using the sequencing test sample to assess the system and the consumable reagents. See [Running the Sequencing Test Sample](#) for more information.

Running the Sequencing Test Sample

The sequencing test sample is used to test the integrity of the GenomeLab GeXP System and the consumable reagents used by the system, particularly when there is a problem with separation current.

1. Thaw three vials of sequencing test sample at room temperature.

Note: It is recommended that the lot numbers of all consumables be recorded during troubleshooting.

2. Pipette 40 μ L of sequencing test sample directly to each of 24 wells of a sample plate (three rows total).

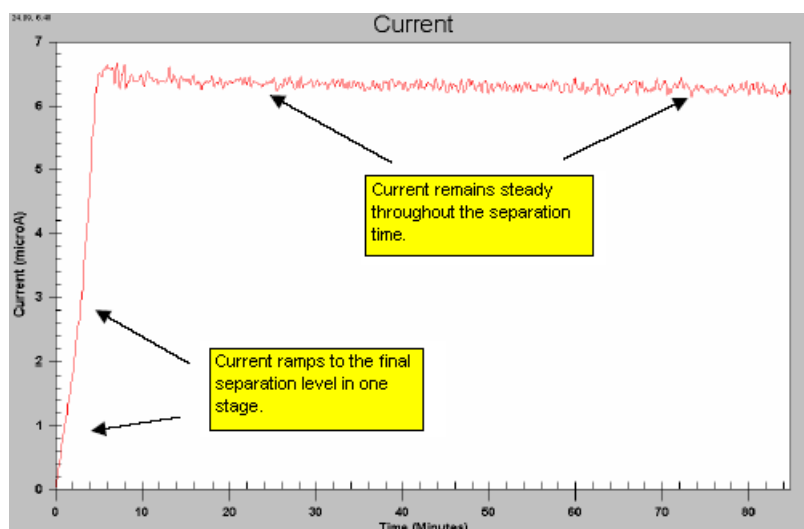
IMPORTANT:

Use non-barrier pipette tips when pipetting sequencing test sample, which contains Sample Loading Solution.

3. Put a drop of mineral oil over the sequencing test sample in each well.
4. Add separation buffer to each corresponding well of a buffer tray, filling each well about $\frac{3}{4}$ full (250 μ L).
5. Perform sample plate setup and select the **LFR-a** separation method, and modify it by increasing the pause time to 5 min.
6. Run the samples according to the standard procedures.
7. Assess the current profile of data obtained with the sequencing test sample in the Sequence Analysis module. Click **File > Open > Sample Data tab**, select sample files from the appropriate project, and then click **OK**.
8. Review the current profile.

The current should ramp in one stage and plateau at 5 μ A to 9 μ A. The current profile should look similar to the current in the example in the figure below.

Figure 3-1 Sequencing Current



Interpreting the Sequencing Test Sample Results

The sequencing test sample provides acceptable results by meeting the following criteria:

- Good raw data signal
- Normal current profile
- Meets the system specifications of 98% accuracy at 700 bases, when the LFR-1 separation method is used

If this is the case, the GenomeLab GeXP System and reagents are fine and further diagnostics of the chemistry are needed.

Note: For more information on Sequence Analysis module and troubleshooting problems with separation current, refer to the *CEQ 8000 Series Sequence Analysis Troubleshooting Guide*, which can be downloaded from sciex.com.

If the sequencing test sample does not yield an acceptable result try testing the other system consumables such as the separation gel, DNA separation capillary array, and another lot of the sequencing test sample. The individual components should be tested in separate experiments to identify the faulty component.

If an acceptable result is still not achieved after substituting all new components, then contact SCIEX Technical Support.

Testing the Chemistry

Once it is determined that the GenomeLab GeXP System is functioning properly, then proceed with troubleshooting the following components:

- GenomeLab GeXP Start Kit
- Experimental process
- Third-party reagents
- Experimental RNA
- Custom multiplex primers

Testing the GenomeLab GeXP Start Kit and Experimental Process

1. Test the components of the GenomeLab GeXP Start Kit and the user's experimental process by performing standard reactions with control reagents.
2. Perform gene expression reactions with Control RNA and a set of multiplex primers previously demonstrated to generate size-specific amplicons.
3. Analyze the reactions with the GenomeLab System software and examine the analyzed results.

Interpreting the Results

- If all of the multiplex peaks and one KAN^r peak are present, this confirms the functional quality of the GenomeLab GeXP Start Kit reagents and third-party-supplied materials. These results also confirm that the user's experimental process is satisfactory.
- These results **do not** confirm the functional quality of any custom-designed multiplex primers and RNA template. See [Testing the Experimental RNA and Custom Multiplex Primer](#) and continue in the troubleshooting process with these reagents.
- If the test does not yield all multiplex peaks and one KAN^r peak, with good raw data signal and normal current profiles, perform the test again using a new GenomeLab GeXP Start Kit.
- Failure to yield all multiplex peaks and one KAN^r peak with a second kit would indicate that third-party reagents or the experimental process are suspect. See [Examining the Experimental Process](#) and [Testing Third-Party Products](#).

Examining the Experimental Process

Review the following as part of a successful GenomeLab GeXP System experimental process:

- Reagent handling and storage
- RNA handling and storage
- Accurate pipetting
- Protocol steps

Refer to the *GenomeLab GeXP Genetic Analysis System Chemistry Protocol* for additional instructions.

Testing Third-Party Products

Some reagents and plasticware, supplied by third parties can have a negative impact on the reverse transcription (RT)-PCR reaction, the raw data signal, and the current profile. Third-party products include the chemicals and plasticware used for suspension and dilution of RNA and primers, the RT-PCR reactions, and the pre-dilution of the PCR Reaction Mix products.

Third-Party Product Dos and Don'ts

- Use high-quality, nuclease-free plasticware.
- Suspend primer multiplexes in 10 mM Tris-HCl, pH 8.0.
- Store stock solutions of RNA samples in a buffered solution, such as THE RNA Storage Solution.
- Store stock solutions of RNA in small aliquots at $-80\text{ }^{\circ}\text{C}$ to preserve RNA integrity.
- Suspend working concentrations of RNA in nuclease-free, non-DEPC treated water, as supplied in the GenomeLab GeXP Start Kit or from Affymetrix or Thermo Fisher Scientific.

Note: Do not use DEPC-treated water or plasticware with the GenomeLab GeXP System process. Residual DEPC can inhibit the reaction and result in low signal strength and a high baseline in the electropherogram.

- Use aerosol-resistant barrier pipette tips with a dedicated set of pipettes for the setup of RT and PCR reactions to prevent cross-contamination of samples.
- Do not allow amplified product to enter the area of RT and reactions setup (amplicon-free zone).
- Use **non-barrier** pipette tips and a separate set of pipettes when handling the PCR products, Sample Loading Solution and DNA Size Standard in a PCR amplicon zone. The Sample Loading Solution reagent may dislodge filter particles of aerosol-resistant barrier tip into the sample and this contamination can cause current failure in the GenomeLab GeXP System.
- Perform pre-dilution of the reactions with 10 mM Tris-HCl, pH 8.0.

Interpreting the Third-Party Products Results

The common symptoms for poor quality reagents or the incorrect reagent concentration are low raw data signal, high baseline, and erratic current profiles.

- If the Control RNA reactions (as outlined in [Testing the GenomeLab GeXP Start Kit and Experimental Process](#)) yield all the multiplex peaks and one KAN^r peak and the raw data signal baseline and current profiles look good, then third-party reagents are satisfactory.
- If the control multiplex reactions did not yield all of the multiplex peaks or the raw data or current profile were abnormal, then replace each third-party reagent one at a time to identify the faulty reagent. Perform the control reactions as described in [Testing the GenomeLab GeXP Start Kit and Experimental Process](#), and analyze them with the GenomeLab System software.
- If the experimental RNA and custom multiplex primers still do not yield acceptable results, and a specific third-party product was not identified as the cause, proceed to the next section.

Testing the Experimental RNA and Custom Multiplex Primer

After confirming that the Control RNA functions correctly with the GenomeLab GeXP System, test the experimental RNA and custom multiplex primers. Test the reagents independently. This is the most efficient means of identifying the source of the problem.

Testing the Experimental RNA

Note: Use high-quality experimental RNA that has a 28S/18S ratio greater than 1.0 with the GenomeLab GeXP System. Confirm that the ribosomal RNA 28S and 18S bands are readily visible and predominant when analyzed on an agarose gel.

Use a set of control multiplex primers that are known to produce size-specific amplicons from the source of experimental RNA in order to test the functional integrity of the experimental RNA.

See [Interpreting the Results](#) for more information.

Testing the Custom Multiplex Primers

Note: Order custom multiplex primers with the universal tag sequences fused to the gene-specific sequence. The oligonucleotides should be of standard desalted, deprotected processing.

During the initial evaluation of the multiplex, the chimeric primers must be evaluated for their ability to produce the expected size amplicon with Control RNA (RNA template known to contain the target transcript).

Note: While the Human Reference Control RNA provides broad gene coverage, not all the gene transcripts are necessarily present. It is highly advisable to have a thorough understanding of the gene expression patterns for the transcripts detected by the custom multiplex primers.

- Develop a custom Control RNA by mixing RNA from several sources to achieve full transcript representation. Generally, this mixture will contain 50% of normal and 50% treated RNA.
-

Note: If more than one treatment is being studied, combine the treatments so that each one is equally represented in the 50% treated RNA.

- Evaluate reverse primers in a multiplex context.
- Evaluate forward primers in both multiplex and singlet reactions. Refer to the *GenomeLab GeXP Genetic Analysis System Chemistry Protocol* for more information.

Interpreting the Results

- If all of the multiplex peaks and one KAN^r peak are present, with no significant undesigned peaks (UDPs), the functional quality of the experimental RNA and custom multiplex primers are confirmed. See [Reference Tables](#) for more information on UDPs.
- If a reaction containing experimental RNA and control multiplex primers yields a KAN^r peak, but no multiplex peaks or multiplex peaks with low signal, then it is likely that the quality of the RNA is poor. Re-evaluate the RNA source or purification process to yield higher quality RNA.
- If a reaction containing custom multiplex primers and Control RNA yields a KAN^r peak, but is missing one or more peaks in the multiplex, then it is likely that redesign is required for the primer(s) of the missing peak(s). See [Multiplex Primer Design](#) for more information on troubleshooting this issue.
- If a reaction containing custom multiplex primers and Control RNA yields a KAN^r peak and all the multiplex peaks, but has significant UDPs, then primer redesign is necessary. See [Multiplex Primer Design](#) for more information on troubleshooting this issue.
- If no reaction yields a KAN^r peak (positive control), then see [Testing the GenomeLab GeXP Start Kit and Experimental Process](#) to re-evaluate the start kit components and experimental process.

By using the previously described process, you should have been able to identify many of the most common causes of problems associated with instrumentation and chemistry in capillary electrophoresis-based gene expression profiling.


Refer to [Reference Tables](#) or [Questions and Answers](#) for additional causes and corrective actions for troubleshooting purposes.

The following tables list issues that might be encountered while analyzing multiplex reactions with the GenomeLab GeXP System.

- [Multiplex Primer Design](#)
- [Gene Expression Chemistry](#)
- [Separation and Fragment Analysis](#)

For specific solutions or examples, look for references to sample figures in [Reference Figures](#).

Multiplex Primer Design

Issue	Cause	Solution
Having difficulties connecting to NCBI Primer-BLAST site.	Internet connection is down.	Work with your local IT to restore the internet connection.
	The NCBI website is offline for maintenance (rarely occurs).	If you can connect to other website but not the NCBI Primer-BLAST site, report the issue by sending an email to blast-help@ncbi.nlm.nih.gov . To use another primer design tool when you cannot access the NCBI Primer-BLAST site, you may use Primer3 at primer3.ut.ee . Please note that primers designed with Primer3 need to be evaluated using a BLAST search to ensure there is no SNP sites in primer region and there is no low-complexity sequences in the amplicon.
The help file content for each search parameter was not shown when the Help button  is clicked. In addition, the Advanced parameters are not available.	The web browser version is too old.	Use a more recent version of the browser or use a different browser. Use browsers that do automatic updates.

Issue	Cause	Solution
No results for primer searches when using the default values of 1,000 to 1,000,000 for Intron length range .	The intron size in the target gene is less than 1,000.	Reduce the minimum intron length based on the sizes of the introns in the target gene.
I used to see exon numbers in the graphical view of primer pairs when I designed primers using NCBI Primer-BLAST. I no longer see the exon numbers. The exons are still shown.	NCBI made some policy changes. Exon numbers are no longer provided in RefSeq transcript records. For details, refer to the article published on <i>Nucleic Acids Research</i> , 2014, Volume 42, Database issue, D756-D763.	Instead of using exon numbers, describe an exon by referencing its location in the genomic sequence.
Multiple peaks for one set of primers in reverse singlet + forward singlet reaction	mRNA isoforms or homologous sequence	<p>The gene sequence used for primer design may have led to amplification of alternative transcripts (isoforms) or a homologous sequence in another gene.</p> <ul style="list-style-type: none"> • Be sure to choose mRNA or cDNA, and not genomic DNA sequence files for primer design. • BLAST designed primer sequences to determine if more than one product will be amplified. • Use sequence alignment software to find a region of the gene that does not share homology with any other gene. Specifically target that region in the Primer Design option. • For transcript variants, design primers to bridge a specific exon-exon junction such that a specific transcript is amplified.

Reference Tables

Issue	Cause	Solution
An undesigned peak (UDP) is present in singlet or multiplex reactions	Non-specific amplification	<p>If a UDP migrates within 3 nt of a designed peak in the multiplex or affects the calculation of a designed gene peak area, then perform singlet reactions to identify the primer(s) causing the UDP. Refer to Undesigned Peak (UDP).</p> <p>Redesign the offending primer by targeting a different sequence or move the affected amplicon(s) to a different location in plex (different size), away from the UDP.</p>
One or more designed gene peaks is absent from multiplex profile but the KAN ^r peak is present	Poor primer design	<p>Perform singlet reactions to determine if each set of primers amplifies the expected fragment size.</p> <p>Redesign primers for those genes that do not yield an acceptable singlet profile.</p>
	RNA sample does not contain the transcript	Use an RNA sample that contains the transcript.
A gene peak disappears from the multiplex profile when it is expected to be present in the RNA sample	Poor primer design	<p>If the peak is present in some wells, but not others prepared from the same RNA sample and Master Mix reagents (RT, PCR), then the primer may not have good binding specificity. Polymorphism(s) in the primer sequence, especially at the 3' end, may result in amplification from one sample RNA but not another.</p> <p>Redesign the primer set to a better region of the gene (not too close to either the 5' or 3' end of the transcript, no repeat sequences, no GC rich regions, no homologous regions, no polymorphisms).</p>
Low or no signal for a particular peak in a multiplex reaction with RNA expected to contain the transcript, using primers that generate a peak in a singlet reaction.	Primers for high expressing genes need attenuation or Primer interference	<p>Reduce the concentration of reverse primers for the high expressing genes.</p> <p>It is most efficient to redesign the affected primer(s).</p> <p>However, if it is necessary to determine which primer is the interfering primer, perform duplex reactions containing the affected primer with each of the other primer sets in the multiplex, then redesign the interfering primer.</p>

Issue	Cause	Solution
A particular amplicon has two or more shoulders or stutter peaks.	Repeats in amplicon sequence cause polymerase slippage Refer to Figure A-2 .	Check the designed amplicon for repeat sequences. Redesign the primers to a region of the gene that does not contain repeat sequences.
Significantly higher %CV in biological replicates compared to technical replicates	There is a SNP in the 3' end of primer sequence	Check the primers for single nucleotide polymorphisms (SNPs). Redesign the primer if any SNPs are present. SNPs can have profound impact on the priming efficiency depending on how much of the instability is introduced. A biological sample with the perfect match to the primers will generate a higher signal than a sample with a mismatch due to the presence of SNPs. This will result in high %CV in biological replicates, but not technical replicates. Biological replicates generally have slightly higher %CV than technical replicates, due to the natural variation between organisms or the variation that was introduced during sample preparation.

Gene Expression Chemistry

Issue	Cause	Solution
A variation in multiplex raw data is seen from build-to-build of custom primer plexes for the same RNA sample	The multiplex formulation has changed	Once optimized, do not change the multiplex formulation (primer concentrations). Carefully assemble each multiplex build for consistency. Remake the multiplex consistent with the optimized formula. Make sure to use the same primer plex formulation throughout a study.
Multiple designed gene peaks are present in reverse multiplex + forward singlet reactions	Primer contamination	The reverse primer stocks or reverse primer multiplex is contaminated with forward primers. Alternatively, forward primer stocks are cross-contaminated with other forward primers. <ul style="list-style-type: none"> Decontaminate the lab bench, pipettes, and other labware with a nucleic acid-destroying solution. Use fresh reagents and remake primer stocks and/or reverse multiplex. Use aerosol-barrier filter tips for making multiplexes and assembling RT and PCR reactions.
Low signal	Primer design	Refer to Multiplex Primer Design .
	DEPC interference	IMPORTANT: Do not use DEPC-treated water as residual DEPC can interfere with PCR amplification. Use Nuclease-free water (Affymetrix 71786) or (Thermo Fisher Scientific 10977-015) when making resuspension buffer (10 mM Tris-HCl, pH 8.0, DNase/RNase-free distilled water) for primer plexes.
	Primer degradation	Use resuspension buffer (10 mM Tris-HCl, pH 8.0, DNase/RNase-free distilled water) for making primer plexes. Store multiplex primers at -20°C .

Issue	Cause	Solution
Low signal (continued)	Primer quality	Primers can contain residual amounts of organic solvent. Order new primers from well-respected oligonucleotide vendors, such as Integrated DNA Technologies.
	Reagents expired and/or improper storage/handling	<ul style="list-style-type: none"> • Check reagent storage conditions and expiration dates. All kit components, except RNA, should be stored at -20°C. • Avoid excessive freeze-thaw cycles. Control RNA and KAN^r RNA should be aliquoted into single use volumes after the first thaw and always stored at -80°C. • Use fresh reagents that have been properly stored. <p>Vortex the RT Buffer 5× containing DTT and the 25 mM MgCl₂ to dissolve any precipitant before use.</p>
	RNA template - quality, quantity	<p>Verify that the RNA is of high quality and adequate quantity. Ribosomal RNA 28S and 18S bands should be prominent on agarose gel and 28S/18S ratio > 1.0.</p> <p>Recheck calculations for amount of input total RNA (25 ng to 100 ng is recommended). Increase the amount of RNA template used.</p>
	Thermal cycler	<p>Check thermal cycler calibration and protocol cycling temperatures.</p> <p>Increase the PCR extension temperature to 70°C to overcome variation in thermal cycler wells.</p> <p>Use a thermal cycler with a heated lid to prevent evaporation and verify that the lid temperature is the same temperature as the incubation chamber.</p>
	Sample degradation or photobleaching occurred	<ul style="list-style-type: none"> • RT reactions can be stored at -20°C for up to one month.

Reference Tables

Issue	Cause	Solution
Low signal (continued)	Sample degradation or photobleaching occurred (continued)	<ul style="list-style-type: none"> Do not expose 5× PCR buffer or PCR reactions to light for an extended period of time. Store PCR reactions at –20°C in the dark (wrapped in foil) for up to one month. Diluted PCR sample may degrade faster in unbuffered water. It is recommended to use fresh 10 mM Tris-HCl, pH 8.0 for pre-dilutions of PCR reactions.
	Capillary electrophoresis	Refer to Separation and Fragment Analysis .
High signal (peak height is greater than 120,000 RFU in analyzed data for one or more fragments)	Reverse primer concentration is too high Refer to Over-range Signal in an Unbalanced Profile .	Attenuate the reverse primer(s) by reducing the concentration in the reverse multiplex
	Too much RNA	Reduce the amount of RNA.
	Too much PCR product loaded	Refer to Separation and Fragment Analysis .
One or more designed gene peaks are absent from multiplex profile but the KAN ^r peak is present	Poor primer design	Refer to Multiplex Primer Design .
	RNA	<p>The RNA sample may not contain the transcript(s) or the gene is down-regulated in that sample.</p> <ul style="list-style-type: none"> Use more RNA in the RT reaction (up to 100 ng per reaction). Use an RNA source known to contain the gene transcript of interest to validate the primer design. Combine RNA samples/sources so that all genes in multiplex are represented at a relatively moderate detectable level and use this RNA as a positive control for gene detection and multiplex optimization.

Issue	Cause	Solution
One or more designed gene peaks are absent from multiplex profile but the KAN ^r peak is present (continued)	RNA (continued)	RNA may be degraded. Verify the RNA quality (Ribosomal RNA 28S/18S ratio should be > 1.0). Store stock RNA samples in THE RNA Storage Solution at –80 °C. Aliquot to working volumes, and thaw aliquots only once to ensure RNA integrity.
	Primer concentration	Increase reverse primer up to 150 nM per reaction (1.5 µM in the reverse multiplex) for each low expresser. Additionally, decreasing the high signal of high expressers through attenuation may bring up the signal of low expressers.
Peaks are present in the RT minus reactions.	Genomic DNA contamination Refer to Genomic DNA Contamination .	Treat RNA with RNase-free DNase during RNA purification.
	Amplification contaminated with another DNA template or DNA amplicons	<ul style="list-style-type: none"> • Use aerosol-resistant barrier tips during RT and PCR reaction preparation to minimize contamination from external sources. • Use fresh tips for each step in preparing the reactions. • Use separate pre- and post-PCR work areas. • Do not bring amplified product into pre-PCR area.
	Well-to-well contamination	Contamination across wells can occur during set up for PCR or separation, especially with single channel pipetting. Use a multichannel pipette to reduce pipetting error and contamination.
Low or no signal for a particular peak in a multiplex reaction using primers that were validated in a singlet reaction	Primer interference	Refer to Multiplex Primer Design .

Reference Tables

Issue	Cause	Solution
A UDP is present in multiplex reactions	Non-specific amplification	Refer to Multiplex Primer Design .
Sloping gene expression profile or Smaller fragments have much higher signal than larger fragments but size standard peaks look normal.	Signal drop-off Refer to Signal Drop-Off .	<p>Thermal cycler temperature fluctuation during PCR extension leads to a disproportionate amplification of shorter fragments over long fragments, called signal drop-off. This commonly occurs in the edge wells of a thermal cycler that is not performing well.</p> <ul style="list-style-type: none">• Repair and/or calibrate the thermal cycler.• Use the center wells of the thermal cycler.• Alternatively, optimize the extension temperature; generally by raising the extension temperature by a degree or two. <p>IMPORTANT: Signal drop-off can lead to high %CV in analyzed data. Do not use results of these wells for data analysis.</p>

Issue	Cause	Solution
<p>Sloping gene expression profile (continued)</p> <p>or</p> <p>Smaller fragments have much higher signal than larger fragments but size standard peaks look normal.</p>	<p>Signal drop-off</p> <p>Refer to Signal Drop-Off.</p>	<p>The incorrect DNA polymerase was used. SCIEX has validated Thermo-Start <i>Taq</i> DNA Polymerase for use with the system.</p>
<p>Unbalanced profile</p> <p>or</p> <p>Reference (housekeeping) genes are not generating the expected high peaks relative to other genes in the plex.</p>	<p>Multiplex is not optimized</p> <p>Refer to Over-range Signal in an Unbalanced Profile.</p>	<p>Attenuate the concentration of reverse primers for high signal peaks and high expressers. The purpose of attenuation is to bring down the peak height of high expressers to the level of moderate expressers in the same plex. It is an approach that balances relative signal strength in the optimal detection range.</p> <ul style="list-style-type: none"> • Select a moderate expresser peak in a moderate signal range (10,000 RFU to 50,000 RFU) as reference upon which the optimal attenuation factor for high expressers will be decided. Then bring down the high expresser peaks to the level of the moderate expresser reference peak by reducing reverse primer concentration. It is not necessary, nor correct, to consider absolute peak height for this approach. • Increase the concentration of reverse primers for low expressers up to 150 nM per reaction to increase signal strength relative to the moderate expresser gene(s).

Separation and Fragment Analysis

Issue	Cause	Solution
Current failure	Air bubble in the manifold	<p>Perform extensive purging of the system with fresh separation gel before each run. Perform a Manifold Purge three times with 0.4 mL gel. Then perform three capillary fills.</p> <p>Run the PCR samples again. Add the recommended amount of separation buffer (250 µL) to each well of the buffer plate that corresponds to a filled well of the sample plate. Keep the buffer evaporation cover over the buffer plate when installed on the GenomeLab GeXP System.</p> <p>Use fresh 10 mM Tris-HCl, pH 8.0 to pre-dilute PCR reactions, if necessary, before adding the diluted sample to Sample Loading Solution with DNA Size Standard.</p>
	No separation buffer or excessive separation buffer	
	Excessive amount of salt in sample	
Low signal or no signal	No separation buffer or excessive separation buffer	<p>Run the PCR samples again. Add the recommended amount of separation buffer (250 µL) to each well of the buffer plate that corresponds to a filled well of the sample plate. Keep the buffer evaporation cover over the buffer plate when installed on the GenomeLab GeXP System.</p>
	Poor injection due to excess salt	<p>Too much salt in a sample can lead to poor injection of amplicons which leads to low signal.</p> <p>Use fresh 10 mM Tris-HCl, pH 8.0 to pre-dilute PCR reactions, if necessary, before adding the diluted sample to Sample Loading Solution with DNA Size Standard 400.</p>
	Chemistry	Refer to Gene Expression Chemistry .

Issue	Cause	Solution
High signal for one or more fragments (peak height is >120,000 RFU in analyzed data)	Too much RNA	Reduce the amount of RNA used in the RT reaction.
	Too much PCR sample was loaded Refer to Figure A-5 .	Prepare serial dilutions of the PCR products (1:5, 1:10, 1:20) in 10 mM Tris-HCl, pH 8.0. Mix 1 µL of the pre-diluted sample to Sample Loading Solution with Size Standard 400 and test each dilution in all capillaries to find the best dilution for analysis. The linear range of detection lies between 370 RFU to 120,000 RFU in analyzed data. It is recommended that data outside this range not be used for analysis.
Additional peaks from different dye channels are present in the data	Too much PCR sample was loaded Refer to Figure A-8 .	If the peak height (signal) is too high (>120,000 RFU), a small peak may be observed in an alternate dye channel either directly adjacent to or underneath the over-ranged peak. Decrease the amount of sample loaded on the GenomeLab GeXP System or pre-dilute the PCR product in 10 mM Tris-HCl, pH 8.0.
Unresolved fragment in a particular size range is present in standard (STD) and no template control (NTC) reactions.	Protein-nucleic acid complex Refer to Figure A-9 .	A non-specific, protein-nucleic acid complex will migrate in a particular size region of electropherogram. This complex is of consistent size, specific to a multiplex and does not affect analysis unless it overlaps with a designed peak. Redesign the primers of any gene peaks that comigrate with the complex.
Valid peaks were not analyzed or called in the Fragment Analysis module	Analysis parameters	Incorrect analysis parameters can lead to poor analysis. Use the default analysis parameters: Slope Threshold = 10%, Relative Peak Height Threshold = 1%, Confidence level = 95%, SizeStd 400, Cubic Model, Dye Mobility Calibration ON = PA ver.1, Calculated Dye Spectra. The Slope and Peak Height Thresholds can be reduced to pick up small peaks.
	Exclusion filter	An exclusion filter in the Fragment List tab of Fragment Analysis module may be active.

Reference Tables

Issue	Cause	Solution
Size shift in designed gene peaks and/or KAN ^r peak	Analysis parameters	Use the default analysis parameters: Slope Threshold = 10%, Relative Peak Height Threshold = 1%, Confidence level = 95%, SizeStd 400, Cubic Model, Dye Mobility Calibration ON = PA ver.1, Calculated Dye Spectra.
	Incorrect DNA Size Standard	Verify which Size Standard chemistry was used. The default analysis parameters are designed to be used with the DNA Size Standard 400. If the DNA Size Standard 600 was used, the fragment sizing will be different for samples analyzed with default analysis parameters. Edit the analysis parameters so that DNA Size Standard 600 and Quartic model are selected and then reanalyze the data. Save this revised analysis parameters with a new name (such as GeXPss600).
The peak for a low expresser or uninduced gene was not detected.	Analysis parameters Refer to Figure A-10 and Figure A-11 .	When the analysis parameters are lowered to Slope Threshold = 1 and Peak Height Threshold = 0, nearly every peak, can be detected. If a very small peak (~370 RFU) with the exact same fragment size as the induced gene is detected, it can be treated as real peak in the un-induced sample. It is best if this very small, un-induced peak resides in an area with a clean baseline (no UDPs or excessive noise) of the multiplex profile so the peak call is accurate. Refer to Figure A-11 .
Too many small UDPs called	Analysis parameters	Set up and apply locus tag and allele IDs through binning and then apply an exclusion filter in Fragment List view: allele ID = (empty space or blank). Alternatively, exclude small, insignificant peaks by establishing an exclusion filter in the Fragment List of the Fragment Analysis module so that the peaks below a particular peak height will not be called.

Issue	Cause	Solution
Analysis error	Over-range data	<p>The data is above the linear range of detection of the GenomeLab GeXP System and the software cannot analyze the data properly.</p> <p>Any over-range data should not be used for expression analysis.</p> <ul style="list-style-type: none"> • Dilute PCR samples in 10 mM Tris-HCl, pH 8.0 to bring data into the linear range (less than 120,000 RFU in analyzed data). • Attenuate high expressers if necessary to balance the profile.
Split peaks with extremely high signals in analyzed data	Over-range data Refer to Split Peaks .	<p>Check the raw data to confirm the results. Do not use over-range data for expression analysis.</p> <p>Dilute the PCR sample in 10 mM Tris-HCl, pH 8.0 to bring the data in the linear range of detection (less than 120,000 RFU in analyzed data).</p> <p>Attenuate the high expressers if necessary to balance the profile.</p>
Inconsistent fragment sizes or unusual peak shapes or low signal	Gel life exceeded	Check the on-board gel life. Gel life is verified for 72 hours on the GenomeLab GeXP System.
	DNA separation capillary array life exceeded	Check the DNA separation capillary array life. DNA separation capillary array life is verified for 100 runs or 30 days on the GenomeLab GeXP System, whichever comes first.
	Failure to maintain proper capillary separation temperature	<p>Check the run log for verification of separation temperature.</p> <p>Run the samples again. If the problem continues, contact SCIEX Technical Support.</p>

Gene Expression Analysis

Issue	Cause	Solution
High %CV	Sample size	<p>Perform at least three technical replicates for each RNA sample.</p> <p>Generally, more technical replicates of the same RNA sample or RT reaction reduces the %CV.</p>
	Sample type	A small number of biological replicates can lead to a high %CV due to inherent diversity between organisms. Low expresser genes tend to have higher %CV.
	Reference gene	<p>Review the choice of reference gene to verify that relative expression of this gene is constant across all samples.</p> <p>If the reference gene expression fluctuates greatly between samples, this could influence the %CV. Choose a reference gene that has constant expression under all conditions that will be examined in the study.</p> <hr/> <p>Note: Normally, the KAN^r peak signal is usually out of range and should therefore not be used as a reference gene for normalization of experimental genes. If it is desired to use KAN^r as a reference gene for normalization, the amount of KAN^r RNA needs to be reduced so that the peak height of KAN^r is similar to that of the median-expressers in the multiplex.</p> <hr/>
	Signal drop-off	Refer to Gene Expression Chemistry .

Issue	Cause	Solution
High %CV (continued)	SNPs in the primer(s)	Refer to Multiplex Primer Design .
Third-party software for gene expression analysis	Data analysis	<p>The following analyses can be performed on data from the GenomeLab GeXP System using Microsoft Excel:</p> <ul style="list-style-type: none">• Multiple reference gene normalization• Calculation of fold-change• Statistical analysis <p>Additional software programs that can be used are:</p> <ul style="list-style-type: none">• geNorm at https://genorm.cmgg.be• DecisionSite at www.spotfire.com• PartekGS at www.partek.com• Prism at www.graphpad.com/prism/Prism.htm

Multiplex Primer Design

Question	Answer
What are the optimal conditions to use when I manually design or redesign primers for use with the GenomeLab GeXP System?	<p>Although these are the optimal conditions for primers, individual primers within a multiplex will vary.</p> <ul style="list-style-type: none">• Approximately 20 nt in length, without universal tag• 50% G+C content• $T_m = 60\text{ }^\circ\text{C}$ (Range: $57\text{ }^\circ\text{C}$ to $63\text{ }^\circ\text{C}$)• Ensure that the Max T_m difference between the forward and the reverse primers is no more than $5\text{ }^\circ\text{C}$• The last 5 nucleotides at 3' end should contain at least 2 As or Ts and not contain any polymorphisms <p>The primer length can vary from 20 nt as long as it meets the other conditions.</p>
Can I design primers to detect alternative transcripts?	<p>Yes.</p> <p>First, define the mRNA isoform(s) of interest and design primers specifically to include or exclude exons or to bridge unique exon-exon junctions.</p>

GenomeLab GeXP System Chemistry and Fragment Analysis

Question	Answer
<p>What is the difference between the pre- and post- PCR areas?</p>	<p>The pre-PCR area is used to set up both the RT and PCR reactions. The post-PCR area is where the PCR reaction goes after amplification.</p> <p>The pre-PCR area is divided into two zones:</p> <ul style="list-style-type: none"> • No Template Zone: Assemble and aliquot the master mixes • Template Addition Zone: Add RNA for the RT reaction or add cDNA to the PCR reaction <p>IMPORTANT: Amplified (PCR) product should never be brought into the pre-PCR area.</p> <p>Use the thermal cycler for PCR in the post-PCR area. Any area that is exposed to amplified PCR product should be considered as an area that contains amplified template in the environment.</p>
<p>What can happen if amplified PCR product is brought into the pre-PCR area?</p>	<p>One symptom of PCR product contamination of the pre-PCR area is peaks in the RT minus and No Template Control reactions. The contamination of RT-PCR sample wells with exogenous template will compromise the relative quantitation.</p> <p>To clean up a pre-PCR area contaminated with amplified product or other nucleic acid template, use a 5% bleach solution or commercially available decontaminant such as DNA Zap (Thermo Fisher Scientific) or DNA AWAY (Molecular BioProducts), to wipe down all surfaces and equipment.</p>

Questions and Answers

Question	Answer
What RNA should I use to evaluate and optimize my custom multiplex?	<p>Initially test the multiplex on a Control RNA that consists of a mixture of the RNA samples that will eventually be tested individually with the multiplex (e.g. untreated + treated or normal + disease). All the gene transcripts must be present in the Control RNA for initial evaluation and validation of multiplex primers. For optimization of the multiplex, ratios of each RNA sample in the Control RNA should reflect a moderate level of expression for most individual genes.</p> <hr/> <p>Note: Levels of expression are relative for each gene.</p> <hr/>
Is a UDP in singlet reactions cause for concern?	<p>If a UDP:</p> <ul style="list-style-type: none">• migrates at the same size as a designed peak in the multiplex• affects the quantitation of a designed gene peak <p>then redesign the primers that cause this UDP.</p> <p>It is best to assess the significance of a UDP in a singlet reaction when the peak height of the designed peak is less than 120,000 RFU.</p>
What do I do if a UDP co-migrates with a designed peak in a multiplex?	<p>If it is possible to determine which primer is causing the UDP from singlet reactions, then redesign this primer by targeting a different sequence. Alternatively, the designed peak can be moved to a different location in the plex by redesigning the primers. This may simply involve moving the primer position a few nucleotides in one direction or the other to shift the designed peak away from the UDP.</p>

Question	Answer
<p>What is attenuation and how will it affect my results?</p>	<p>Attenuation is the process by which the reverse primer concentration of high expressers is reduced. This brings the high expresser gene signals into range of the moderate expressers.</p> <p>Attenuation is used to balance the signal of the gene expression profile within the linear range of detection. The GenomeLab GeXP System has a lower and upper limit in its range of detection (370 RFU to 120,000 RFU in the analyzed data). During multiplex optimization, it is important to ensure that all peaks fall within that limit, ideally within a range between 2,000 RFU to 50,000 RFU.</p> <p>Attenuation performed during the optimization of a particular multiplex has no effect on results, because this optimization is carried out on Control RNA. The goal is to establish the baseline levels of expression and gene specific reverse primer concentration with Control RNA before testing other RNAs. Attenuation affects only the detection of mRNA of the particular transcript. This is usually a high expresser, such as a housekeeping gene that needs to be brought into the linear range of detection. The concentration of reverse gene-specific primers are changed only during the plex optimization stage. Once concentrations for all primers in a reverse multiplex have been optimized, the primer concentrations remain fixed for all subsequent experiments. Thus, the relative quantitation of gene expression for any particular gene will remain constant in a particular sample. Further the fold change in expression for this gene between treatments is always calculated using the relative quantitation.</p> <p>For more information on attenuation, refer to Applications Information Bulletin A- 2049A <i>Multiplexed, Quantitative Gene Expression Analysis for Lettuce Seed Germination on GenomeLab GeXP Genetic Analysis System</i> available on the SCIEX web site.</p>

Questions and Answers

Question	Answer
What is the best way to detect the peak area of an uninduced or low expresser gene after primer optimization?	<p>To detect the peak area of an uninduced/low expresser gene:</p> <p>Change the analysis parameters in the Fragment Analysis module to Slope Threshold = 1 and Peak Height Threshold = 0, and reanalyze. With these settings, nearly every peak, can be detected. If a very small peak (~370 RFU) with the exact same fragment size as the induced gene is detected, it can be treated as real peak in the uninduced sample.</p> <p>For the most accurate peak call, it is best if this very small, uninduced peak resides in an area with a clean baseline (no UDPs or excessive noise) of the multiplex profile.</p>
What is the best way to detect a large fold induction?	<p>Induced genes will usually have a relatively low signal (small peak) in the uninduced or basal state and high signal (tall peaks) in the induced state. Attenuate the reverse primer concentration and pre-dilute the PCR products such that uninduced samples generate a very small, yet detectable peak and induced samples generate a peak within linear range of detection (370 RFU to 120,000 RFU in analyzed data).</p> <p>Separate the PCR reactions from uninduced RNA samples in middle CEQ capillaries (C, D, E, F) and reactions from induced samples in the outer capillaries (A, B, G, H). Detection of an approximately 1,000-fold change in expression can be achieved with this method.</p>

Question	Answer
What is the minimum and maximum relative limit in fluorescence units (RFU) for a designed peak to remain in the linear range of detection?	<p>370 RFU to 120,000 RFU in the analyzed data. This is the recommended signal range for quantitation with the GenomeLab GeXP System.</p> <p>In order to detect variations in gene expression from sample to sample, design gene peaks within the range of approximately 2,000 RFU to 50,000 RFU, during multiplex optimization.</p>
Can I use the KAN ^r peak as a reference gene for normalization?	<p>Yes, under certain conditions.</p> <p>KAN^r RNA is an independent template that is designed to serve as a positive, internal control for the RT and PCR reactions.</p> <p>Normally, the KAN^r peak signal is out of range and should not be used as a reference gene for normalization of experimental genes. If it is desired to use KAN^r as a reference gene for normalization, the amount of KAN^r RNA needs to be reduced so that the peak height of KAN^r is similar to that of the median-expressers in the multiplex.</p>

Gene Expression Analysis

Question	Answer
Are there any third-party software programs that I can use with data from the GenomeLab GeXP System for gene expression analysis?	<p>Yes.</p> <p>The following analyses can be performed on data from the GenomeLab GeXP System using Microsoft Excel:</p> <ul style="list-style-type: none"> • Multiple reference gene normalization • Calculation of fold-change • Statistical analysis <p>Additional software programs that can be used are:</p> <ul style="list-style-type: none"> • geNorm at https://genorm.cmgg.be • DecisionSite at www.spotfire.com • PartekGS at www.partek.com • Prism at www.graphpad.com/prism/Prism.htm

Reference Figures

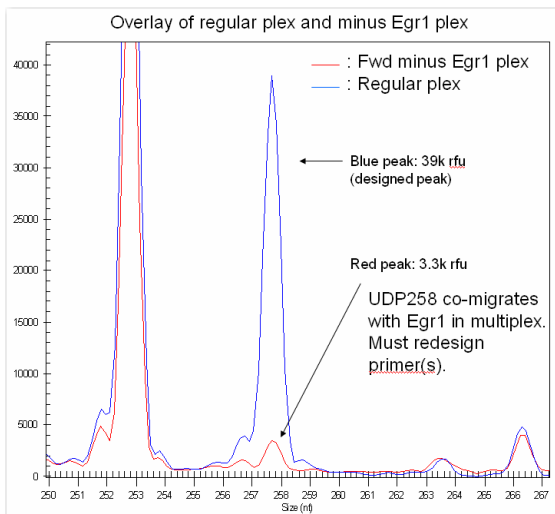
A

The following images are for use with the [Reference Tables](#) and [Questions and Answers](#) of this guide.

Undesigned Peak (UDP)

A UDP that co-migrates with a designed peak will affect quantitation of the designed peak.

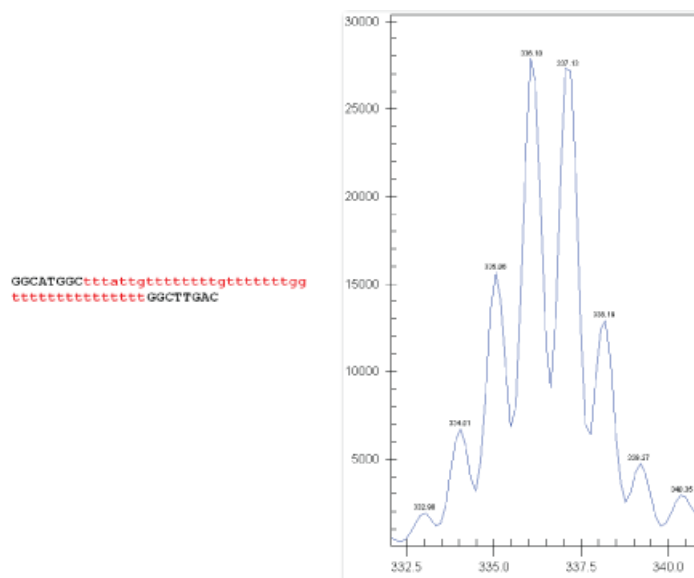
Figure A-1 Co-migrating UDP



Repeat Sequences

Repeats in the amplicon sequence can cause DNA polymerase slippage resulting in stutter peaks.

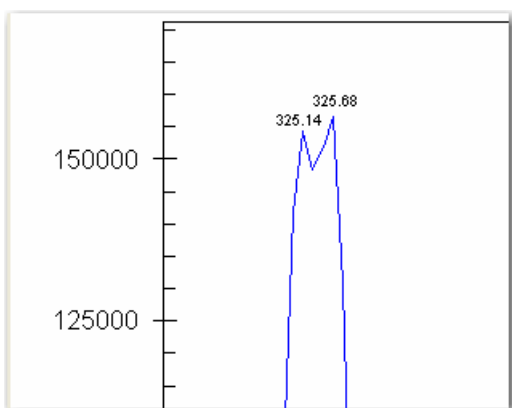
Figure A-2 Repeat Sequence and Resulting Stutter Peaks



Split Peaks

Peaks that are significantly over-range tend to split and be called as two peaks in the analyzed data.

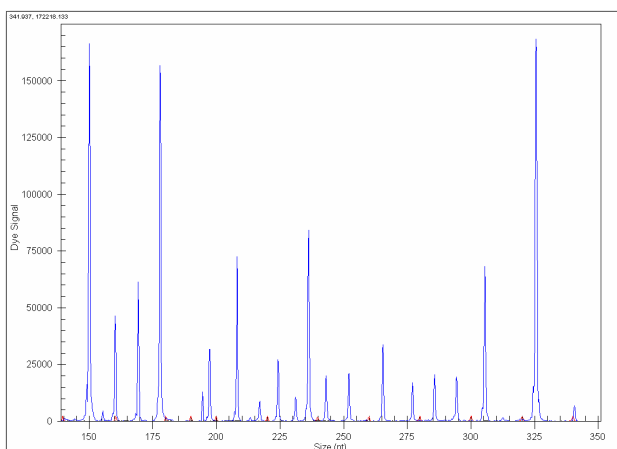
Figure A-3 Split Peak



Over-range Signal in an Unbalanced Profile

A balanced multiplex profile is achieved by attenuating the reverse primer concentration of the high expressers. See [Over-range Signal in a Balanced Profile](#) for a balanced profile.

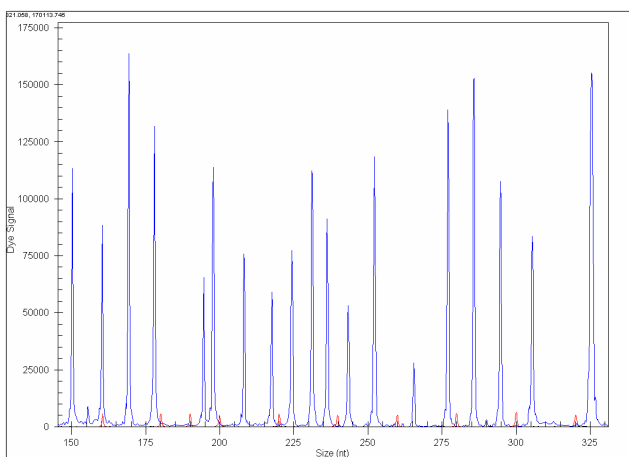
Figure A-4 Unbalanced Profile - Over-range



Over-range Signal in a Balanced Profile

Any signal greater than 120,000 RFU in analyzed data is outside the linear range of detection with standard data analysis. Pre-dilute the PCR product to bring all fragments in the linear range.

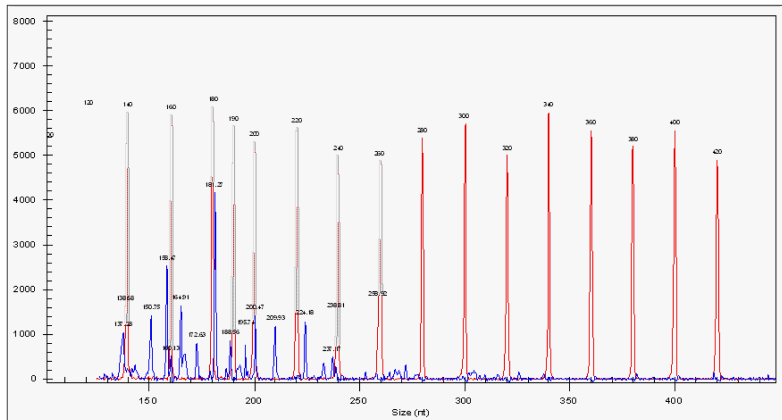
Figure A-5 Balanced Profile - Over-range



Genomic DNA Contamination

Genomic DNA contamination of RNA samples leads to the production of noise peaks in the RT Minus Reaction Mix.

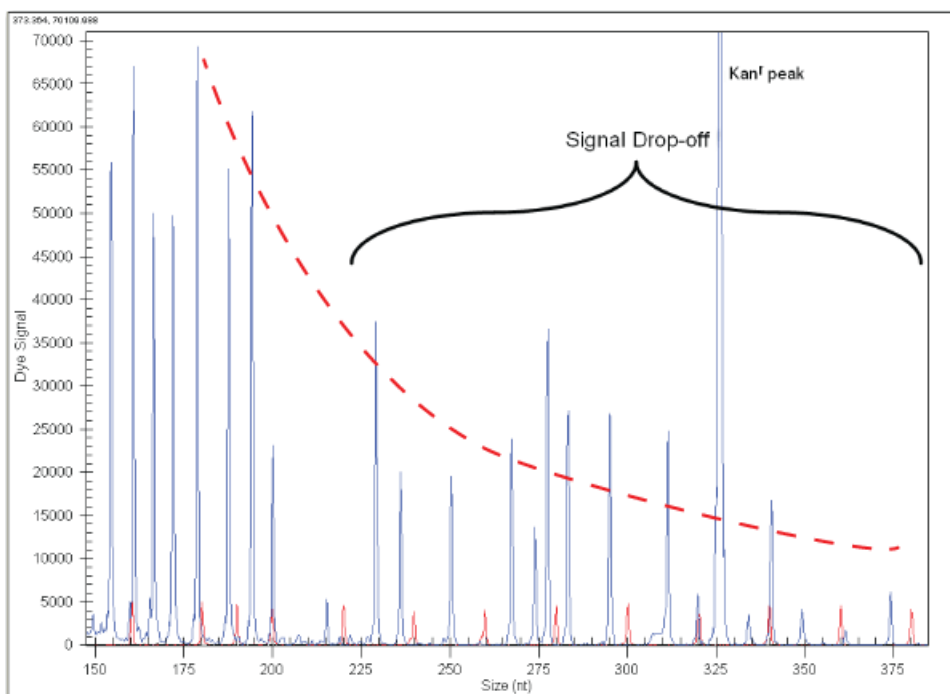
Figure A-6 Noise Peaks in RT Minus Reaction Mix



Signal Drop-off

Smaller fragments have a much higher signal than larger fragments, due to thermal cycling temperature fluctuation or incorrect DNA polymerase.

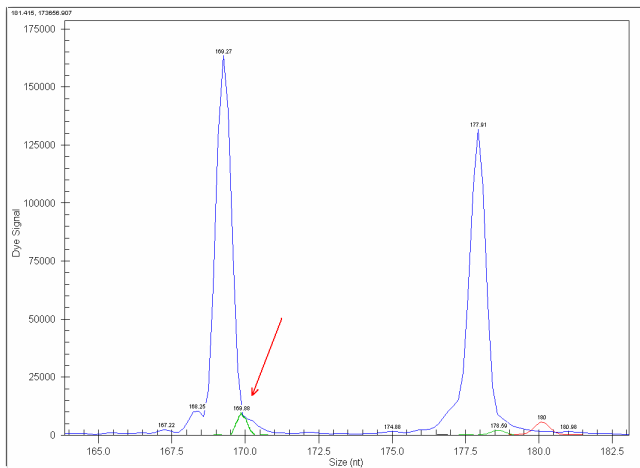
Figure A-7 Electropherogram with Signal Drop-off



Dye-Channel Pull-up

If the D4 (blue) signal is too strong, a small green D3 peak may be observed underneath the over-ranged peak.

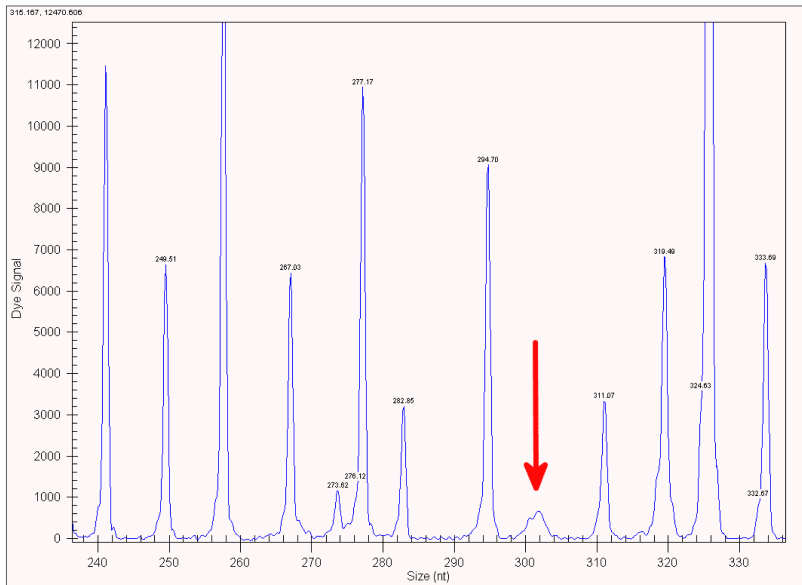
Figure A-8 D3 Channel Pull-up



Unresolved Protein-Nucleic Acid Complex

A non-specific protein-nucleic acid complex will consistently migrate in a particular size region, specific to the multiplex.

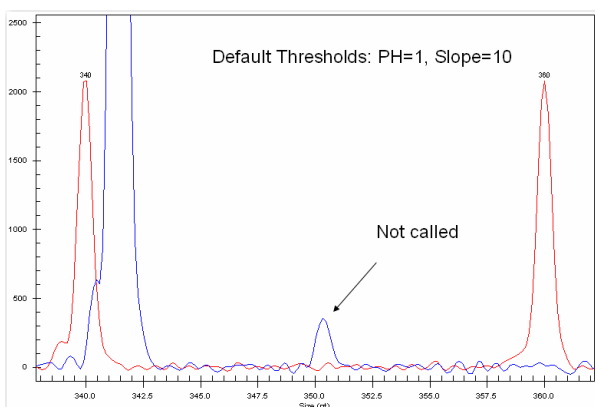
Figure A-9 Unresolved Fragment



Modifying Analysis Parameters

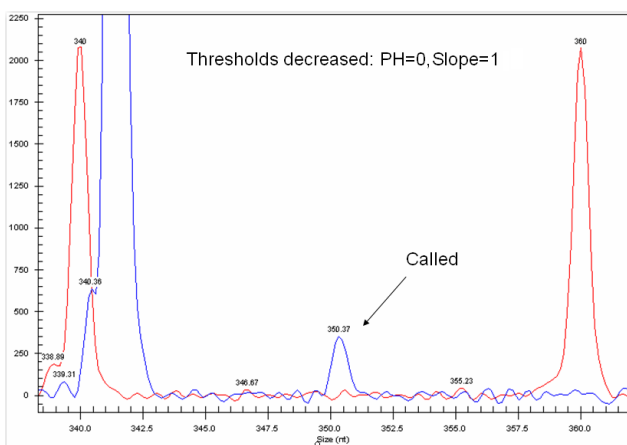
The default analysis parameters are too stringent to allow a peak to be called.

Figure A-10 Default Analysis Parameters



Modified analysis parameters allow the peak to be called.

Figure A-11 Modified Analysis Parameters



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SCIEX and its representatives maintain a staff of fully-trained service and technical specialists located throughout the world. They can answer questions about the system or any technical issues that might arise. For more information, visit the SCIEX website at sciex.com or contact us in one of the following ways:

- sciex.com/contact-us
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Documentation

This version of the document supercedes all previous versions of this document.

To view this document electronically, Adobe Acrobat Reader is required. To download the latest version, go to <https://get.adobe.com/reader>.

To find software product documentation, refer to the release notes or software installation guide that comes with the software.

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