DEVYSER

RESULTS FOR LIFE

DEVYSER QF-PCR

TROUBLESHOOTING GUIDE

CONTENTS

1. CAPILLARY ELECTROPHORESIS DATA

1.1. PCR Artefacts

1.1.1. Stutter peaks

Stutter peaks are detected as minor extra peaks that are one or a multiple of repeats smaller than the actual short tandem repeat (STR) allele (Figure 1.1). Stutter peaks may be included in the ratio calculation. The stutter peak area is typically less than 15% of the corresponding STR peak area for tetranucleotide STR markers but might increase at low levels of input DNA. Overloaded peaks will also show relatively high stutter because truncated peaks result in false peak stutter ratios.

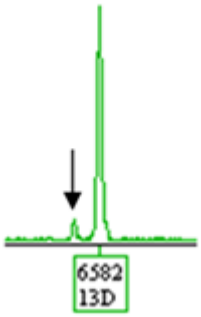


Figure 1.1: Stutter peak as indicated by the arrow

1.1.2. A peaks

Minus A (-A) peaks are detected as extra peaks that are one base pair shorter than the full length (+A peak) PCR product (Figure 1.2). -A peaks result from incomplete addition of the terminal A base by the polymerase, commonly occurring at sub-optimal PCR conditions. For example, -A peaks may be more pronounced at low (section 1.4) and high levels (section 1.6) of input DNA, the ramping rate in the PCR being too fast or slow, and the use of incompletely mixed activated reaction mix (see section 1.10). -A peaks may be included in the ratio calculation.

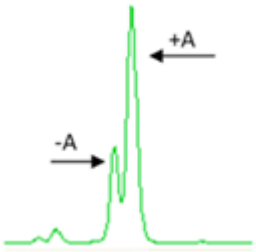


Figure 1.2: -A and +A peaks as indicated by the arrows.

1.2. Electrophoretic Artefacts

1.2.1. Crosstalk

Crosstalk/bleed-through between dye channels may occur during detection (Figure 1.3 and 1.4). Crosstalk appears as equally sized peaks with lower peak heights in neighboring dye channels and should be excluded from the analysis. The level of crosstalk is related to the spectral calibration of the instrument. Normally up to 10 % crosstalk is observed between the dye channels. Increased crosstalk indicates the need for a new spectral calibration. Spectral calibrations are routinely performed when using a new dye set, changing capillary array, changing polymer type or performing instrument service of laser or CCD camera. The dyes that are used for PCR products and size standards must match the dyes used for spectral calibration. The dyes must also be compatible with the dye set (filter set used) on the capillary device.

Saturation of the CCD camera (indicated by off-scale peak indicators in the electrophoretic trace) normally cause increased levels of crosstalk.

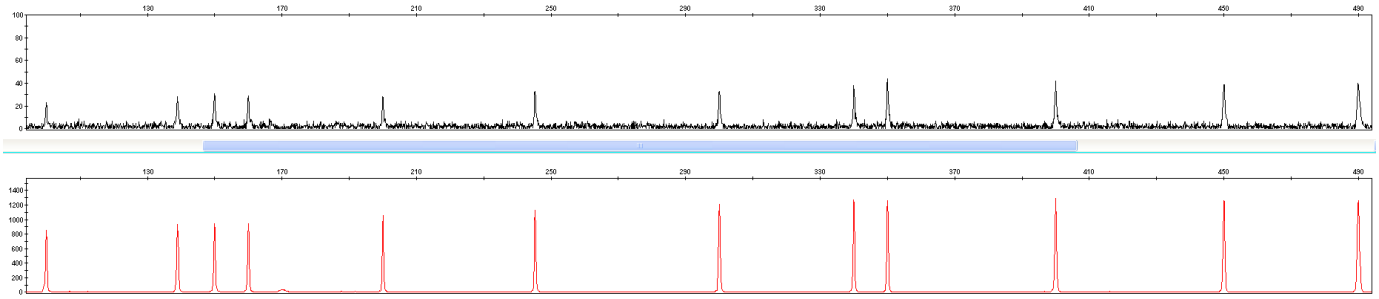


Figure 1.3. Crosstalk from red to yellow channel (dye channels shown separately).

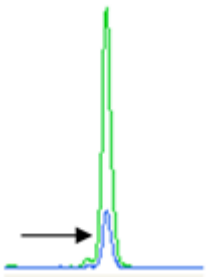


Figure 1.4. Crosstalk from green to blue channel as indicated by the arrow (superimposed dye channels).

1.2.2. Dye blobs

Dye blobs are PCR artefacts that may appear in the sample analysis range (Figure 1.5). In general, dye blobs appear as broad, undefined peaks of a single dye and tend to occur relatively early in the data. Dye blobs may however be observed in the overall electrophoretic trace at low levels of input DNA. Dye blobs can easily be observed in the negative control.

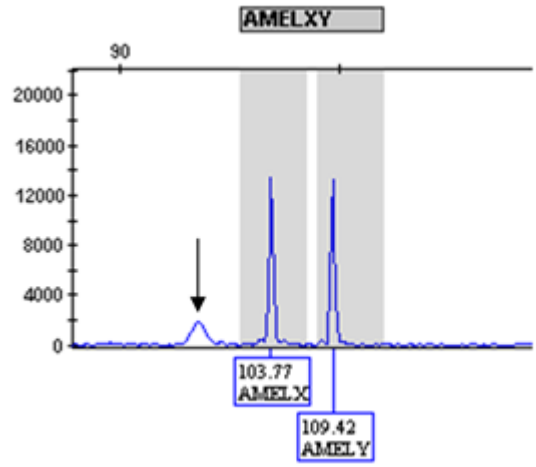


Figure 1.5. Dye blob as indicated by the arrow.

1.2.3. Electrophoretic spikes

Electrophoretic spikes are artefacts observed as sharp peaks in all dye channels (Figure 1.6). These artefacts are commonly corrected for and do normally not affect the data interpretation. If falling in a marker allele, a spike might however affect the ratio calculations. Electrophoretic spikes might be caused by old buffer, urea crystals in the polymer or by air bubbles or dust in the capillary electrophoresis system.

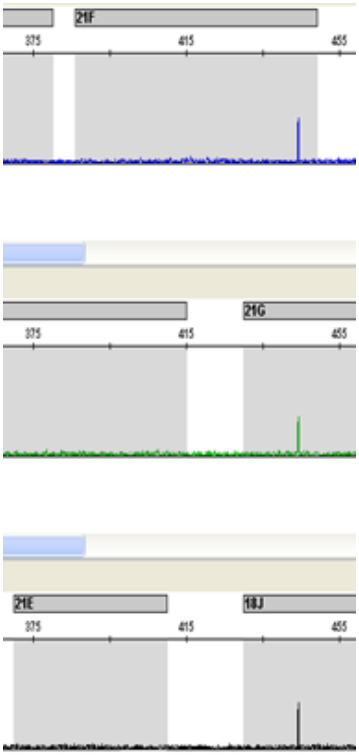


Figure 1.6. Electrophoretic spikes observed in the blue, green and yellow dye channel.

Possible solutions

Inspect the system for air bubbles. If present, use the appropriate wizard to remove the bubbles. Install fresh polymer and electrophoresis running buffer on the instrument.

1.3. Signal sloping

Signal sloping is commonly observed as peak heights declining with increased fragment size (indicated by black arrow in Figure 1.7). Compare the peak heights of the smaller fragments with the longer fragments in both the chromosome markers and the size standard. Signal sloping can be observed in the Samples Plot but is more easily observed in the sample raw data tab in GeneMapper.

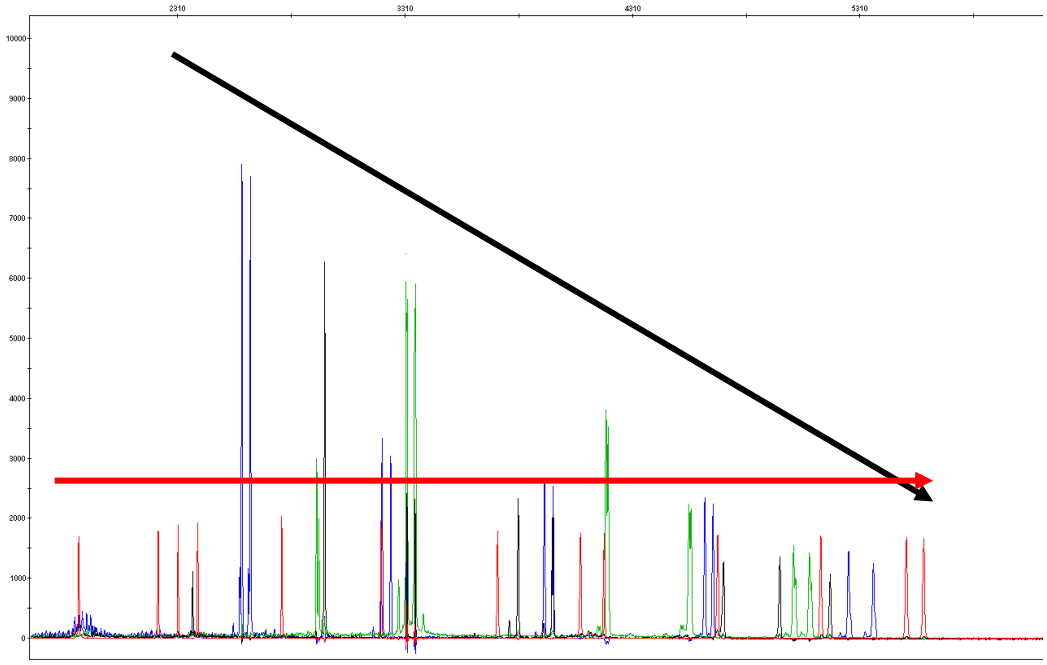


Figure 1.7. Signal sloping of marker alleles shown in the Raw Data tab in GeneMapper. Black arrow demonstrates the signal sloping of shorter to longer length alleles while the red arrow displays a consistent peak height of the size standard (red peaks).

Signal sloping in marker alleles

In case the signal sloping is visible in the marker alleles only (black arrow in Figure 1.7), and not in the size marker (red arrow in Figure 1.7), the signal sloping might have been caused by inefficient amplification of the longer length alleles. The signal sloping pattern might appear because of 1) DNA overload/high input DNA in the PCR, 2) DNA degradation, 3) PCR inhibitors or 4) Incomplete mixing of the reaction mix (-A peaks usually observed).

Possible solutions

1. DNA overload/high input DNA in the PCR: Rerun the PCR reaction with diluted sample DNA.
2. DNA degradation: Re-extract DNA using a validated DNA extraction method (see section 1.12). Repeat the PCR.
3. PCR inhibitors: Re-extract DNA using a validated DNA extraction method (see section 1.13). Repeat the PCR.
4. Incomplete mixing of the reaction mix: Make sure that the PCR Activator is thoroughly mixed with PCR Mix by pipetting manually

Signal sloping in marker alleles and size marker

If signal sloping is visible in the marker alleles as well as the size marker fragments (Figure 1.8), the injection conditions are not optimal in the capillary system. Signal sloping of the size marker might be explained by low quality formamide, old polymer/capillary array or a high injection voltage (>2,2 kV). Old polymer and high injection voltage cause a slow injection rate of larger fragments, thereby resulting in wider peaks. The signal sloping in the size marker might also be caused by overloaded capillaries.

Possible solutions

Use fresh HiDi Formamide, electrophoresis running buffer and polymer as well as an injection voltage of approximately 1,5 kV (recommendations may vary depending on instrument used). Use less PCR product, e.g. 1 µL to 20 µL HiDi Formamide and re-inject the PCR product.

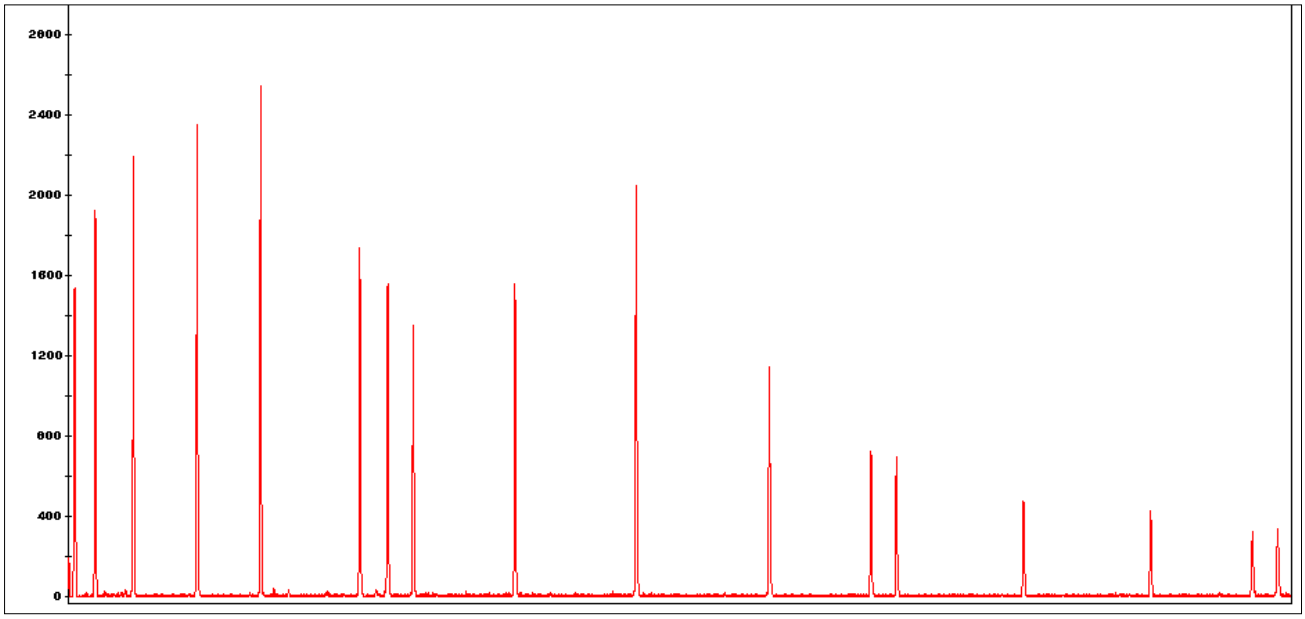


Figure 1.8. Signal sloping of the size marker GS-500 ROX.

Unexpected size marker pattern

In case the size marker peaks display unequal signal intensities (Figure 1.9), the injection mixture might have contained a high salt concentration. This may cause re-annealing of PCR fragments and will result in unequal signals or unexpected signal loss of size marker fragments. This might also affect the marker alleles. Observe that the GS-600 LIZ size standard normally has peaks of varying intensities and is not as easy to troubleshoot as 560 SIZER ORANGE and GS-500 ROX. In the last two size markers, the fragments have been designed to correct for the normal injection bias which favors smaller above larger fragments, generating marker peaks of similar height throughout the chromatogram.

Possible solutions

The total volume of PCR product in the injection mixture should not exceed 10 %, e.g. 1,5 µL PCR product to 15 µL loading cocktail. Signal intensities will not increase by adding more PCR product, but rather decrease and cause problems with reannealing. Alternatively, salt levels can be reduced by post-PCR purification using spin columns.

Replace the electrophoresis running buffer and rerun the failed runs with a lower PCR product concentration.

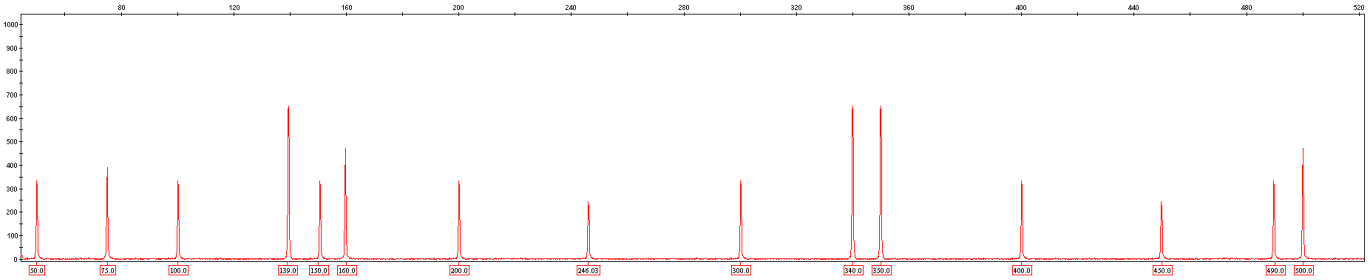


Figure 1.9. Unequal signal intensities for the size marker fragments in GS-500 ROX.

1.4. Low peak signals

Low input DNA in the PCR reaction will generate low signals and usually skewed and inconclusive ratios as seen in markers 13B and 21C in Figure 1.10 (red boxes). The ratio skewing can be explained by the low copy problem causing random PCR amplification, i.e. peak imbalance (uneven amplification) and allelic dropout (see section 1.5). This phenomenon might also be caused by DNA degradation (see section 1.12). Other issues related to low levels of input DNA are presence of PCR artefacts, electrophoretic artefacts, -A peaks and dye blobs in the overall electrophoretic trace. Occasionally, peak signals are below the limit for labeling, causing problems in the data interpretation. In addition, low peak signals might cause a noisy baseline and a low signal to noise ratio.

When low peak signals display skewed and inconclusive ratios, a proper quantification might not be possible. Generally, peak signals should exceed values three times the baseline for a specific capillary electrophoresis instrument.

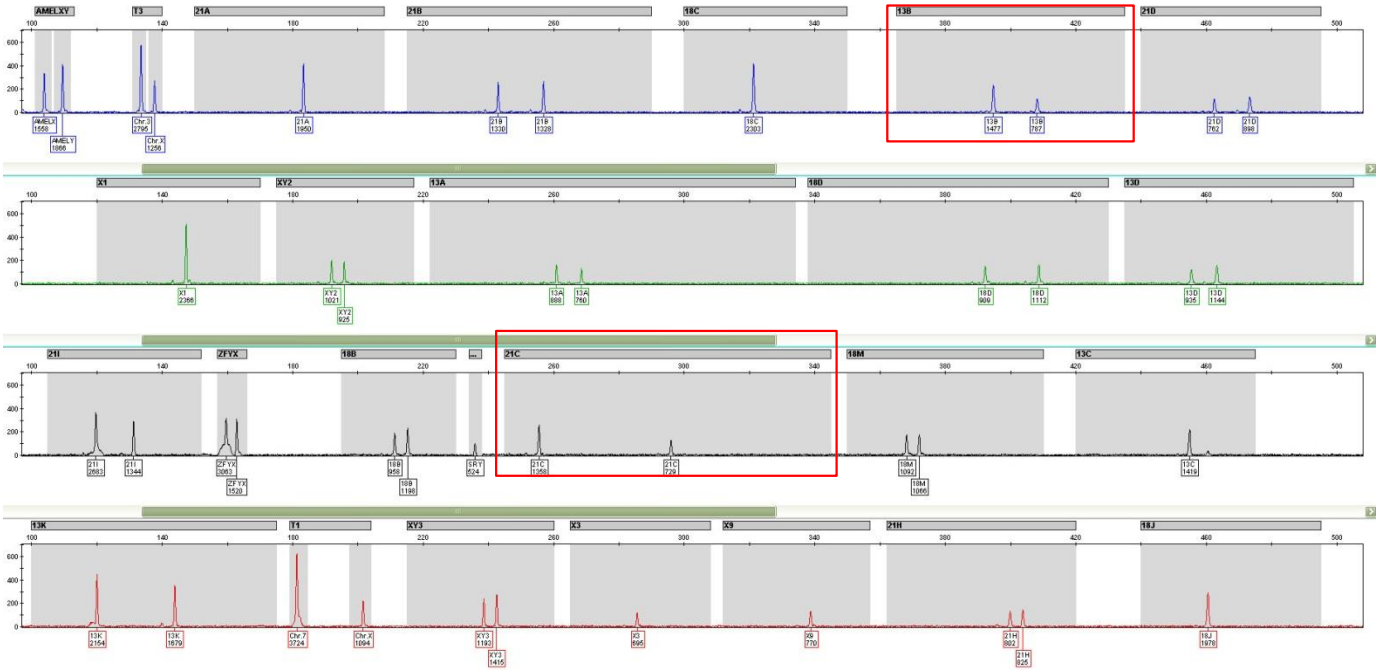


Figure 1.10. Low input DNA in Devyser Compact v3 causing marker ratio skewing as seen in markers 13B and 21C, as indicated by red boxes.

Possible solutions

Random PCR amplification that causes inconclusive marker ratios is best avoided by rerunning the PCR at higher input DNA. DNA should therefore be re-extracted and diluted in a lower volume. In case of DNA degradation, a re-extraction of sample DNA is recommended, using a validated DNA extraction method (see section 1.12).

In some cases, low peak signals might be caused by low quality HiDi Formamide in the capillary electrophoresis. Reinject the samples using fresh HiDi.

1.5. Allelic drop-out

Complete allelic drop-out may be observed as absent or low amplification of an individual marker or as a single peak detected in heterozygous markers. In trisomic markers, complete allelic drop-out may generate a disomic marker pattern. Skewed, inconclusive or abnormal marker ratios in a single chromosome specific marker (1:2, 2:1 or 1:1:1) may be caused by partial allelic dropouts.

Allelic dropouts may be observed at low peak signals as a result of a low input DNA in the PCR reaction. In this case, the allelic drop-out appears as a result of inadequate amounts of DNA template that in turn cause random PCR amplification (described in section 1.4).

If the DNA input is adequate and peak signals are within recommended ranges, allelic drop-outs may be caused by suboptimal PCR conditions such as incorrect annealing temperatures, ramping rates being too low or too fast, or because the ramping rates have been set according to block temperature and not to the calculated sample temperature.

Possible solutions

Consult the kit-specific Instructions for Use (IFU) to confirm that the correct ramping rates have been applied on the thermal cycler and that the ramping rates have been set to the calculated sample temperature mode. Make sure that an adequate amount of DNA is used in the PCR. Recommendations on sample DNA concentrations can be found in the kit specific IFU.

Verify that the recommended instrument service and calibration has been performed on the thermal cycler and that the instrument meets the temperature requirements. If the allelic drop-out does not seem to be related to the performance of the thermal cycler, the presence of a primer-binding site mutation might be possible. To investigate this further, repeat the PCR reaction with a decreased annealing temperature (0,5 - 1 ˚C). Please observe that modifying the PCR profile might impair the overall marker quality in the mix.

1.6. Saturated/overloaded peak signals

Saturated/overloaded peak signals may be caused by too high input DNA in the PCR and/or by a too high injection voltage/time during electrophoresis. Overloading may result in difficulties in data interpretation and incorrect results. Generally overloading of DNA/PCR product is characterized by saturated/overloaded signals, heavy crosstalk and truncated peaks. Overloaded peaks will also generate increased levels of PCR and electrophoretic artefacts, such as stutter, -A peaks, non-specific amplification and elevated baseline (Figure 1.11). Too high signals may also result in size calling errors and saturation of the capillary electrophoresis instrument (CCD camera), which makes it difficult to perform quantitative calculations. Importantly, excessive input DNA in the PCR may appear as signal sloping (explained in section 1.3). Longer fragments may be within the recommended signal level but should not be used for data interpretation.

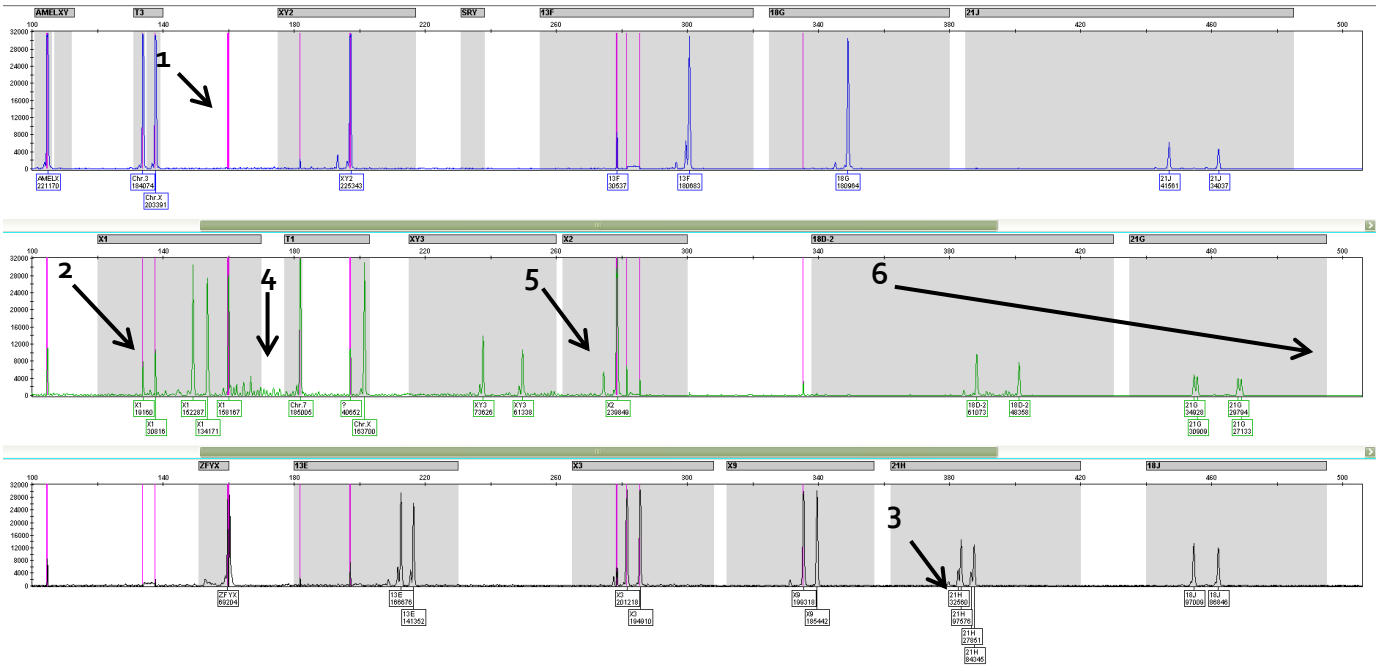


Figure 1.11. Results from a heavily overloaded PCR reaction using Devyser Complete v2 Mix 2.

1. The pink off-scale peaks indicate saturation of the CCD camera (GeneMapper Software)
2. Crosstalk from the blue to the green channel
3. - A peaks
4. Elevated/noisy baseline
5. Increased stutter with false peak stutter ratio
6. Signal sloping (easily observed in the sample Raw Data tab in GeneMapper)

Possible solutions

When signs of a suboptimal PCR are detected as a result of high input DNA in the PCR, e.g. -A peaks, truncated peaks and signal sloping, the original sample should be diluted and tested in a new PCR reaction. If CCD saturation is observed without signs of signal sloping, signal intensities can be reduced by decreasing the injection time or injection voltage which typically are proportional to the amount of injected PCR product (linear correlation). If signal sloping is detected after re-injecting the samples for a shorter time or with a lower injection voltage, a new PCR reaction is required to improve the data quality.

1.7. Marker ratio skewing

Marker skewing in heterozygous markers may generate inconclusive ratio calculations (consult the Best Practice Guidelines for QF-PCR at www.devyser.com/downloads or www.acgs.uk.com/quality/best-practice-guidelines for more information). The amplification of shorter length alleles is normally slightly more efficient than the amplification of longer length alleles. This phenomenon is known as preferential amplification of shorter length alleles and is commonly observed in fragment analysis. The degree of preferential amplification is normally low and does not affect the outcome of ratio calculations. In rare cases, extensive skewing might however occur in widely spaced alleles (>24 bp) because of a more pronounced preferential amplification. Occasionally this might cause inconclusive marker results (Figure 1.12).

A general skewing of markers might be caused by low input DNA in the PCR or by using too slow or too fast ramping rates in the PCR. Stutter peaks or crosstalk peaks falling in the allele bin may also cause marker skewing.

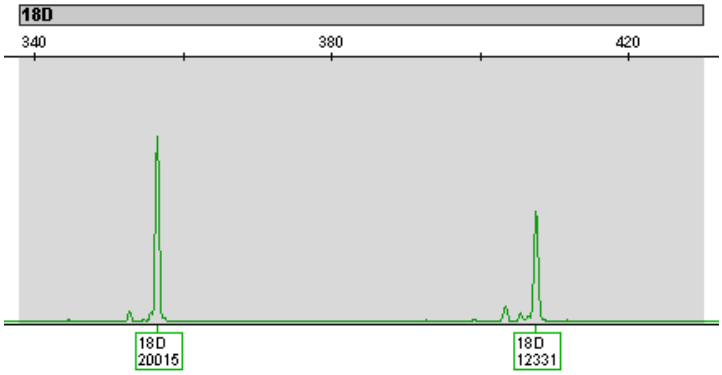


Figure 1.12. Results from marker 18D with widely spaced alleles causing inconclusive marker ratio in Devyser Compact v3.

Possible solutions

Consult the kit-specific Instructions for Use (IFU) to confirm that the correct ramping rates have been applied on the thermal cycler. Make sure that an adequate amount of DNA is used in the PCR. Recommendations on sample DNA concentrations can be found in the kit specific IFU.

1.8. Split peaks

Split peaks appear as two peaks of similar length, commonly originating from a single PCR product that has been altered or has a different structure (Figure 1.13). Split peaks affecting all markers might be caused by DNA degradation whereas split peaks in one single marker might be related to the design of that specific marker, e.g. microdeletion in the primer site. High salt concentrations may also generate split peaks because of secondary structure formation. Further, split peaks may be a result of a pronounced incomplete addition of the terminal A base, generating a -A and a +A peak of equal heights.

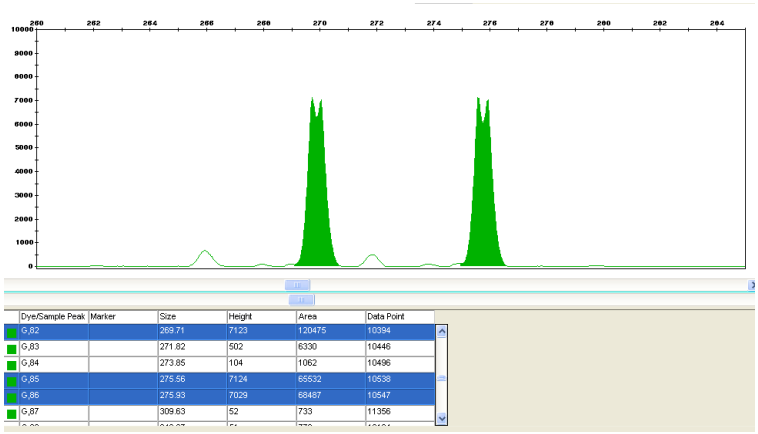


Figure 1.13. A heterozygous diallelic marker that is reported as a triallelic marker due to peak splitting.

Possible solutions

In case of DNA degradation, re-extract the DNA using a validated DNA extraction method (see section 1.12) and repeat the PCR. Salt concentrations can be reduced by purification of the PCR products. To avoid -A peaks, make sure that adequate amount of DNA is used in the PCR and that the ramping rates are correctly given in the PCR.

1.9. Shoulder peaks

Shoulder peaks are small peaks that are one nucleotide shorter or longer than the real marker peak (Figure 1.14). Shoulder peaks may appear because of light exposure or repeated freeze-thaw cycles that adversely affect the quality of the fluorescent primers. The quality of the amplified PCR fragments might also be compromised by prolonged storage in HiDi Formamide.

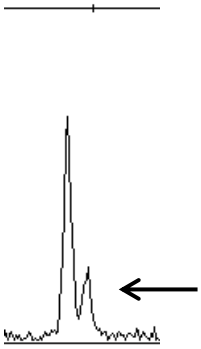


Figure 1.14. Results from a marker showing a shoulder peak that is one nucleotide longer than the real marker peak (indicated by the arrow).

Possible solutions

Avoid prolonged light exposure and repeated freeze-thaw cycles of the PCR mix and the PCR products. Start the electrophoresis immediately after adding the PCR product to the loading cocktail.

1.10. Incomplete mixing of reaction mix

Incomplete mixing of the activated reaction mix will show peak heights declining with size (black arrow in Figure 1.15) and extensive -A peaks generating a peak appearance equal to that of split peaks (red box).

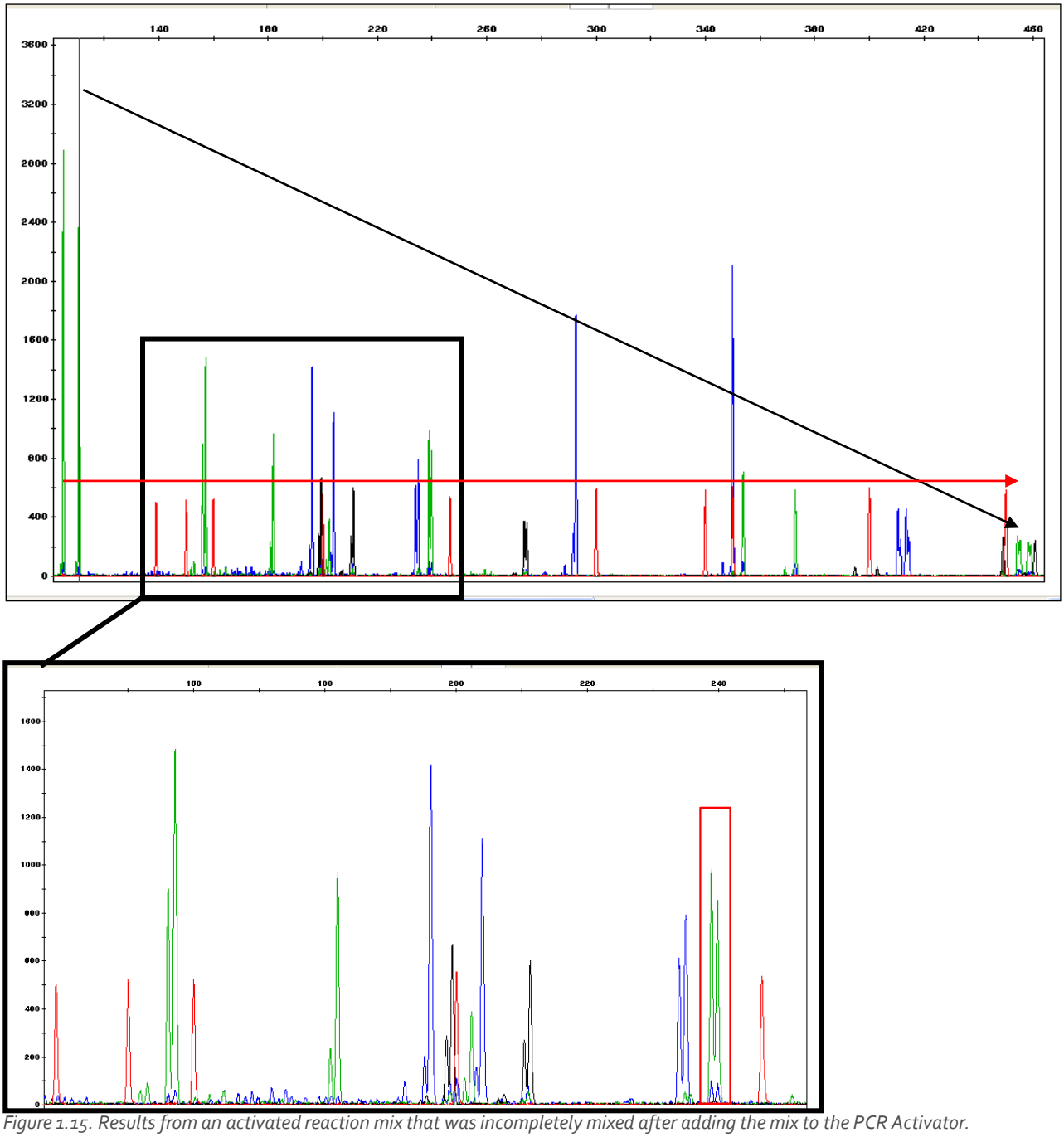


Figure 1.15. Results from an activated reaction mix that was incompletely mixed after adding the mix to the PCR Activator.

Possible solutions

Make sure that the PCR Activator is thoroughly mixed with PCR Mix by pipetting manually several times from the bottom of the tube (see kit specific IFU).

1.11. Incorrect sizing

Although the sizing quality in GeneMapper is good, incorrect identification of the size marker fragments by the software may sometimes occur. This might be caused by sample overloading, spectral overlap or insufficient run times. In Figure 1.16, the sizing is incorrect due to insufficient run time. The longest fragment of the size marker can therefore not be identified and consequently the sizing pattern is incorrect (see labeling and compare to the expected pattern in Figure 1.17). As a result, the sizing of the marker alleles in Devyser Complete v2 did not appear as expected, seen by markers falling outside or into wrong bins in Figure 1.18.

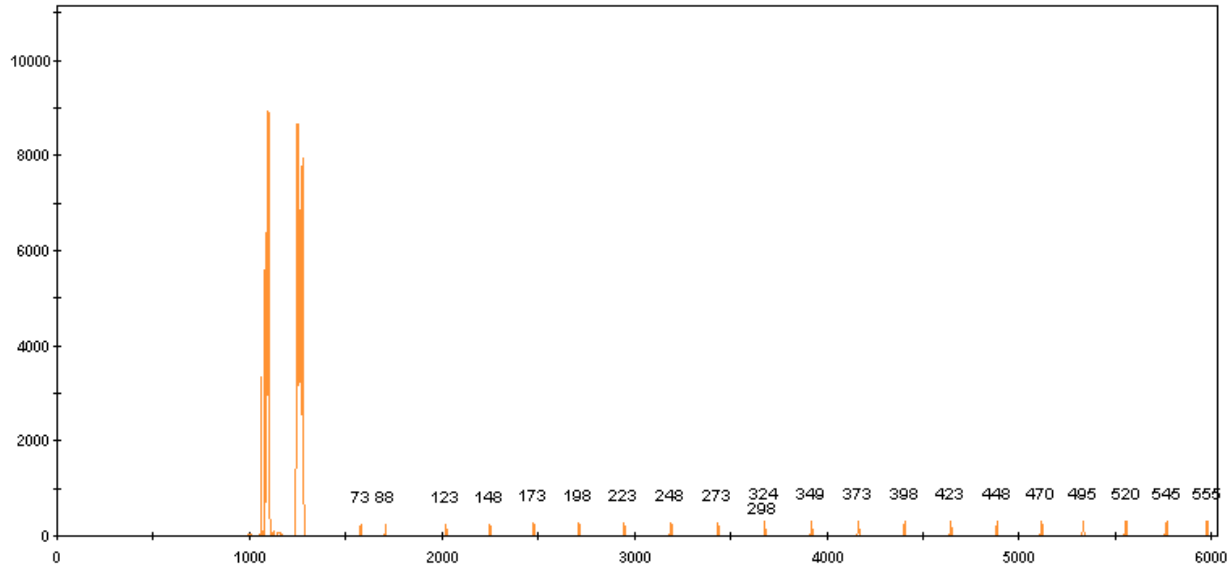


Figure 1.16. Incorrect sizing of the Devyser 560 SIZER ORANGE size marker due to insufficient run time (20 out of 21 fragments detected).

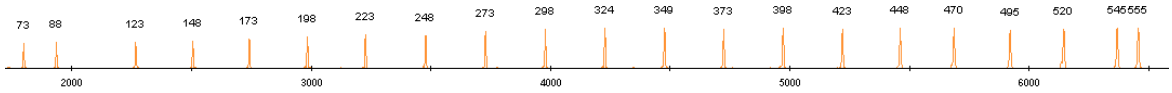


Figure 1.17. The expected size standard pattern for Devyser 560 SIZER ORANGE (21 fragments)

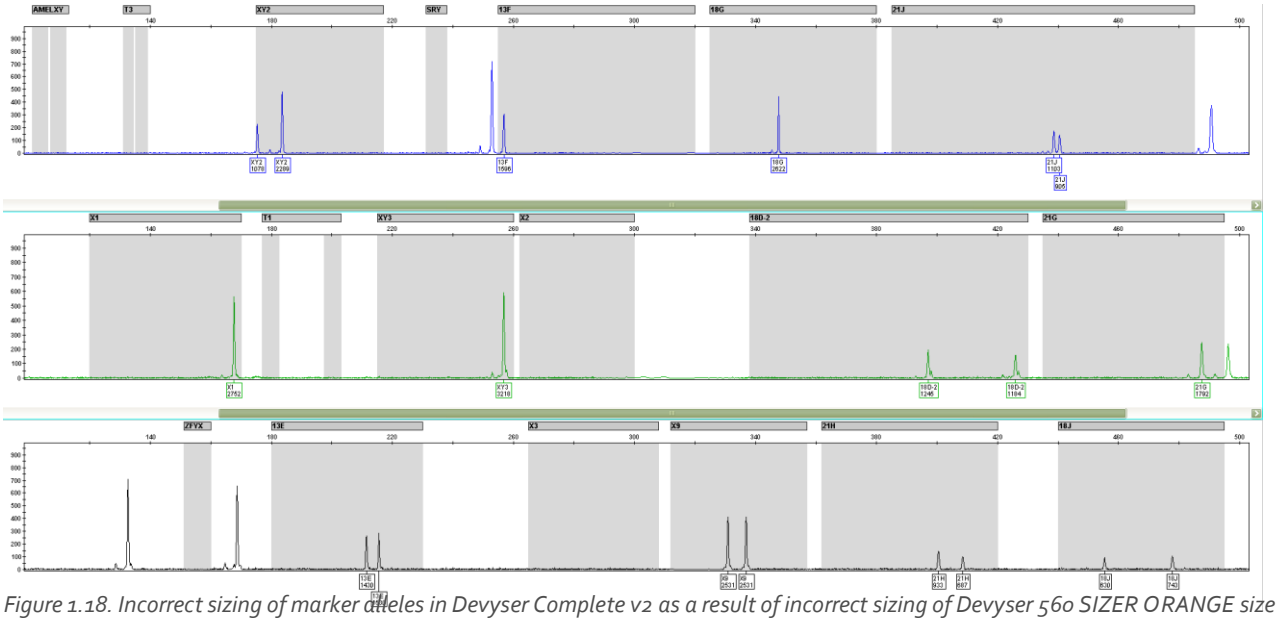


Figure 1.18. Incorrect sizing of marker alleles in Devyser Complete v2 as a result of incorrect sizing of Devyser 560 SIZER ORANGE size marker.

Possible solutions

Adjust the size standard manually in the Size Match Editor in GeneMapper according to the expected size marker pattern and re-analyze the sample. Always inspect the size standard in the Samples Plot in order to check the quality of the electrophoresis.

1.12. DNA Degradation/Poor DNA quality

Degraded DNA may result in inefficient or no amplification of the longer length alleles (allele dropout). Capillary electrophoresis data from a degraded DNA sample commonly shows the signal sloping pattern of the marker alleles as described in section 1.3. DNA degradation may also cause uneven amplification.

Possible solutions

Repeat the PCR reaction and detection with purified or re-extracted DNA.

1.13. DNA Inhibition/PCR Inhibition

DNA inhibition may result in inefficient or no amplification as well as allele dropout from any location. Hence no specific pattern can be expected in case of DNA/PCR inhibition.

Possible solutions

Determine the DNA quality. Repeat the PCR reaction and detection with purified or re-extracted DNA. PCR inhibitors may also be diluted by diluting the DNA.

1.14. DNA Contamination

Contamination by other samples will result in marker alleles located inside and/or outside marker ranges. Contamination may occur in any of the steps in the QF-PCR workflow, i.e. sampling, DNA extraction, DNA dilution, PCR setup and detection. If the DNA is contaminated with another sample, two genotypes will be observed in the electrophoretic trace. If present in equal amounts, the marker signals from both genotypes will be of similar height. Normally this is not the case and the resulting peak areas may be inconclusive if the contaminating peaks are included in the analysis. In other words, the contaminating genotype commonly has lower peak signals (see Figure 1.19). An extra genotype might result in detection of four alleles in one marker (see markers 18C, 13A, 18D, 21I, 21H and 18J in Figure 1.19, red boxes). If the contaminating peaks have the same allele sizes as the original sample, inconclusive ratios may be observed.

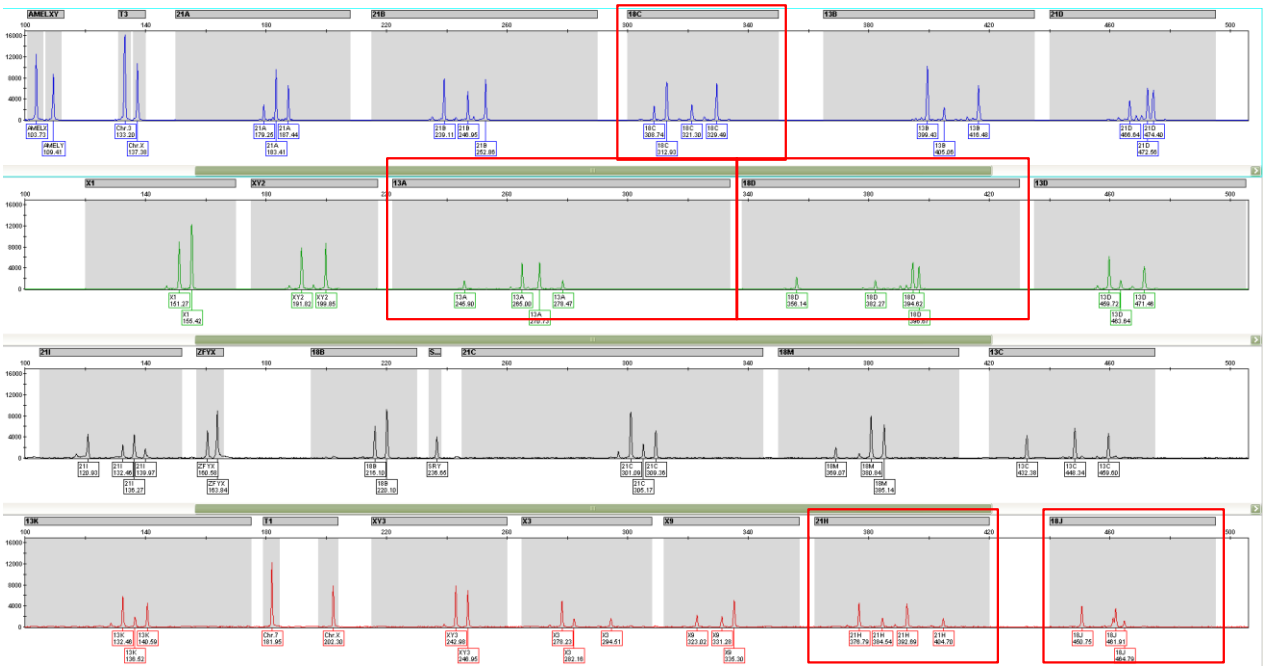


Figure 1.19. Contaminated sample showing two different genotypes in Devyser Compact v3.

Possible solutions

Contamination of the PCR mix might be excluded by including a negative control in the PCR reaction (e.g. water). If contamination of the extracted DNA is suspected, repeat the PCR reaction and detection with re-extracted DNA. If contamination of the original sample is suspected (i.e. blood, AF or CVS) a new sampling is required.

If contamination is suspected to have occurred in the PCR setup and/or detection, make sure to use fresh reagents (PCR Mix, PCR Activator, HiDi Formamide and electrophoresis running buffer), clean instrumentation, and to work in a unidirectional manner as described in the kit-specific IFU.

1.15. High baseline/background

The baseline, i.e. the minimal intensity of each dye, is the observed intensity when the laser is activated and the starting amount of fluorescence is measured by the instrument. The baseline is expected to be low. A high baseline will decrease the sensitivity of detection by narrowing the dynamic range.

The baseline can be estimated from the signal intensity observed at the start of the run. A high baseline might be caused by bad quality of the spectral calibration, overloading of the instrument or by a dirty capillary window. A high baseline might in turn generate high background signals. Background signals are non-specific peaks that may be observed inside or outside marker ranges as well as in the spectral calibration run. These non-specific peaks are normally caused by contamination of the electrophoresis instrument.

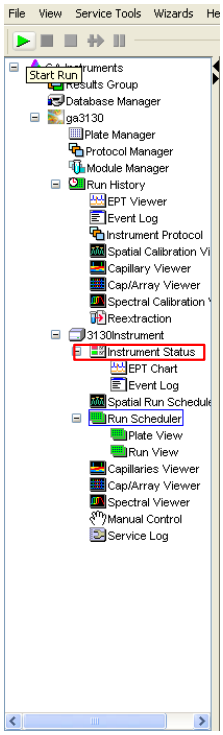
Possible solutions

Contamination of the electrophoresis instrument can be investigated by running HiDi Formamide with or without size marker. If non-specific peaks are observed, the instrument can be cleaned by performing a water wash. Consult the technical support at Life Technologies for further instrumental support.

1.16. Failed spectral calibration

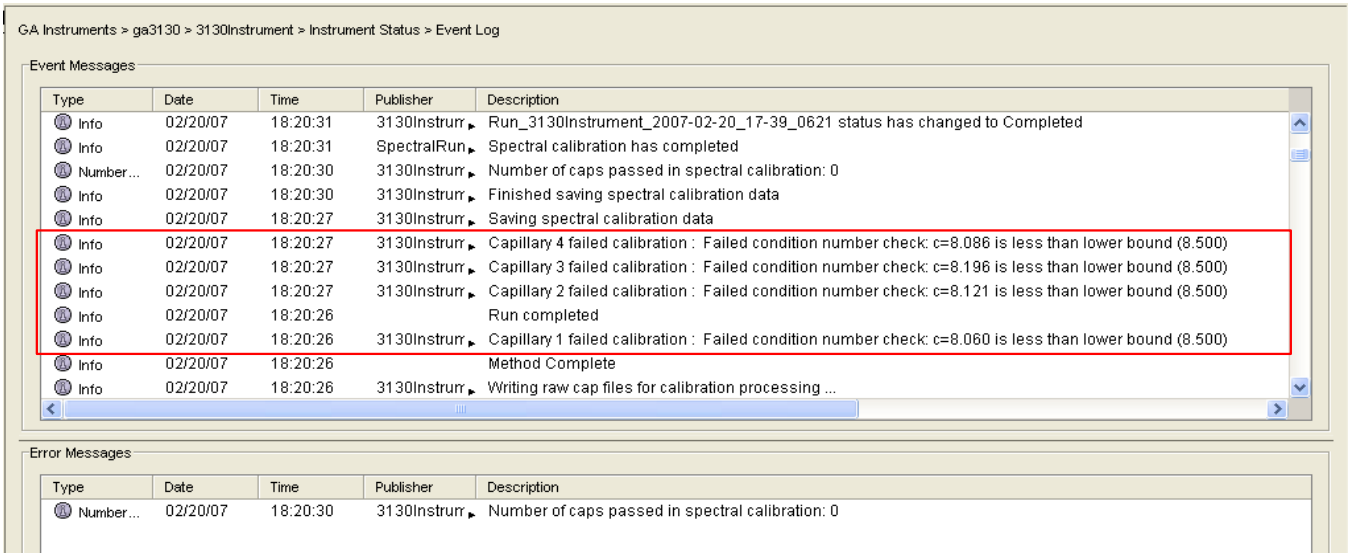
In a failed spectral calibration, one or more capillaries did not meet the requirements for passing the spectral calibration. The spectral calibration might fail because of too high or low signals of the DNA fragments, failed identification of the peaks or peak order, extra peaks or condition or quality values falling outside the recommended ranges. For further details refer to the Instrument specific user guide, DEV-5 Spectral Calibration Protocols and the DEV-5 Dye Set MultiCap kit insert. DEV-5 Spectral Calibration Protocols and kit inserts can be downloaded from the Devyser homepage at www.devyser.com/downloads.

1. To find out more information about the failed spectral calibration, select Instrument Status on the left navigation window in the Instrument Data Collection Software. The Event Log appears.



2. The Event Log shows details about the failed spectral calibration in the Event Messages box. In the Description field below, the erroneous spectral calibration run is described (marked in red).

In this example the calibration failed due low condition number falling outside the recommended range.



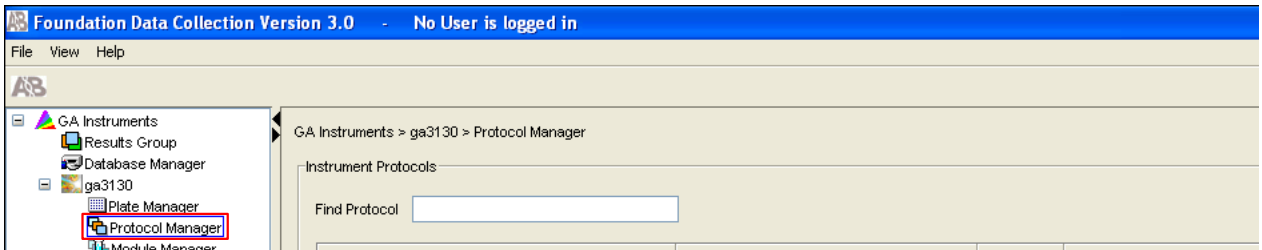
Possible solutions

As described in the kit insert for DEV-5 Dye Set MultiCap kit, the DEV-5 dilutions may need to be optimized due to instrument-to-instrument variation in the sensitivity of detection. If the spectral calibration fails due to saturated peak signals, perform a rerun of the spectral calibration with a new DEV-5 dilution containing half the amount of DEV-5. If signals are too low, dilute the DEV-5 reagent 1:10 instead of 1:20 in HiDi Formamide.

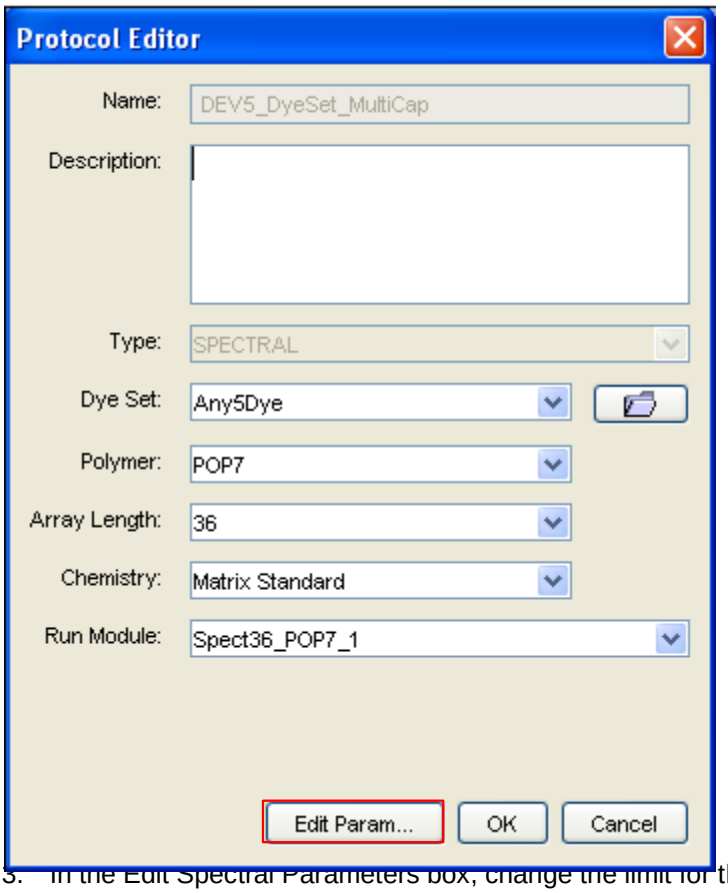
If extra peaks are detected in the spectral calibration, this might indicate a contamination in the Genetic Analyzer instrument. In this case, perform a water wash according to the instrument specific user guide. Contact technical support at Life Technologies for further details.

If the spectral calibration fails because of the condition value or the quality value, as exemplified on page 18, the spectral parameters can be adjusted before rerunning the spectral calibration. Follow the steps below.

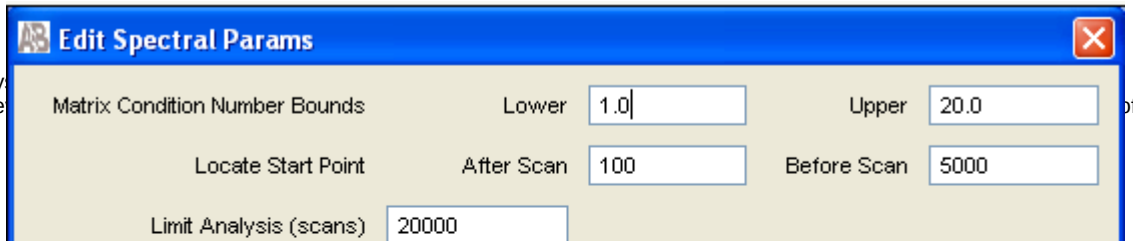
1. Select Protocol Manager on the left navigation window in the Instrument Data Collection Software. Highlight the spectral calibration module to be modified and select Edit. The Protocol Editor appears.



2. In the Protocol Editor, select Edit Param…



3. In the Edit Spectral Parameters box, change the limit for the condition number or the Minimum Quality Score according to the spectral calibration results. Click OK.



4. Rerun the spectral calibration using the new parameters.

2. RESULTS INTERPRETATION

2.1. Maternal cell contamination (MCC)

Maternal cell contamination (MCC) is commonly observed in blood stained amniotic fluid samples and low-quality chorionic villi samples (CVS). In MCC, two genotypes are observed where one allele is shared between the mother and the fetus (see Figure 2.1). No more than 3 alleles are observed in the case of MCC. In the case of external sample contamination, such as twin, external DNA contamination or chimerism, 4 alleles may be observed (see section 1.14). Where three alleles are detected, the shared allele typically has the same peak area as total area of the maternal allele and the fetal allele together.

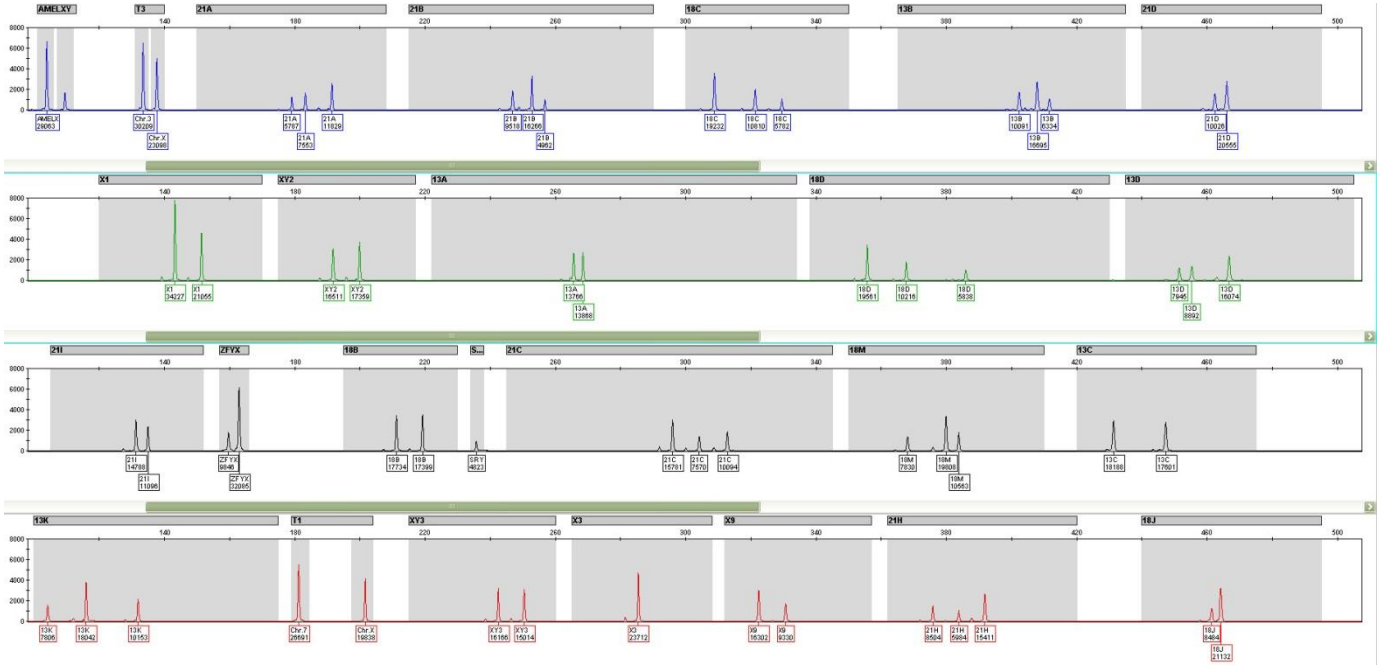


Figure 2.1. Amniotic fluid sample displaying maternal cell contamination, Devyser Compact v3.

Considerations

In the case of MCC, it should be possible to identify the fetal genotype but should not be interpreted if inconclusive allele ratios are observed. Interpretation of the results might be possible when the level of MCC is low and there are no inconclusive allele ratios in the fetal genotype. It is however recommended to run the maternal sample in order to identify the maternal alleles and, if possible, exclude them from the analysis and perform data interpretation of the fetal genotype. Consult the Best Practice Guidelines for QF-PCR at www.devyser.com/downloads or www.acgs.uk.com/quality/best-practice-guidelines for more information. A more in depth whitepaper called “QF-PCR and maternal cell contamination” by Dr. Kathy Mann can be found at the following location https://devyser.com/resources/downloads/whitepapers/.

2.2. Mosaicism

Mosaicism can be detected as extra peaks (third allele) and/or skewed allele ratios in markers located on the same chromosome.

Mosaicism can be detected by QF-PCR in cases when the abnormal cell line constitutes at least 15% of the total sample. The mosaicism is likely to have meiotic origin, i.e. non-disjunction at meiosis I or II, if trisomic markers with three differently sized alleles are observed (see Figure 2.2). If the trisomic markers show two differently sized alleles with skewed allele ratios, the origin of the mosaicism is more likely to be mitotic, i.e. non-disjunction after fertilization. Discrepant results between QF-PCR analysis and karyotyping, especially in CVS samples, might suggest confined placental mosaicism (CPM). In CPM the cytogenetic abnormality is confined to the placental tissue whereas the fetal tissue is normal (2).

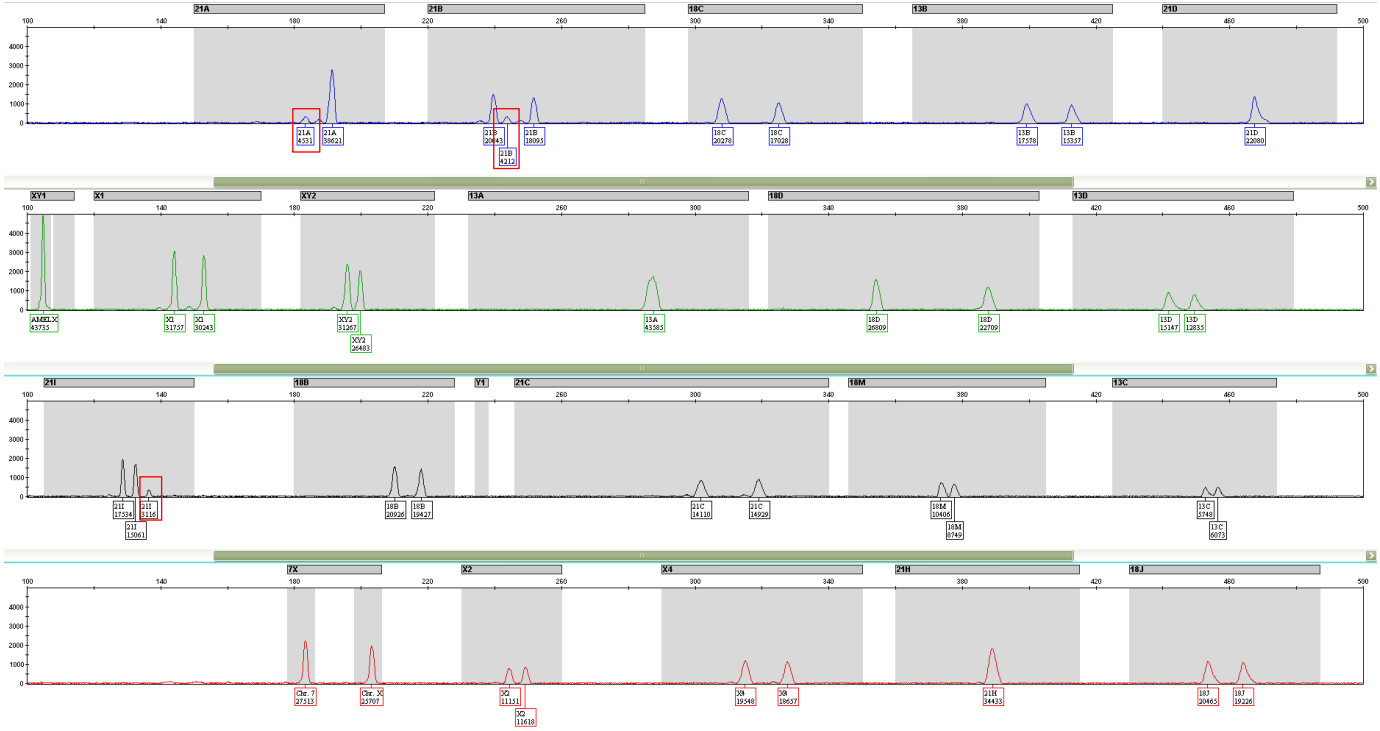


Figure 2.2. Mosaic trisomy 21, Devyser Compact (abnormal genotype marker in red boxes)
Electrophoretic trace kindly provided by Dr. A. Reparaz-Andrade, Complexo Hospitalario Universitario de Vigo, Spain.

Considerations

Consult the Best Practice Guidelines for QF-PCR at www.devyser.com/downloads or www.acgs.uk.com/quality/best-practice-guidelines for more information. A more in depth whitepaper called “Detecting mosaicism by QF-PCR” by Dr. Kathy Mann can be found at the following location https://devyser.com/resources/downloads/whitepapers/.

2.3. Copy number variation (CNV)

Copy number variants (CNV) are inherited changes that may be observed as single abnormal markers flanked by normal markers. CNVs are duplications or deletions that can occur as a result of unequal chromosomal crossover.

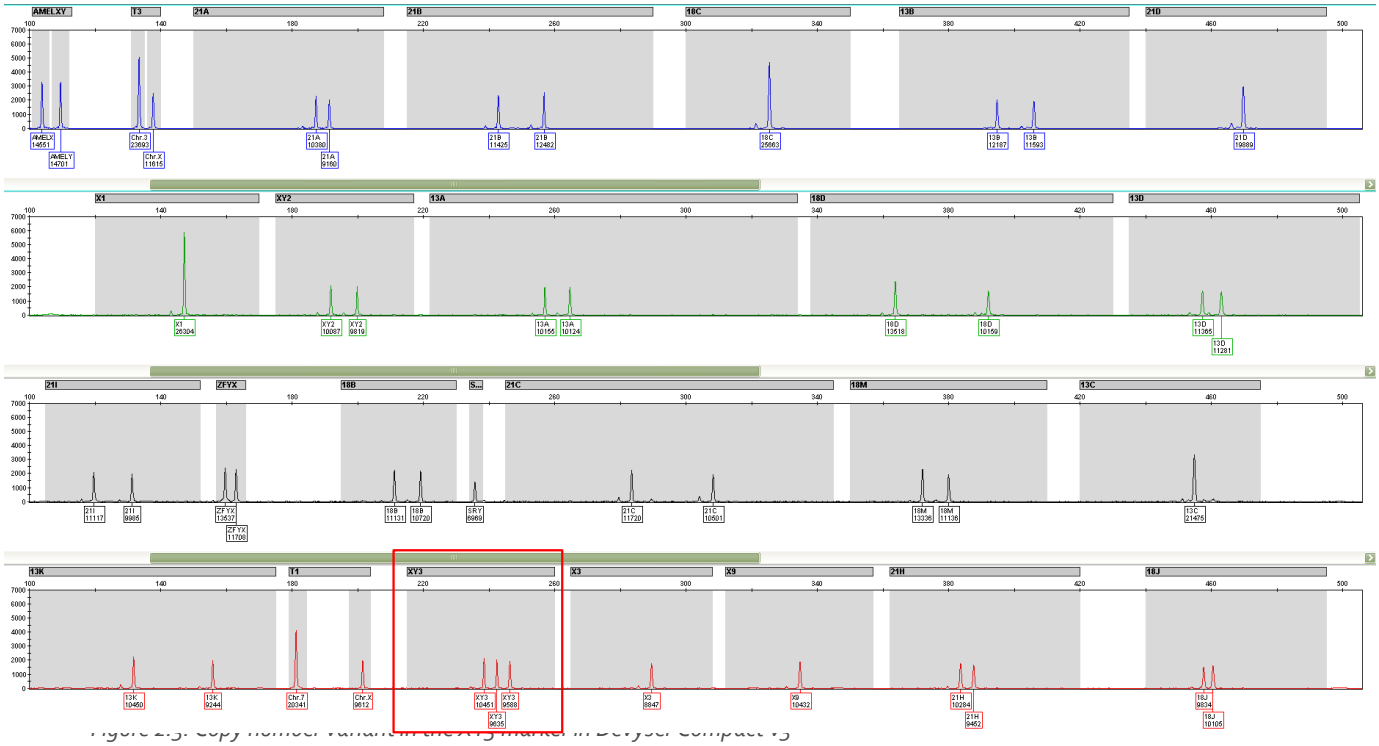


Figure 2.3. Copy number variant in the XY3 marker in Devyser Compact v3

Considerations

Analysis of parental samples may provide additional information when investing a potential CNV. Consult the Best Practice Guidelines for QF-PCR at www.devyser.com/downloads or www.acgs.uk.com/quality/best-practice-guidelines for more information.

2.4. Partial chromosome trisomy

Partial chromosome trisomy might be suspected when informative markers for a single chromosome demonstrate normal ratios (1:1) on one chromosomal arm and abnormal ratios (1:2, 2:1 or 1:1:1) ratios on the other chromosomal arm. Since Devyser aneuploidy products have not been validated for detection of partial chromosome abnormalities, other techniques are recommended to confirm a suspected partial trisomy.

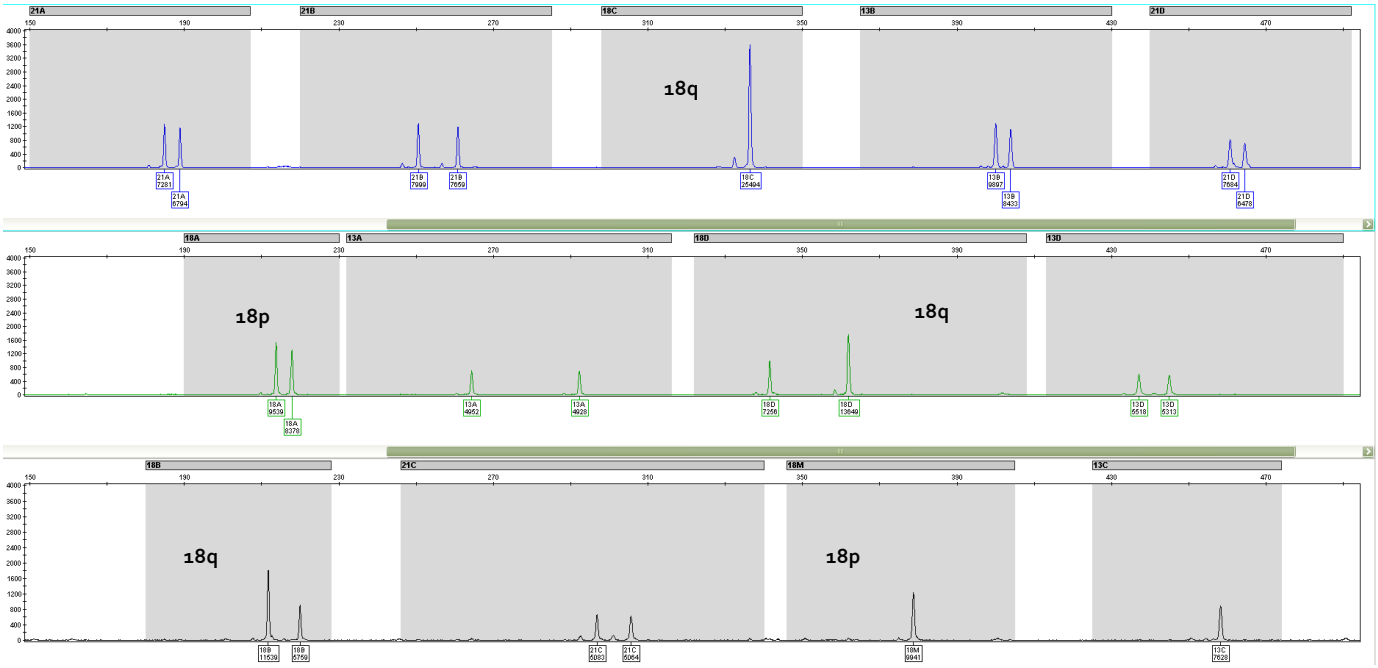


Figure 2.4.1. Sample with suspected partial chromosome 18q trisomy, Devyser Complete Mix 1

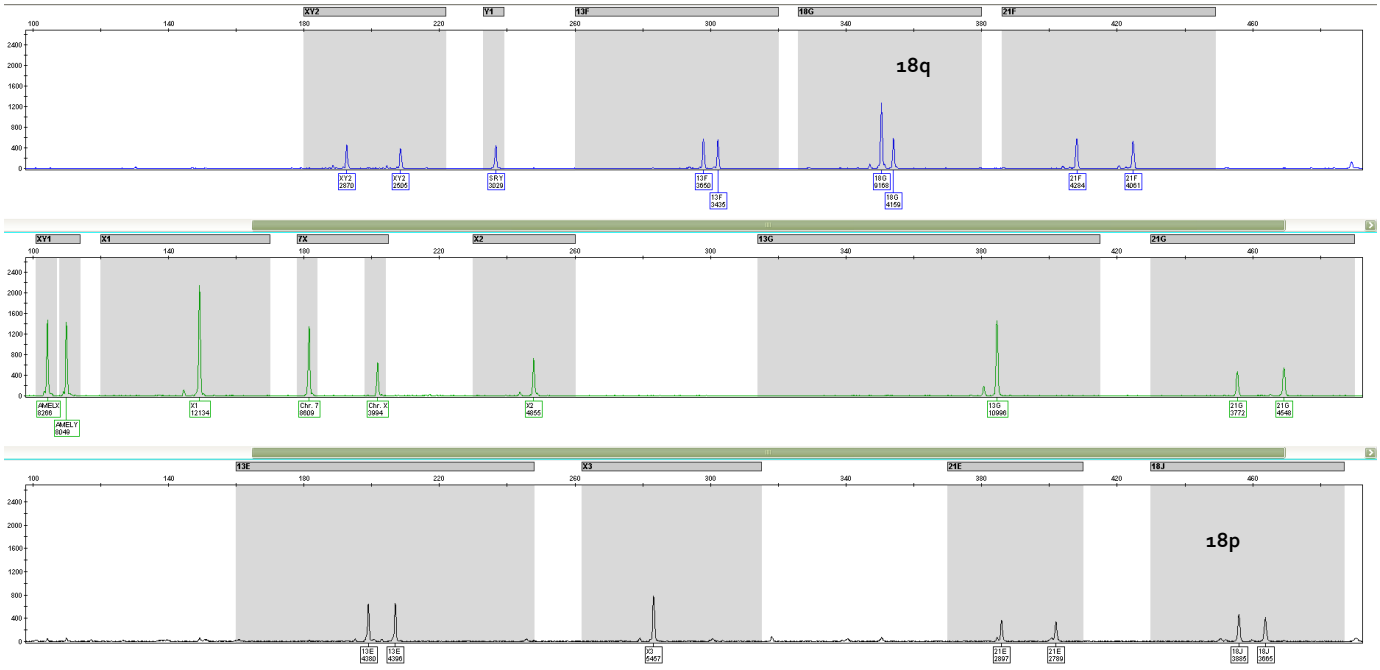


Figure 2.4.2. Sample with suspected partial chromosome 18q trisomy, Devyser Complete Mix 2
Electrophoretic traces kindly provided from Brno, Czech Republic.

2.5. Somatic microsatellite mutation (SMM)

SMM's usually involves an increase of 1-2 repeats in the repeat unit (microsatellite) and can be identified in a profile when present in a subpopulation of cells (mosaic). Somatic microsatellite mutations (SMMs) are more frequently observed in chorionic villi samples (CVS) than in amniotic fluid (AF) samples. SMM is detected as a novel allele at a single locus and is probably caused by a mitotic replication error. In CVS samples, SMM may affect only one of several villi investigated.

SMM appears either as 3 alleles where A+B=C (A and B are the peaks with reduced height), as exemplified in Figure 2.5, or as a skewed di-allelic ratio. The sum of A and B should give a normal ratio when compared with the unaffected allele, C.

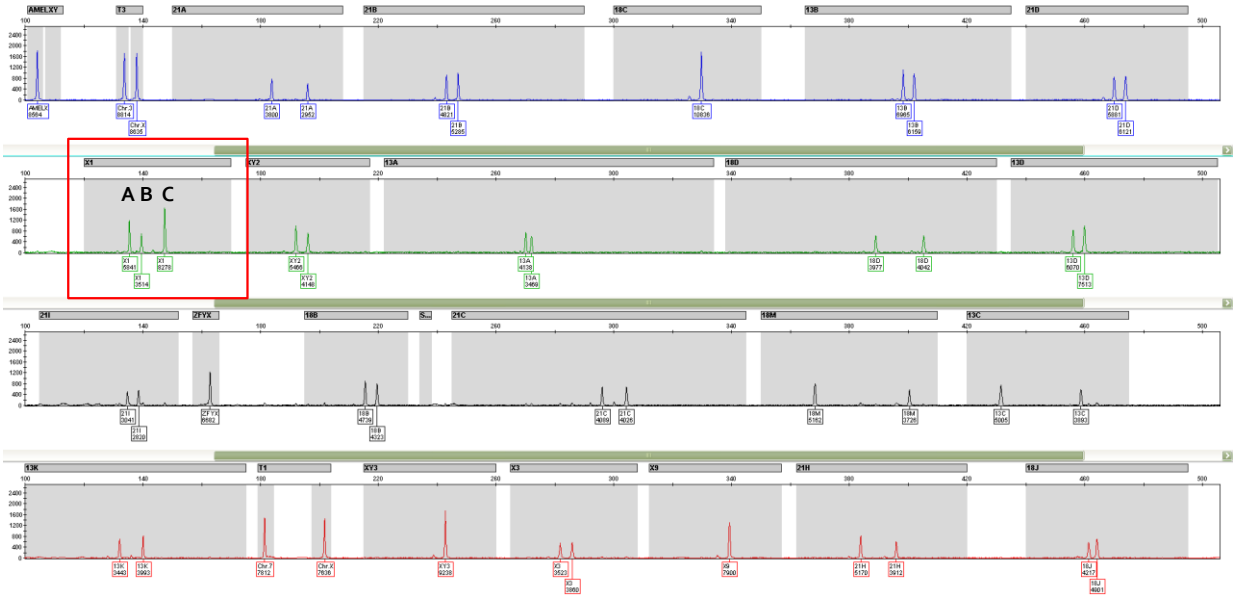


Figure 2.5. Sample with a somatic microsatellite mutation observed in marker X1, Devyser Compact v3 Electrophoretic traces kindly provided from Prague, Czech Republic.

Considerations

SMMs are mosaic and may therefore be classified by testing a different cell population from the same patient, i.e. other villi or cultured cells. Further, if the SMM displays 2:1 or 1:2 ratios, testing a different population may also make it possible to distinguish SMM from a CNV (see section 2.3).

Consult the Best Practice Guidelines for QF-PCR at www.devyser.com/downloads or www.acgs.uk.com/quality/best-practice-guidelines for more information.

2.6. Microvariant

Microvariants are alleles containing partial repeat units. Tetranucleotide STR markers normally have four bases in the repeat units of the core repeat. Occasionally it is however observed that that the marker peaks are not separated by a multiple of four. The partial tetranucleotide repeat in the microvariant can be 1, 2, or 3 bases long. Alleles with incomplete repeat units exist in most STR loci.

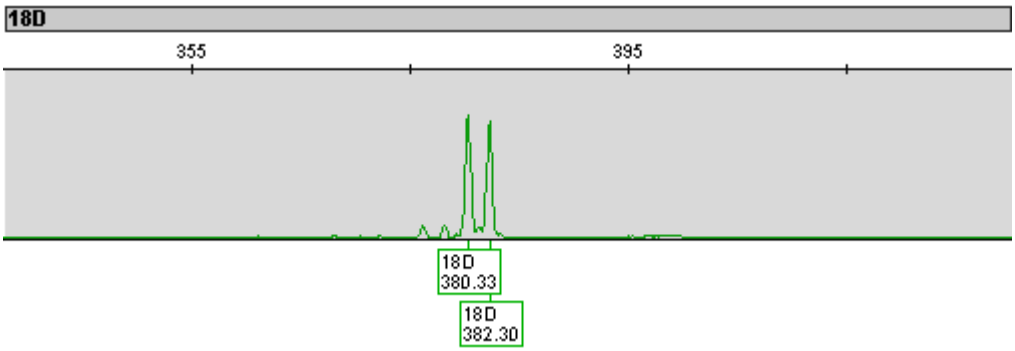


Figure 2.6. Microvariant in a heterozygous 18D marker (tetranucleotide STR). The alleles are separated by two base pairs, and not four which is expected if separated by one tetranucleotide repeat.

3. REFERENCES

1. ACGS best practice guidelines for use of Quantitative Fluorescence-PCR for the detection of aneuploidy v4 (2018).

2. Detection of Mosaicism for primary trisomies in prenatal samples by QF-PCR and karyotype analysis, Prenat Diagn 2005; 25:65-72