Troubleshooting Sanger sequencing data

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Introduction

Effective minor variant detection with Minor Variant Finder Software requires high quality data with minimal noise.

While standard Sanger sequencing data is generally of high quality, the precision of detecting minor variants and the nuances of high quality data traces, specifically sources of baseline noise, become more important for an application of this nature.

This document provides guidance for the review of your data and troubleshooting tips for improving sequencing data quality, if needed.

Instructions and examples in this guide were generated with Applied Biosystems™ Sequence Scanner Software, available for free download following on-line registration. See “Before you begin“ on page 2.

Before you begin

Download software that allows you to visualize and evaluate your electropherograms. We recommend one of the following:

- Sequence Scanner Software.
  A free download of Sequence Scanner Software can be obtained at: **http://resource.thermofisher.com/pages/WE28396/**, following registration at Thermo Fisher Scientific.
- Minor Variant Finder Software.
  Minor Variant Finder Software is available for purchase at: **www.thermofisher.com/mvf**.

Review your data

To review the quality of your data or to troubleshoot sequencing issues:

1. Import the .ab1 files into Sequence Scanner Software.
2. Open files in Trace Manager by selecting **View** ▸ **Thumbnails**.
3. Set Y Scaling to **Individual** to look for major quality issues (see Figure 1). Alternatively, set Y Scaling to **Uniform** to compare an entire data set for relative signal strength.
4. Examine traces for potential quality issues and raw signal variability among samples.

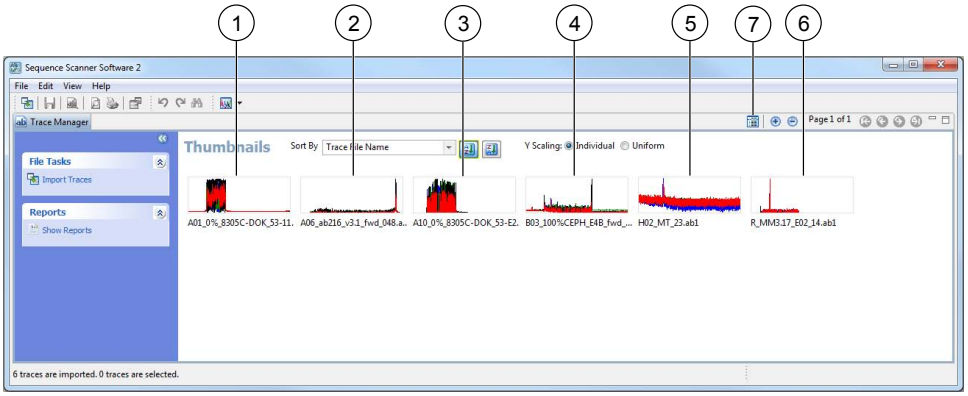

Figure 1 Thumbnail view of imported .ab1 files

① Major signal saturation (most peaks are above the recommended signal range)
② Signal is within the recommended signal range and showing a normal reptation peak. Reptation peaks are large peaks observed at the end of long runs.
③ Minor signal saturation (a few peaks are above the recommended signal range)
④ Within the recommended signal range
⑤ Low signal intensity
⑥ Dye blob
⑦ View Details icon

5. Click the **View Details** icon at the top right corner, then confirm that the instrument, polymer, and dye set match the experimental set-up.

   Note: If you have not used the correct mobility file, you can re-basecall the data with the correct mobility file using Sequencing Analysis Software. Sequencing Analysis Software is available for purchase at: **http://www.thermofisher.com/order/catalog/product/4474950**.

   The mobility file name is formatted as KB_instrument_polymer_dye set.mob.

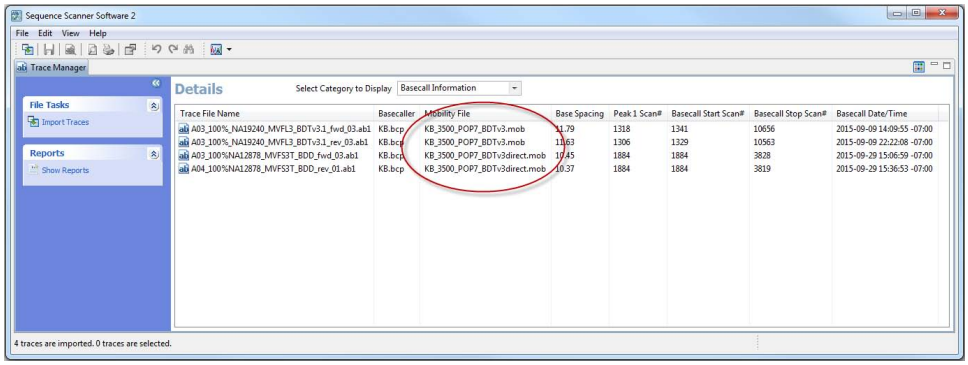

Figure 2 Mobility file format

6. Double-click a thumbnail or trace file name to open the electropherogram.

7. Review the data in the following tabs:
   - Analyzed – Review for issues such as dye blobs, primer dimers, mixed sequence content, peak compressions, and G/C degradation.
   - Raw – Review for issues such as signal saturation or low signal, dye blobs, and primer dimers.
   - Analyzed + Raw – Review for pull-up peaks. Pull-up peaks can be caused by off-scale peaks visible in the raw data and can cause spurious secondary peaks in the analyzed data.

     Note: This tab is useful in determining the impact pull-up peaks have on the analyzed data.
   - Annotation – Review parameters set during data collection or while setting up the run. Information is provided on: trace identification, data analysis, instrument and data collection software and the run configuration used.

     Note: Use this tab if poor peak spacing or mobility issues are suspected to identify parameters set during data collection or setting up the run.
   - EPT – Review for abnormal fluctuations in power, temperature, or voltage.

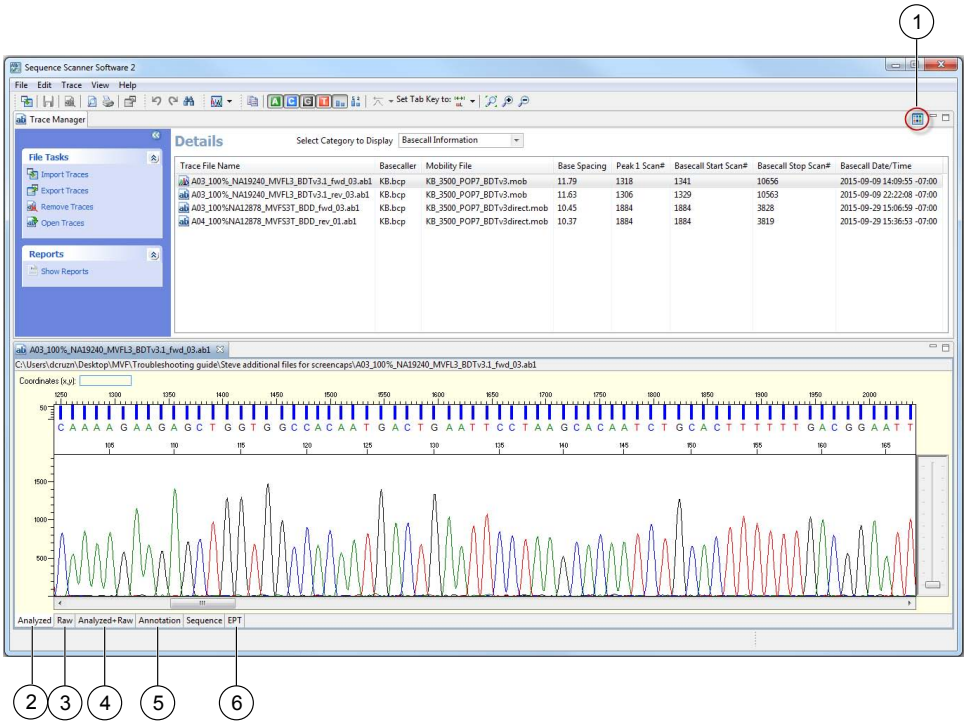

Figure 3 Parts of the screen

1. Icon to return to thumbnail view.
2. Analyzed tab
3. Raw tab
4. Analyzed + Raw tab
5. Annotation tab
6. EPT tab

Recommended raw signal ranges and signal-to-noise ratio for minor variant detection

Instrument	Recommended raw signal range in relative fluorescent units (RFU)			Recommended signal-to-noise ratio	Trace score [1]	Recommended peak under peak (PUP) value [1]
	Lower limit	Upper limit (annotation tab average)	Upper limit (individual peaks)			
3130/3130xl	1,000	3,000	7,900	>150	>40	>20
3500/3500xl	1,000	10,000	30,000			
3730/3730xl	1,000	10,000	26,000			

[1] Trace score and PUP values are metrics used in Minor Variant Finder Software.

Figure 4 is an example electropherogram within the recommended raw signal range and signal-to-noise ratio. High-quality data, within the ideal raw signal range, allows for minor variant detection above baseline (background) noise. A 5% variant in a trace with an average 3,350 RFU should produce a variant (at ~170RFU) that is distinguishable from the system noise in high-quality sequencing data.

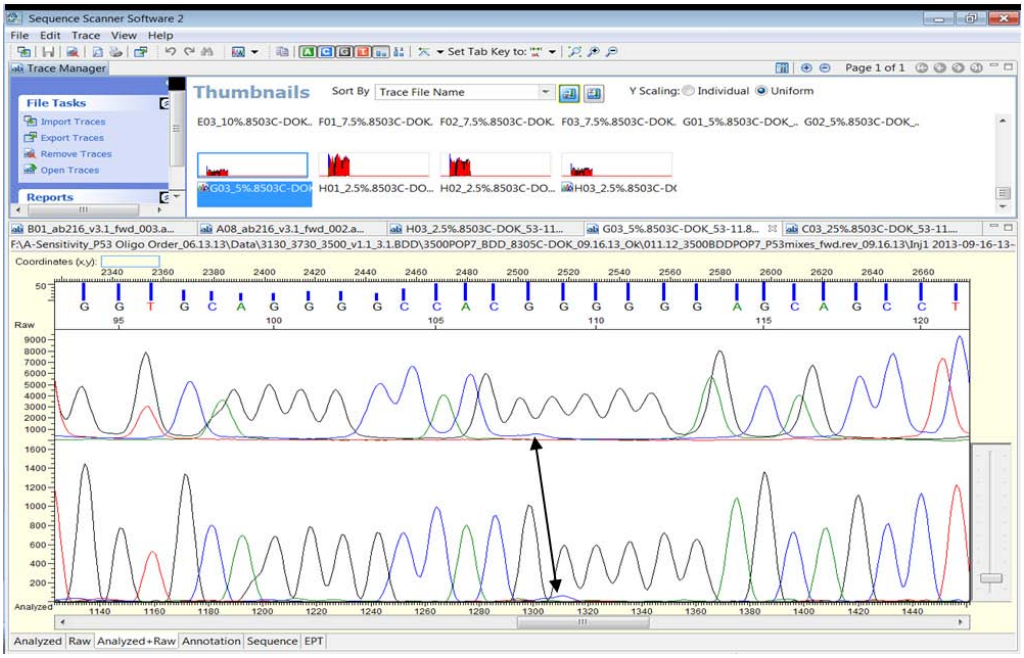

Figure 4 Visual minor variant detection in an electropherogram of high-quality Sanger sequencing data

In this example, the average 4-color raw signal across the entire electropherogram is ~ 3,350 RFU. The average raw signal-to-noise value is ~ 1,300. The arrow indicates the potential variant in the raw data in the upper trace and the corresponding basecalled data in the lower trace.

Common sources of Sanger Sequencing noise

Common sources of noise	How to recognize the source	Figure Number(s)
Signal saturation	The raw signal exceeds the recommended maximum RFU. **Note:** Excessive raw signal causes pull-up peaks in the analyzed data, which can incorrectly be identified as mixed bases.	Figure 1 (Thumbnail #1), Figure 5, Figure 6, and Figure 7
Low signal intensity	The raw signal is below the recommended minimum RFU.	Figure 1 (Thumbnail #5), Figure 8 and Figure 9
Dye blobs	Large broad peak normally seen at 85–90 bp or 125–130 bp.	Figure 1 (Thumbnail #6) and Figure 10
G/C compression	Subtle G or C peak shoulders or unresolvable GC-rich regions.	Figure 11 and Figure 12
G/C degradation	Decreased signal, increased baseline noise, and minor n+1 secondary peaks.	Figure 13
Mixed sequence content		
Primer impurity	Secondary peaks throughout the trace.	Figure 14
Contamination by a second sequence	Mixed sequence content throughout the length of the trace.	Figure 15
Off-target amplification	Mixed sequence content after the primer region.	Figure 16
Homopolymers	Long stretch of one base type leads to mixed sequence or excessive baseline noise (typically observed in stretches >9 bases)	Figure 17
Heterozygous insertions or deletions	Mixed sequence content starting at a specific point.	Figure 18
Primer dimers	The presence of a secondary sequence in the 5′ end, roughly the length of the two PCR primers added together.	Figure 19

Signal saturation

High sample signal causes saturation of the CCD camera. Signal saturation causes pull-up spectral peaks that cannot be corrected by spectral calibration. These pull-up spectral peaks are mobility corrected in the Analyzed sequence and can be incorrectly identified as minor peaks (see “Example of the impact of minor signal saturation on minor variant detection“ on page 8. Extreme signal saturation will appear as mixed sequence content (see Figure 7).

Any degree of signal saturation can impact minor variant detection.

Note: The 3500 Data collection software flags .ab1 files with off-scale peaks. You must manually check for off-scale peaks from data generated with the 3130 or 3730 Genetic Analyzer platforms.

Examples of signal saturation

The following figure shows examples of signal saturation. The red line indicates the maximum raw signal recommended.

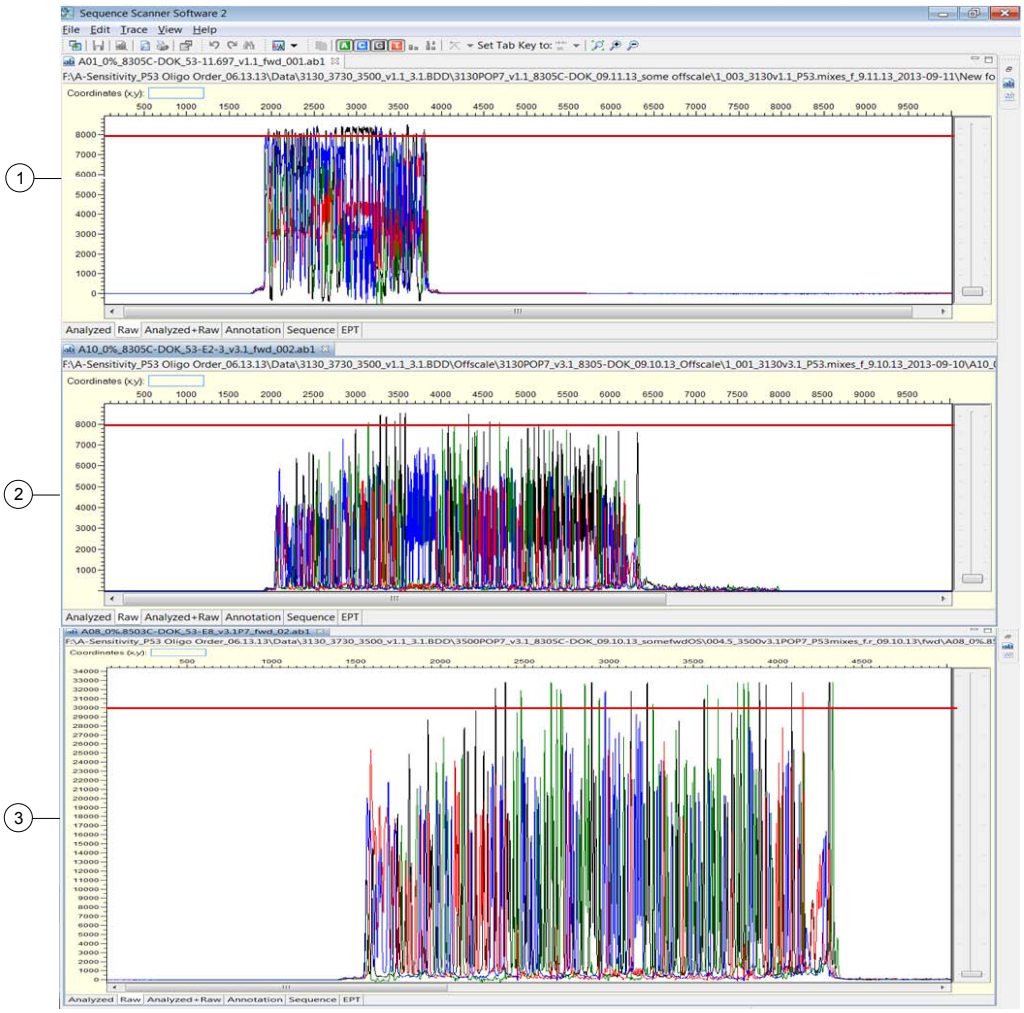

Figure 5 Signal saturation – Raw data view

1. Severe signal saturation on a 3130 Genetic Analyzer
2. Minor signal saturation on a 3130 Genetic Analyzer
3. Minor signal saturation on a 3500 Genetic Analyzer

Example of the impact of minor signal saturation on minor variant detection

Figure 6 is an example of signal saturation that causes spectral pull-up peaks that can be incorrectly identified as a minor variant.

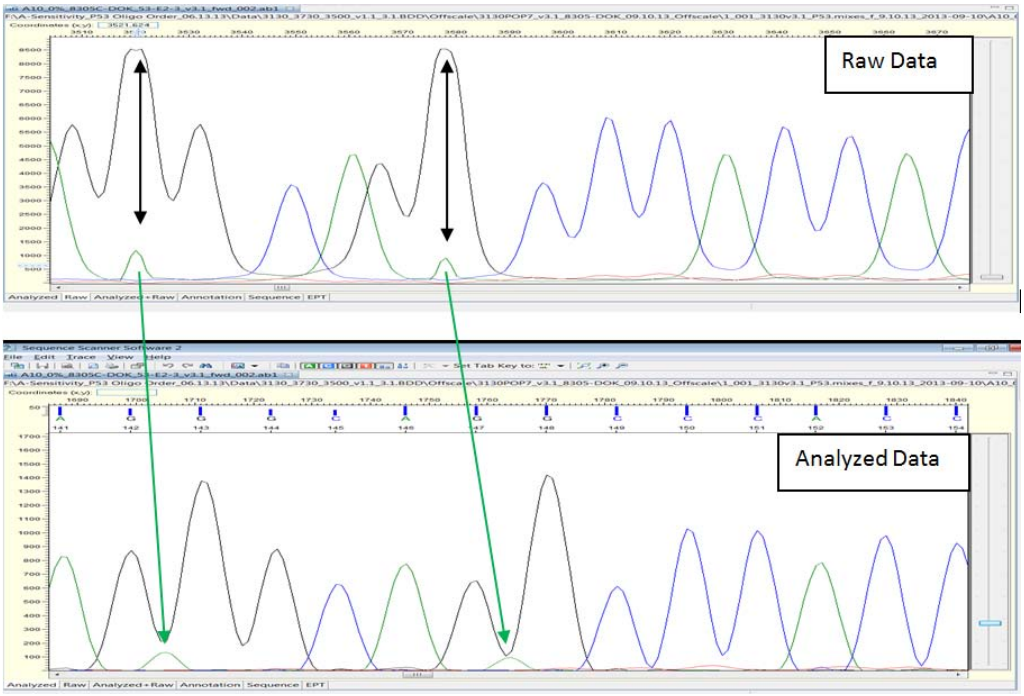

Figure 6 Pull-up peaks in raw vs. analyzed data from a sample with minor signal saturation on the 3130 instrument

The black arrows in the top panel highlight two G off-scale peaks with flattened tops that have saturated the camera and caused the pull-up peaks. The green arrows point to the pull-up peaks in the raw data (top) and in the analyzed data (bottom) that could be mistaken for true minor variants depending on their location in the electropherogram and the basecalled position after mobility correction.

Example of extreme signal saturation

Figure 7 shows what appears as mixed sequence caused by extreme signal saturation.

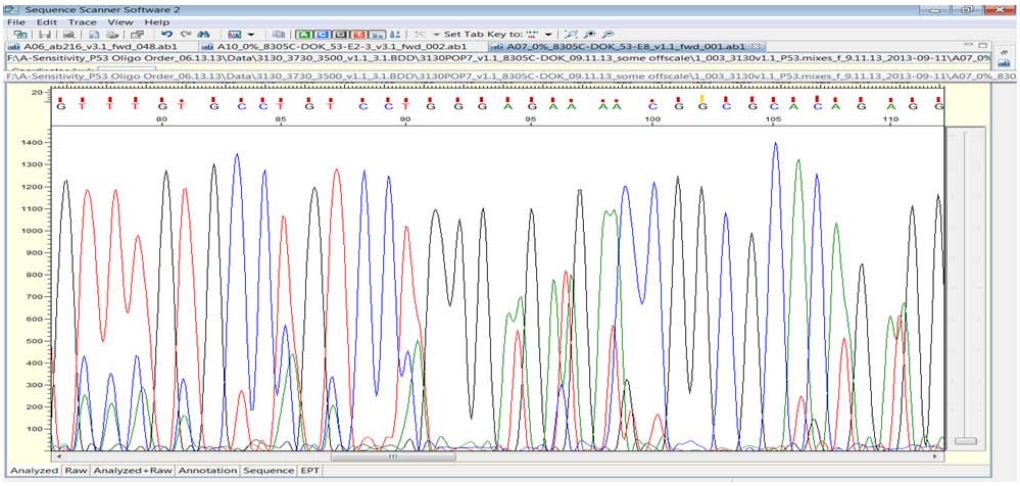

Figure 7 Extreme signal saturation

Mixed sequence in the Analyzed view due to extreme signal saturation. A quick review of the raw data can help diagnose a scenario such as this; the raw data view of the analyzed sample shown here is shown in the top panel in Figure 5.

Signal saturation: possible causes and recommended actions

Possible cause	Recommended action
Too much template was used in the sequencing reaction resulting in too much sequencing product.	If the sample has been on instrument <24 hours, reduce injection time in run module, then re-inject the sample.
	If the sample is purified with the BigDye XTerminator™ Purification Kit and has been on instrument <24 hours, carefully remove 10 µL of sample off the BigDye XTerminator™ beads in the plate, then add 10 µL of 0.1 mM EDTA to dilute the sample. Re-inject the sample using a standard run module (non-BigDye XTerminator™ module).
	Repeat the sequencing reaction using less template.
Water was used as the injection solution.	Use Hi-Di™ Formamide or a 0.1 mM EDTA injection solution for samples. **Note:** Using water as an injection solution causes highly variable quantities of DNA to be injected, because there is no competition for the charged DNA/salts.

Low signal intensity

Low signal intensity can be caused by many factors including thermal cycler malfunction (in the case of an entire plate failure) and insufficient sequencing template quantity/quality. Raw signal <500 RFU makes detection of minor variants more difficult.

Examples of low signal intensity

The examples below show moderately and severely low signal traces.

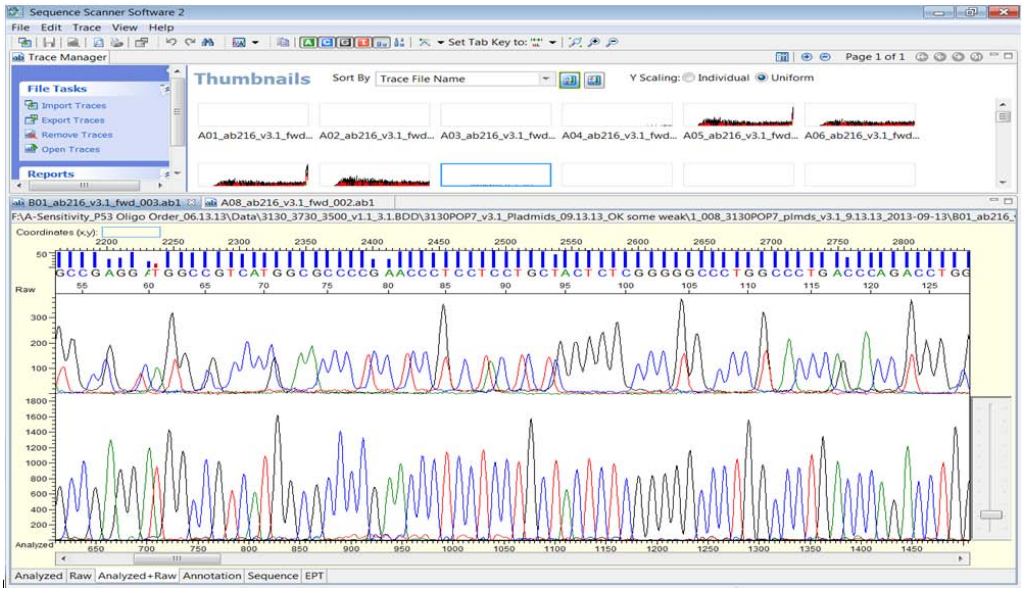

Figure 8 Moderately low signal intensity in a sample with an average raw signal-to-noise ratio of ~ 75

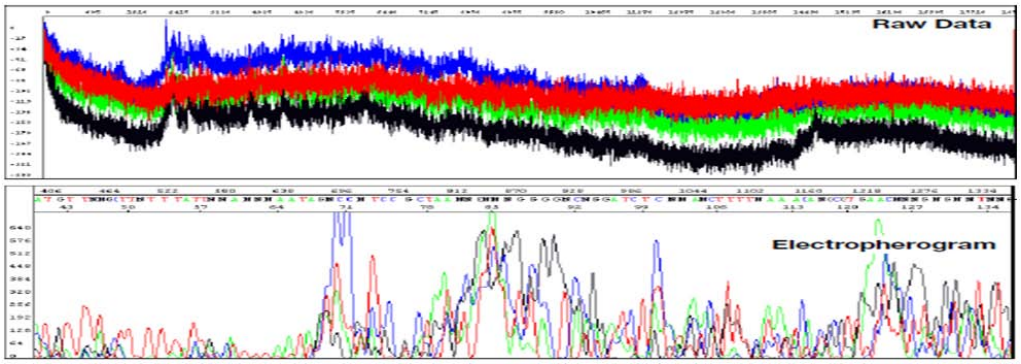

Figure 9 Severely low signal intensity due to hardware failure or a failed reaction

More severe signal issues are often related to poor injection, failed reaction, or a blocked or broken capillary.

Low signal: possible causes and recommended actions

Note: When sequencing signal is weak, increasing the injection time (re-injecting sample) or increasing primer and/or template in the cycle sequencing reactions can improve signal strength if DNA quality, PCR purification, and sequencing reaction purification steps have been performed properly.

Possible cause	Recommended action
Poor template quality.	Check DNA quality. If necessary, clean up the templates.
	Check the sequencing reaction for the DNA template control in order to check sequencing reaction quality.
Insufficient primer or template in the cycle sequencing reaction.	Check DNA quantity. Use the amounts recommended per PCR and sequencing kits. Check the DNA template control to determine sequencing reaction quality.
The amount of BigDye™ Reaction Mix in the reactions was insufficient; the sequencing chemistry was too dilute.	Follow recommended procedures to prepare sequencing reactions with BigDye™ Reaction Mixes.
Sample contains salts from insufficient purification of templates, PCR products, or sequencing reactions with ethanol precipitation. Salts in the sample interfere with proper electrokinetic injection.	Review DNA quality, PCR purification, and sequencing reaction purification steps.
Sample volume is too low.	Resuspend samples using sufficient volumes (10 µL).
Instrument run buffer is old.	Replace the buffer according to the procedures in your instrument user guide.
Injection failed.	Verify correct run module was used.
	Verify correct volume in well.
	Verify capillaries are not broken.
	Verify that data quality for capillaries is consistent and not trending downward. A decrease in data quality from a specific capillary can indicate blockage.
Sample evaporated because water was used as the injection solution.	Use Hi-Di™ Formamide to resuspend sample.
	Use new plate septa or check plate septa for wear.
	Add more resuspension solution to the samples before loading them.
	Inject soon after plate is placed on the instrument.
Autosampler alignment is off and the tips did not enter the sample.	Verify the correct run module was used.
	If you are using samples purified with the BigDye XTerminator™ Purification Kit and your auto sampler was recently calibrated, run the BDX Update utility. Select: **Start> All Programs> AppliedBiosystems> BDX Updater**. (The utility is installed with the BigDye XTerminator™ run modules.)

Possible cause	Recommended action
Autosampler alignment is off and the tips did not enter the sample.	Contact Technical Support to arrange a service engineer visit if alignment problem is not solved by the actions above.
Thermal cycler failure.	Contact Technical Support.

Dye blobs

Dye blobs are caused by unincorporated dye terminators remaining in solution after purification of the cycle sequencing reactions. Unincorporated dye terminators from the BigDye™ Terminator v3.1 Cycle Sequencing Kit and BigDye™ Direct Cycle Sequencing Kit are most commonly seen to co-migrate with the ~ 85–90 bp labeled fragments. In more severe instances, these blobs can also be detected at ~ 60–65 bp and within 125–140 bp regions. Dye blobs are typically seen as broad "C" or "T" peaks, but can also show up as "G" blobs. Dye blobs are more common when first testing new sequencing purification methods.

Example of dye blobs

Figure 10 shows severe dye blobs in the 60–65bp, 85–100bp, and 125–140bp regions. Although the sequence quality appears high, the blobs obscure nearly 40 bp of the 100 bases displayed. This would make the sequence unsuitable for variant detection.

Figure 10 Severe dye blobs in the 60–65bp and 125–140bp regions

Dye blobs: possible causes and recommended actions

Possible cause	Recommended action
Sample bypassed the purification material when using spin columns/spin plates for sequencing clean-up.	Ensure transfer of the sample to the center of the purification material. Sample dispensed along the walls of the clean-up column may bypass the purification material. Use a single channel pipette and/or position the tip directly above the spin column/plate while dispensing at low speed.
Ethanol concentration is too high during ethanol precipitation. This leads to unincorporated dye terminators and salts precipitating with the sequencing product.	Repeat procedure with correct ethanol concentration.
Incorrect ratio of BigDye XTerminator™ reagents.	Vortex theBigDye XTerminator™ Solution bulk container at maximum speed for at least 10 seconds before dispensing. If you pre-mix the SAM/BDX solution, ensure that the solution is well mixed before each sample well dispense step to maintain the appropriate ratio of reagents.
Insufficient mixing during the vortexing step when using the BigDye XTerminator™ Purification Kit.	Verify that the plate is firmly attached to the vortexer. Follow the protocol for vortexing.

G/C compression

G/C compression is often a result of too much sequencing template or incomplete denaturation of GC-rich regions of sequencing template, leading to subtle G or C peak shoulders or un-resolvable regions of GC bases.

Example of G/C compression

The following examples of G/C compression are often a result of too much sequencing template, a potential by-product of too much input DNA, and can show up near the 260–270 bp region of the electropherogram when using BigDye™ Direct.

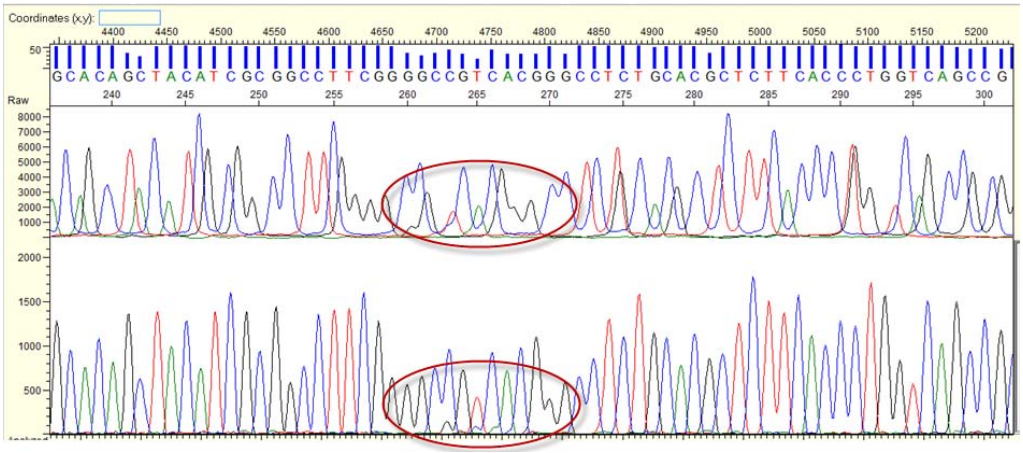

Figure 11 G/C compression due to an excess of sequencing template

A BigDye™ Direct sequencing sample with the raw data trace on top and the basecalled/analyzed data trace on the bottom. Subtle G/C peak shoulders are observed with poor resolution of a triplet of G peaks.

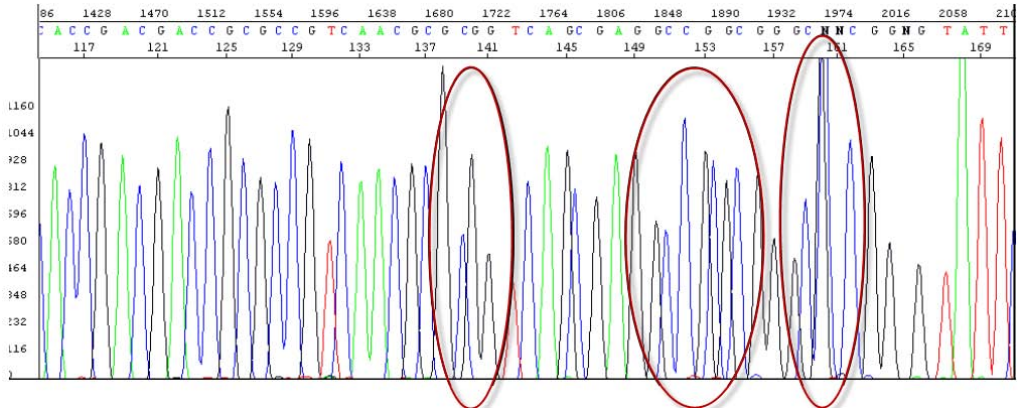

Figure 12 G/C compression due to GC-rich templates

Compressions encountered using the dGTP BigDye™ Terminator Kit, an alternative non-standard kit for GC-rich templates.

G/C compression: possible causes and recommended actions

Possible cause	Recommended action
Too much input gDNA during PCR, leading to excessive sequencing template when using the BigDye™ Direct Cycle Sequencing Kit.	If using the BigDye™ Direct Cycle Sequencing Kit, reduce the amount of input DNA; ensure ≤20 ng is used.
	Reinject the sample. In most cases, compressions are eliminated after the first injection.
GC-rich regions, especially when sequencing with dGTP sequencing chemistry. Possibly caused by incomplete denaturation of the synthesized DNA.	No corrective action is known at this time. Using a different sequencing primer located closer to the GC-rich region may help to resolve G/C compression.

G/C dye terminator degradation

Sequencing reactions covered by septa or MicroAmp™ Clear Adhesive Film and resuspended in Hi-Di™ Formamide are generally stable for up to 12–24 hours at room temperature when protected from light, heat, acidic conditions, bleach, and air. However, prolonged exposure to environmental conditions, such as prolonged storage at room temperature, leads to degradation of the dye terminators, especially the G and C dyes. G and C dye degradation can lead to decreased signal, increased baseline noise, and minor n+1 secondary peaks that can impact the ability to detect minor sequencing variants.

Example of G/C dye terminator degradation

Figure 13 shows examples of G and C dye terminator degradation.

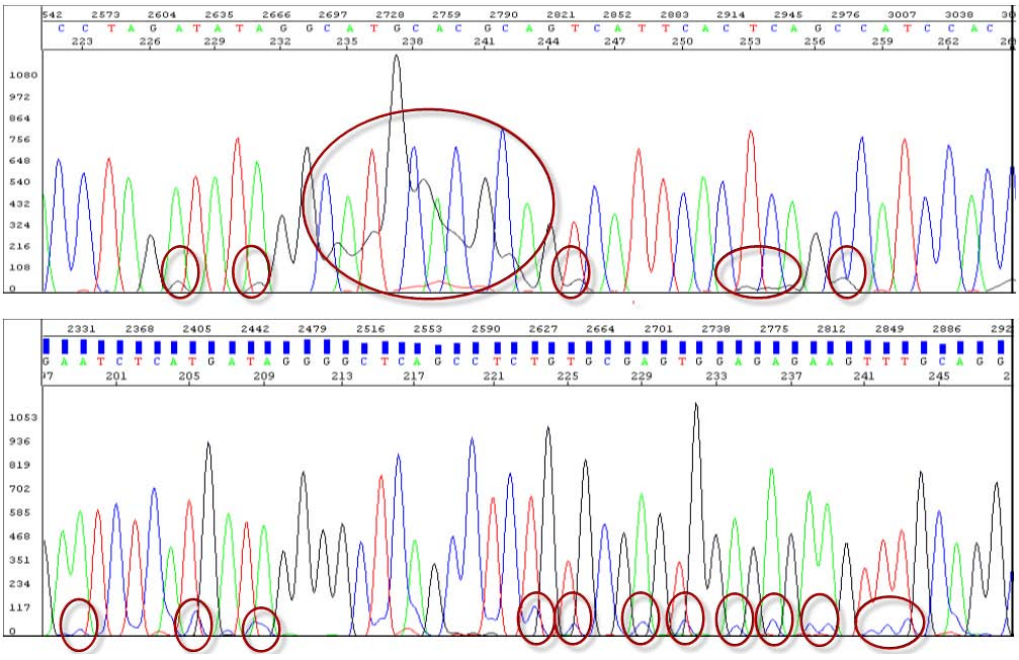

Figure 13 G and C terminator degradation

The top panel shows G dye terminator degradation, while the bottom panel shows C dye terminator degradation.

G/C degradation: possible causes and recommended actions

Possible cause	Recommended action
The dye labels attached to the dd-terminators are degraded. Initial degradation results in shoulders on C and/or G peaks that can be mistaken as secondary peaks. With further degradation, the C and/or G peaks appear small or rough or disappear completely.	Protect the fluorescently-labeled DNA from light, heat, acidic conditions, and oxygen.
	If no C peaks are visible, repeat the sequencing reactions with fresh reagents.
Sequencing reactions were exposed to light, heat, acidic conditions, bleach, and/or oxygen before they were loaded onto the instrument.	Use tube septa or a heat seal to prevent exposure to air and evaporation of samples.
	Do not leave samples on the instrument for more than 24 hours.
Water was used as the injection solution. **Note:** Resuspending samples in water leads to breakdown of C and/or G-labeled fragments.	Resuspend the samples in Hi-Di™ Formamide or 0.1 mM EDTA.
The Hi-Di™ Formamide is degraded.	Resuspend the samples using a newer lot of Hi-Di™ Formamide.
	Aliquot large lots of Hi-Di™ Formamide into smaller tubes to minimize freeze/thaw cycles and store at –20°C.

Mixed sequence content overview

Contaminating mixed sequence content, in which a secondary sequence contaminates the primary sequence, has many causes, including:

- "Primer impurity" on page 17
- "Contamination" on page 17
- "Off-target amplification" on page 18
- "Homopolymers" on page 19
- "Heterozygous insertions or deletions" on page 20
- "Primer dimers" on page 21

Note: Mixed sequence can also be caused by signal saturation and low peak intensity. See "Signal saturation" on page 6 and "Low signal intensity" on page 10 for troubleshooting information.

Primer impurity

Figure 14 shows mixed sequence caused by the intentional introduction of a 10% (n-1) forward primer impurity.

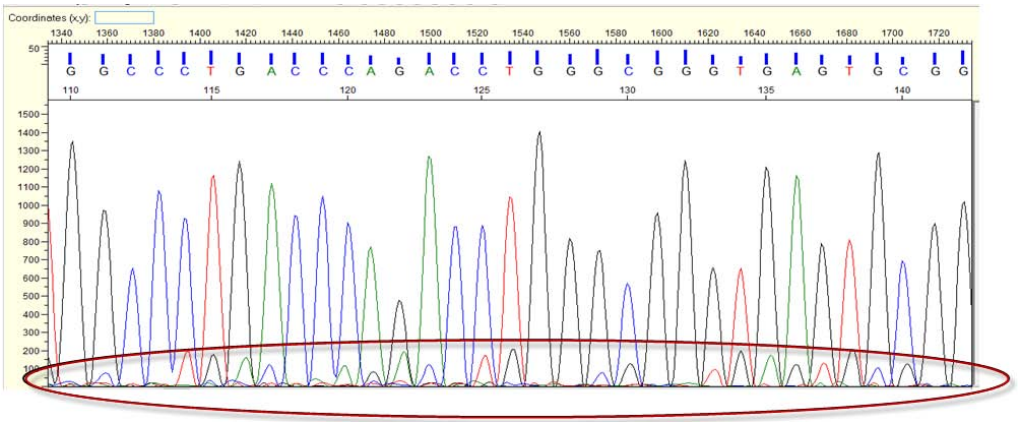

Figure 14 Example of mixed sequences due to a 10% primer impurity

Two sequencing primers were pooled 1:9. Standard sequencing primers (90%) were mixed with primers that had the 3′ base removed (10%). The secondary peaks exhibit a minor n-1 peak at roughly the same % as the primer impurity introduced.

Primer impurity: possible causes and recommended action

Possible cause	Recommended action
Insufficient PCR primer purification.	Repeat using HPLC-purified primers.
Insufficient sequencing primer purification.	

Contamination

Secondary sequence contamination results in mixed sequence content as shown in Figure 15.

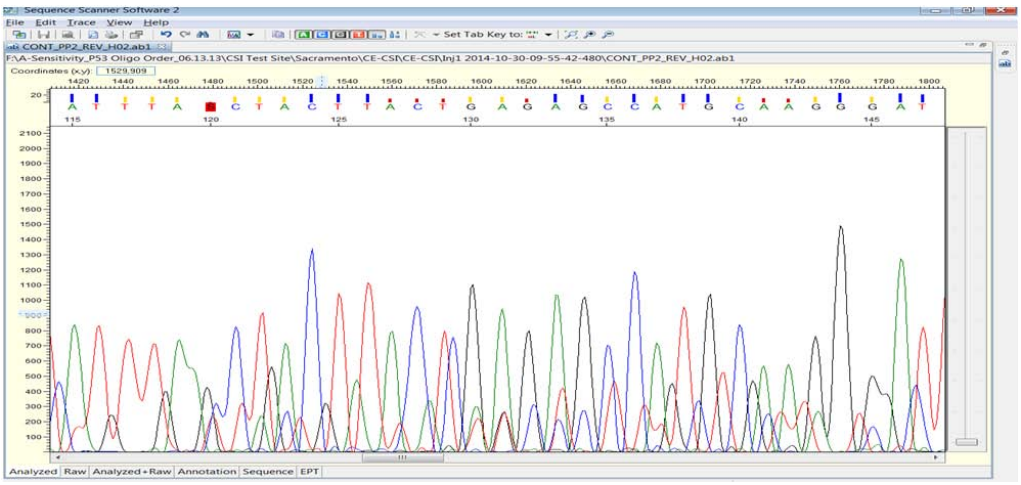

Figure 15 Secondary sequence contamination caused by well-to-well contamination of one sample into another

Contamination: possible causes and recommended actions

Possible cause	Recommended action
Carryover from contaminated septa.	Replace septa, then change the buffer/water/ waste.
Cross-contamination of the specimen, amplicons, or primers.	Review DNA quality.
	Replace pipette tips before aspirating primers.
	Carefully remove adhesive seal post-PCR to avoid the amplicon contaminating adjacent wells.
	Replace pipette tips each time amplicons are dispensed for sequencing reactions.
Incomplete PCR product clean-up.	Remove PCR primers completely before using PCR products as sequencing templates.
Secondary amplification product in the PCR product used as a sequencing template.	Use gel purification to isolate the desired product or design new PCR primers to obtain a single product. We recommend the Primer Designer™ Tool at **http://www.thermofisher.com/primerdesigner**.

Off-target amplification

The entire PCR-specific primer region can be clearly seen when using the BigDye™ Direct Cycle Sequencing Kit with the BigDye™ Direct forward M13 primer. Off-target amplification can clearly be seen after the gene-specific priming region.

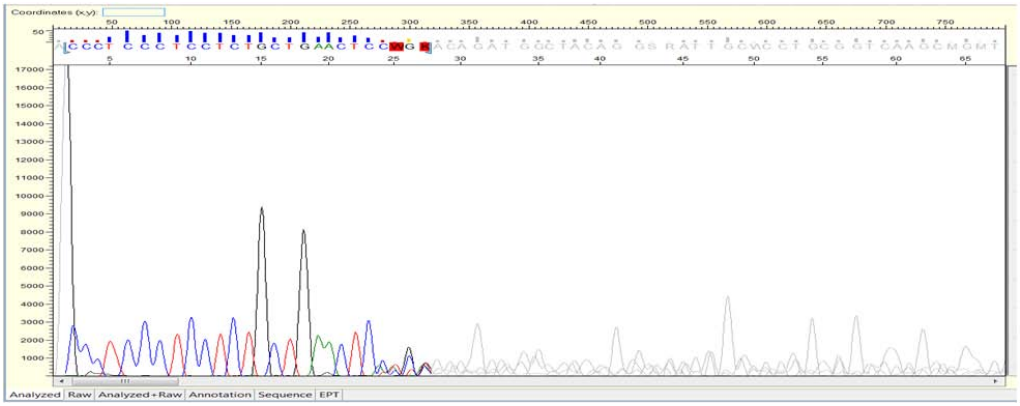

Figure 16 Example of off-target or secondary amplification product
Off-target amplification after the primer.

Off-target amplification: possible causes and recommended actions

Possible cause	Recommended action
PCR product with secondary amplification (>1 sequencing product signal) used as a sequencing template.	Use gel purification to isolate the desired product or design new PCR primers to obtain a single product. We recommend the Primer Designer™ Tool at **http://www.thermofisher.com/primerdesigner**.
	Increase the PCR specificity (increase annealing temperature, lower $MgCl_2$, or change primer designs.)

Possible cause	Recommended action
PCR product with secondary amplification (>1 sequencing product signal) used as a sequencing template.	Re-examine the sequence for primer site homology.
More than one priming site (either upstream or downstream) is present on the sequencing template.	

Homopolymers

Homopolymer stretches longer than 8–9 consecutive bases can lead to n+/-1 peaks (one too many or few of the base type) and cause mixed sequence content and increased baseline noise after the homopolymer.

Excessive 2–3 base repeats may also result in similar noise patterns.

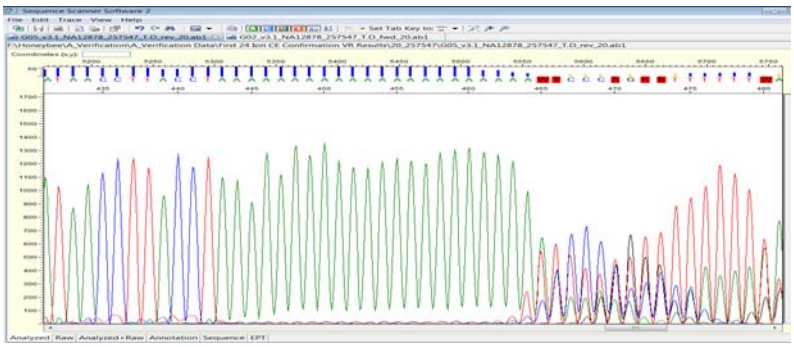

Figure 17 Example of a mixed sequence after a homopolymer
A string of "A" bases leads to mixed sequence content.

Homopolymers: possible cause and recommended actions

Possible cause	Recommended action
A partially extended primer and template dissociate and then re-anneal improperly before extension continues.	Use anchored sequencing primers during PCR amplification.
	Design PCR primers to exclude homopolymer regions greater than- 8–9 bp long if possible.

Heterozygous insertions or deletions

A heterozygous insertion or deletion is a scenario in which one allele contains a specific insertion or a deletion that the other allele lacks, resulting in mixed sequence content following the point of the particular insertion or deletion. This is a true biological event and can usually be confirmed through alignment of both forward and reverse trace files against a reference sequence.

Figure 18 Example of mixed sequence content following a heterozygous insertion or deletion. Mixed sequence content is seen in both forward and reverse traces.

Heterozygous insertions or deletions: possible cause and recommended actions

Possible cause	Recommended action
Heterozygous insertion or deletion mutation.	Assemble both forward and reverse sequence data to determine sequence of insertion or deletion.
	Re-design PCR target to exclude the region where the insertion or deletion occurs if additional minor variants are suspected to be present.

Primer dimers

Primer dimers are an unwanted by-product of poorly-designed PCR primers. These forward and reverse PCR primers hybridize to each other because of complimentary 3′ bases that enable elongation to occur for the length of each primer.

Figure 19 shows the presence of two secondary sequences roughly the length of the two PCR primers added together. This is typical of a primer dimer event.

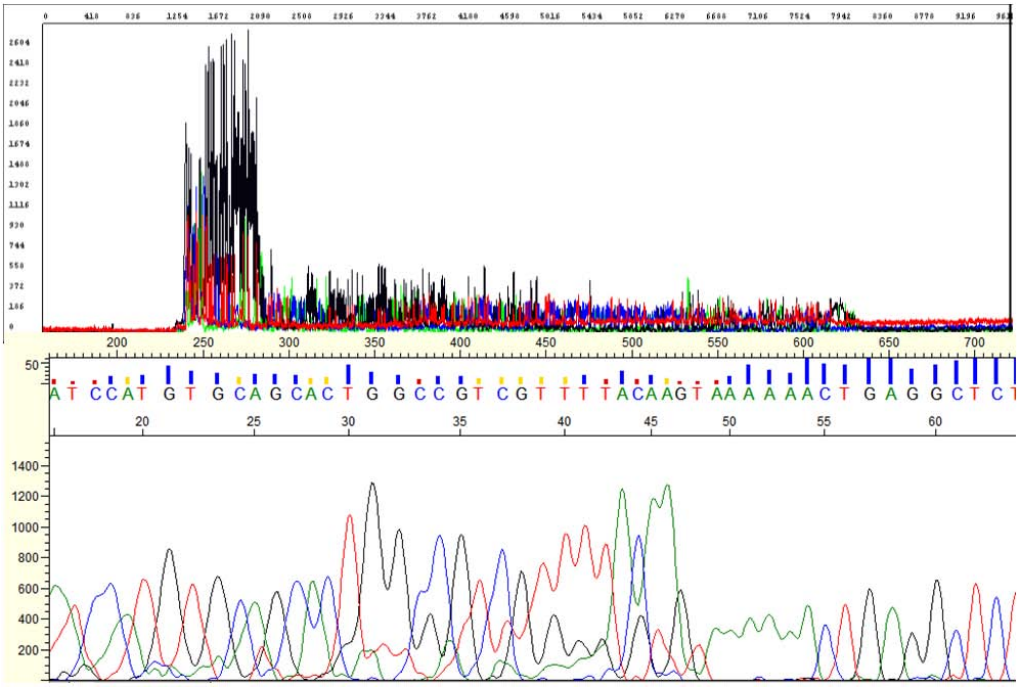

Figure 19 Example of mixed sequence due to primer dimers

The top panel shows the Raw data and the bottom panel shows the Analyzed data. The primer dimer in the Analyzed data is a relatively clean example. However, some amount of excess mixed sequence noise can sometimes be seen even beyond the PCR-specific primer region.

Primer dimers: possible cause and recommended actions

Possible cause	Recommended action
Primer dimers are formed when PCR primers anneal and amplify to create short PCR products incorporating only the two primer sequences. They can then act as templates, albeit very short, in the subsequent sequencing reaction.	Redesign the PCR primers to eliminate the sequences that allow primer-dimer formation.
	Use a "hot start" PCR enzyme to inhibit primer-dimer formation.

Sequence analysis tools

Minor Variant Finder Software

The Minor Variant Finder Software is a simple, easy-to-use desktop software designed for the accurate detection and reporting of minor variants (<25% of a major peak) or 50:50 mixtures as found in a germline heterozygous positions by Sanger Sequencing.

By comparing test specimen and control traces, the software generates a noise-minimized electropherogram for confirmation of minor variants in forward and reverse sequences. The software can detect variants (SNPs or SNVs) with a Limit of

Detection (LOD) of 5% with high-quality data in amplicons of lengths 150 to 500 bp. LOD is defined as the lowest level at which sensitivity ≥95% and specificity ≥99% within the overlapping region of forward and reverse test and control .ab1 files.

Note: LOD was determined using 5% mixtures that were experimentally created with physical mixtures of molecules, and is not based on peak height ratios in electropherograms.

The software also includes an optional NGS confirmation function.

The Minor Variant Finder Software runs in a web browser window, but does not require connection to the internet in order to run. Data is secure on your desktop computer.

Sequence Scanner Software

Sequence Scanner 2 is free software for viewing electropherograms. It provides an easy way to perform a high-level sequencing data quality check or general data review that includes summary tables and electropherograms as well as a general .ab1 file raw/analyzed data view.

To obtain the software, go to: **http://resource.thermofisher.com/pages/WE28396/**.

Next-generation confirmation (NGC) module

The Applied Biosystems™ Analysis Module Next-Generation Confirmation (NGC) is CE Sanger sequencing software hosted on the Thermo Fisher Cloud environment. The software allows you to examine variants from a CE electropherogram to confirm the variants detected by Next Generation Sequencing (NGS) platforms. The software analyzes CE sequencer-generated .ab1 files and performs SNP detection and analysis, SNP discovery and validation, and sequence confirmation, all on the cloud. NGC software can automatically retrieve reference sequences from genomic databases, report variants in genomic coordinates, and report genomic annotations for SNPs. The software analyzes NGS variant .vcf files and analyzes NGS variants and Sanger variants in the same alignment view. The software can also generate a Venn diagram, allowing you to visually compare and confirm variants generated from NGS. In addition, the NGC software generates and exports variants in standard variant call format (VCF).

Variant Reporter™ Software

This software performs comparative sequencing, also known as direct sequencing, medical sequencing, PCR sequencing, and resequencing with DNA sequencing files. The software is designed for reference-based and non-reference-based analysis such as mutation detection and analysis, SNP discovery and validation, and sequence confirmation. The robust algorithms will call SNPs, mutations, insertions, deletions, and heterozygous insertions or deletions for data generated using the Applied Biosystems™ genetic analyzers.

To obtain the software, go to: **https://www.thermofisher.com/order/catalog/product/4475006**.

Related documentation

Document	Publication number	Description
DNA Sequencing by Capillary Electrophoresis Chemistry Guide	4305080	This chemistry guide is designed to familiarize you with Applied Biosystems™ genetic analyzers for automated DNA sequencing by capillary electrophoresis, to provide useful tips for ensuring that you obtain high-quality data, and to help troubleshoot common problems.
Generating high-quality data using the BigDye™ Direct Cycle Sequencing Kit	MAN0014436	Effective minor variant detection with Minor Variant Finder Software requires high-quality sequencing data with minimal noise. This document provides a demonstrated protocol for generating high-quality data for use in minor variant detection using: • BigDye™ Direct Cycle Sequencing Kit • Applied Biosystems™ genetic analyzers with POP-7™ polymer
Generating high-quality data using the BigDye™ Terminator v3.1 Cycle Sequencing Kit	MAN0014628	Effective minor variant detection with Minor Variant Finder Software requires high-quality sequencing data with minimal noise. This document provides a protocol for generating high-quality data for use in minor variant detection using: • BigDye™ Terminator v3.1 Cycle Sequencing Kit • Applied Biosystems™ genetic analyzers with POP-6™ or POP-7™ polymer

The information in this guide is subject to change without notice.

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