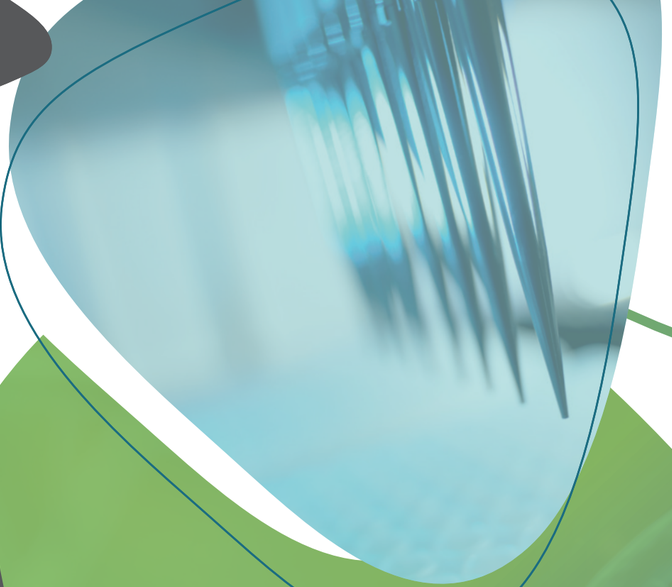
Promega

DNA STR ANALYSIS

TROUBLESHOOTING GUIDE

DNA STR ANALYSIS TROUBLESHOOTING GUIDE



Dear Scientist,

Have you ever wished you had a guide to help you resolve some of your analysis problems? Well, now you have it. I have collected the most common problems that occur during STR analysis and created this STR Troubleshooting Guide. In it, you will find many examples of mishandling during the analysis workflow, and how that mishandling negatively impacts STR analyses. Of course, this guide also provides details on the correct technique for each step.

I hope this guide will help you optimize your analysis workflow and make your workday a little easier.

Kindest regards, *Rita*

Rita Weispfenning Ph.D.
Technical Support and Training Manager
Genetic Identity EMEA

Troubleshooting STR Analysis

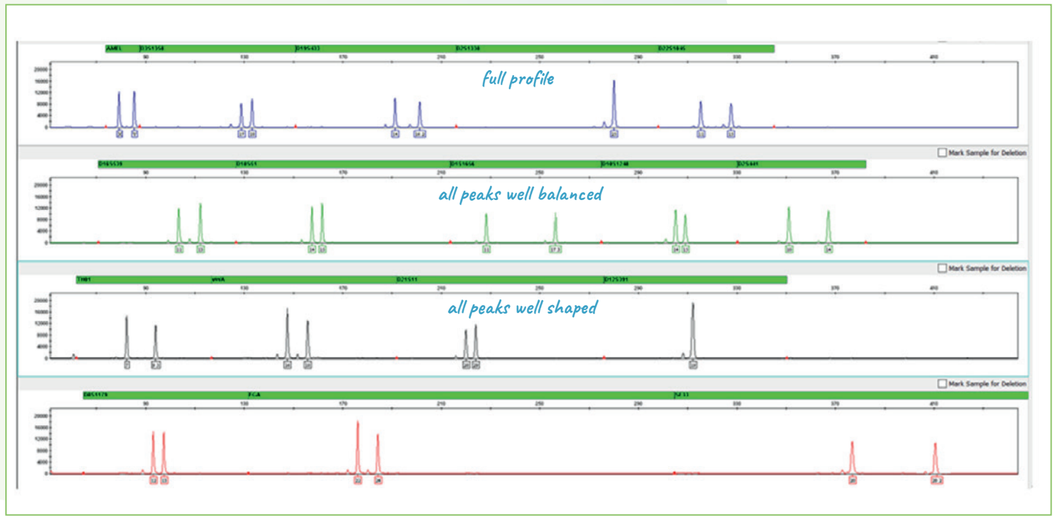
Consequences of Improper Handling in the Analysis Workflow

STR Analysis Workflow – each step is important

- DNA Extraction
- DNA Quantification
- DNA Amplification
- DNA Separation and Detection

STR Profile

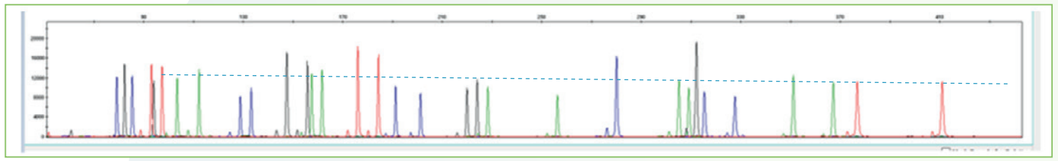
"In an Ideal World..."



PowerPlex® ESI 17 Fast System. Amplified product was separated using an Applied Biosystems® 3130xl Genetic Analyzer (3 kV, 5-second injection).

STR Profile

"In an Ideal World..."



PowerPlex® ESI 17 Fast System. Amplified product was separated using an Applied Biosystems® 3130xl Genetic Analyzer (3 kV, 5-second injection).

- Full profile
- Good intra-locus balance — *sister allele balance*
- Good balance within dye channels — *intra dye balance*
- Good balance between dye channels — *inter dye balance*
- Good peak morphology
- Peak heights at optimal level

Overview of recommended peak heights levels at various CE instruments

Instrument	Recommended signal level	Fluorescence saturation
Spectrum Compact CE System	175–12,000 RFU	30,000 RFU
Applied Biosystems® 3500 Genetic Analyzers	175–10,000 RFU	30,000 RFU
Applied Biosystems® 3730 Genetic Analyzers	150–10,000 RFU	30,000 RFU
Applied Biosystems® 3130, 3130xl Genetic Analyzers	150–4000 RFU	8000 RFU
ABI PRISM® 310 Genetic Analyzers	150–4000 RFU	8000 RFU

STR

DNA EXTRACTION

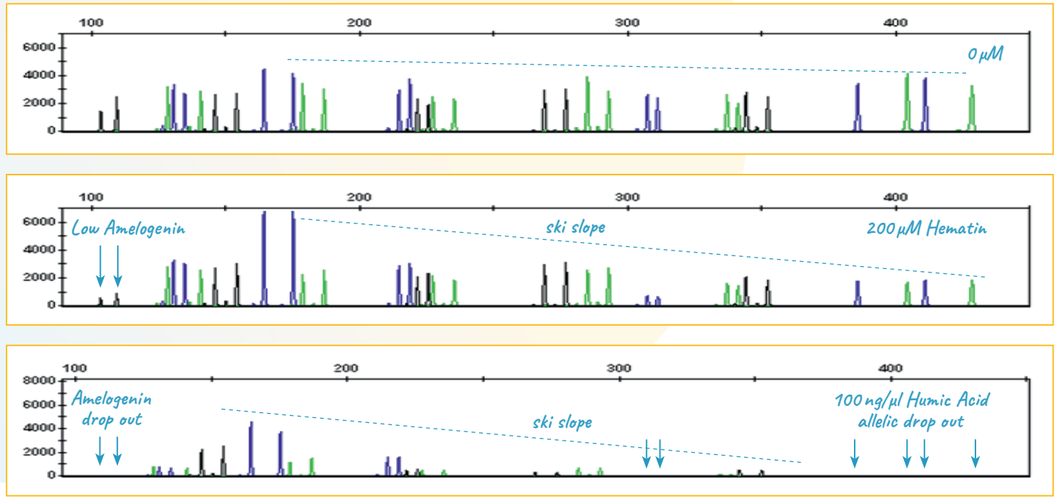
STR Analysis Troubleshooting

DNA Extraction

- Use an extraction method that efficiently removes PCR inhibitors and provides pure and clean DNA
- Elute DNA in TE-4 buffer *e.g. elution buffer in DNA IQ™ system*
- Prevent ethanol carry over

Ski Slope and Reduced Amelogenin in STR Profile

PCR Inhibitors

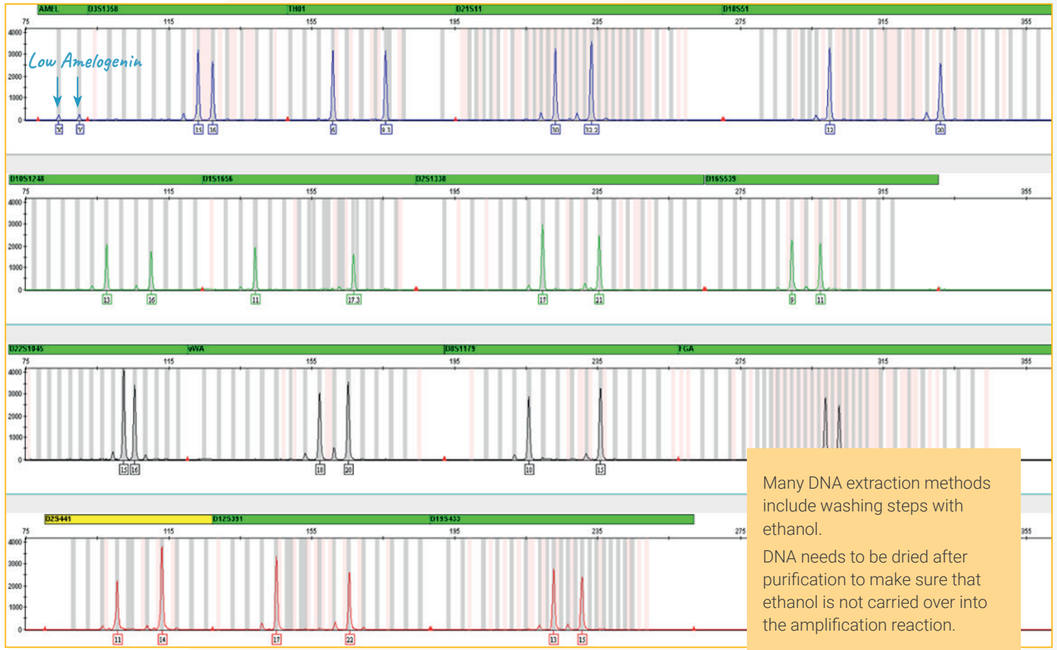


PowerPlex® 16 HS System. Amplified product was separated using an Applied Biosystems® 3130xl Genetic Analyzer (3 kV, 5-second injection).

Use an extraction method that is able to remove PCR inhibitors!

Low Amelogenin in Otherwise Good STR Profile

Ethanol Carry Over



Many DNA extraction methods include washing steps with ethanol.

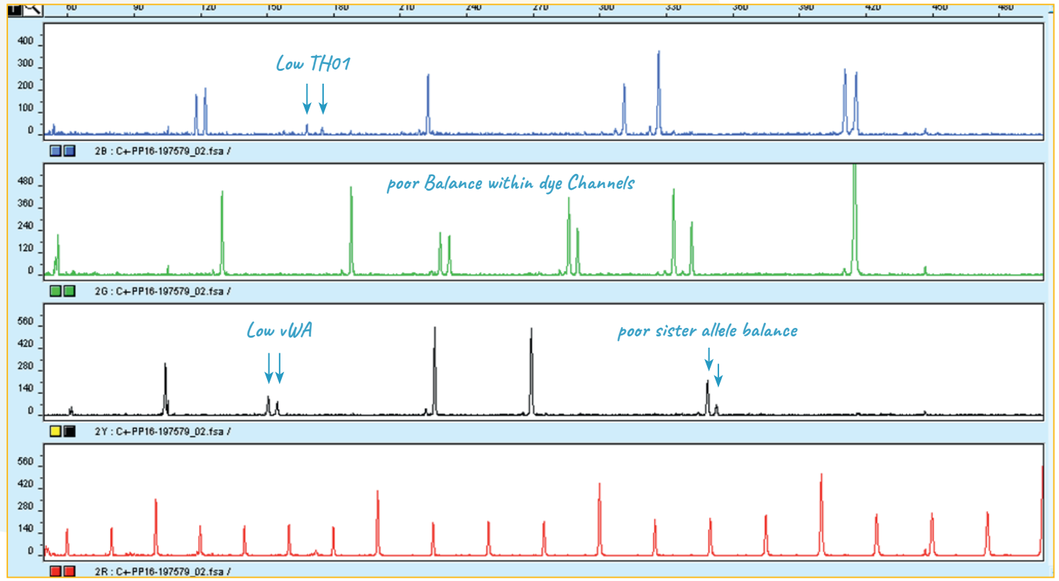
DNA needs to be dried after purification to make sure that ethanol is not carried over into the amplification reaction.

PowerPlex® ESX 17 Fast System. Amplified product was separated using an Applied Biosystems® 3500 Genetic Analyzer (1.2 kV, 24-second injection).

Do not omit or shorten the drying step in the DNA extraction workflow!

Imbalanced STR Profile

TE Buffer ($T_{10}E_1$) Used for DNA Elution



PowerPlex® 16 System. Amplified product was separated using an Applied Biosystems® 3130xl Genetic Analyzer (3 kV, 5-second injection).

Elute DNA in TE-4 buffer!

Good to know about TE ($T_{10}E_1$) Buffer

Key Details

TE ($T_{10}E_1$) buffer contains a high EDTA concentration.

Consequence:

- EDTA chelates or binds with magnesium

  Amount of available Mg^{2+} ions is reduced
  Polymerase needs Mg^{2+} ions to function well

- Polymerase activity is reduced

  Some loci are more sensitive to reduced polymerase activity than others

Imbalanced STR Profile

TE Buffer ($T_{10}E_1$) Used for DNA Elution

If profiles show signs of imbalance due to the use of $T_{10}E_1$ buffer, use TE-4 buffer instead.

TE^{-4} buffer
- 10 mM Tris-HCl
- **0.1 mM** EDTA (pH 8,0)

$T_{10}E_1$ buffer
- 10 mM Tris-HCl
- **1 mM** EDTA (pH 8,0)

STR

DNA

QUANTIFICATION

STR Analysis Troubleshooting

DNA Quantification (qPCR)

- Do not blindly trust the HID Real-Time PCR Analysis Software to evaluate the dye spectra. Inspect the calibration spectra yourself
- Use the recommended plates and adhesive films
- Pay attention to superior sealing of plates to prevent evaporation
- Let PowerQuant® System results show you the next step

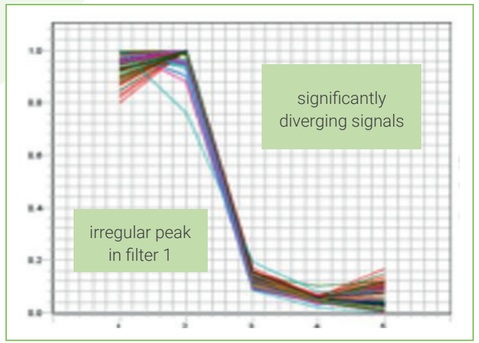
Autosomal DNA not Quantified

Poor Dye Calibration

Well	Sample Name	[Auto]	[Deg]	[Y]	IPC Cq
C12	Sample 2	0,0000	2,6157	2,2108	21,00
D12	Sample 2	0,0000	2,1735	3,2051	21,22
Average	Sample 2	0,0000	2,3946	2,7080	21,06
A12	Sample 50	0,0000	50,1331	50,4322	35,55
B12	Sample 50	0,0000	62,3636	46,6547	32,73
Average	Sample 50	0,0000	56,2484	48,5435	34,14

Failure of PowerQuant® autosomal target (FAM) channel to be detected

Poor spectral of CFG540 (Y-target), which should not have been accepted



Inspect the calibration spectra yourself.

Poor Dye Calibration

Adhesive Films of Non-Optical Grade

Poor spectral of CFG540 (Y-target)

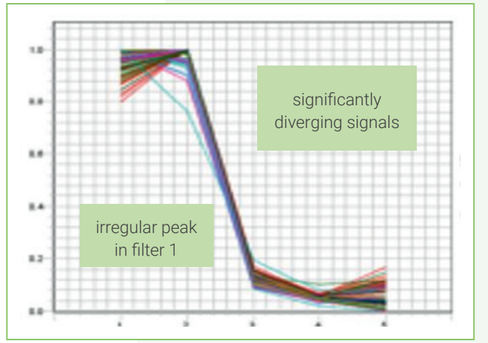


Plate sealed with **Eppendorf PCR clean adhesive film** (non optical grade)

Good spectral of CFG540

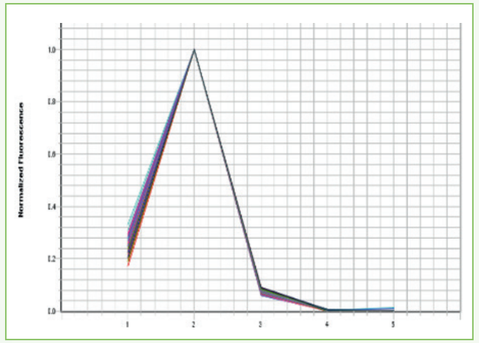


Plate sealed with recommended **MicroAmp® optical adhesive film** (Applied Biosystems)

Please also note our recommendation for plates: MicroAmp® optical 96-well reaction plate (Applied Biosystems)

Use the recommended plates and adhesive seals.

Confusing IPC Values

Evaporation Due to Careless Plate Sealing

	Settings							
	IPC Shift Threshold	M/F mixture Threshold	Deg Threshold		Autosomal	Degradation	IPC	Y
	0,3	2	2		Autosomal	Degradation	IPC	Y

Well	Sample Name	[Auto]	[Deg]	[Y]	IPC Cq	IPC Shift	IPC Threshold	[Auto]/[Y]	[Auto]/[Y] Threshold	[Auto]/[D]	[Auto]/[D] Threshold
G3	2FM_P_dj3_G	0,0052	0,0026	0,0002	21,46	0,50	At or Above	26,48	At or Above	2,02	At or Above
G4	2FM_P_dj3_G	0,0055	0,0034	0,0002	21,03	0,07	Below	31,71	At or Above	1,63	Below
Average	2FM_P_dj3_G	0,0054	0,0030	0,0002	21,25	0,29	Below	28,94	At or Above	1,80	Below

Unusually large differences in IPC values in PowerQuant® System duplicate samples due to evaporation.

Ensure proper plate sealing to prevent evaporation.

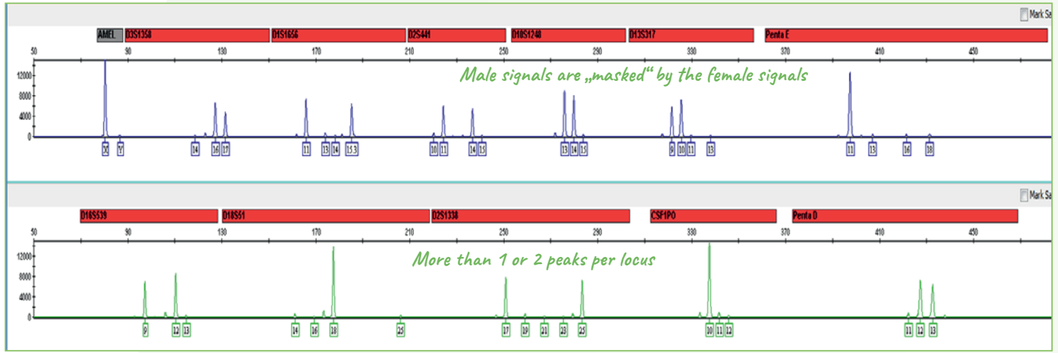
Let PowerQuant® System Results Show You the Next Step

PowerQuant® System Provides Directions for the Next Step

PowerQuant Result	Preferred Next Step
Good quality DNA	Autosomal STR Amplification
Female-male mixture with high excess of female DNA	Y-STR Amplification
Light to medium degradation	Amplification with higher than normal template
Light to medium inhibitor presence	Dilution of the DNA
High inhibitor presence	Repurification of the DNA
Zero Quant, or high degradation	No further processing. Don't waste your time and reagents

"Masked" Male Alleles in Mixed STR Profile

High Excess of Female DNA in Male-Female DNA Mixture

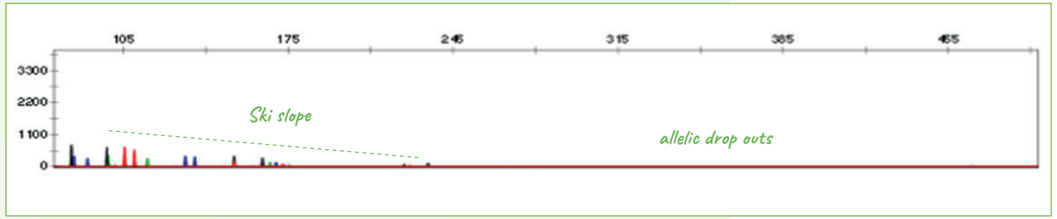


PowerPlex® Fusion System (blue and green channel only). Amplified product was separated using an Applied Biosystems® 3130xl Genetic Analyzer (3 kV, 5-second injection).

Better: Perform Y-STR Amplification to focus on male alleles only.

Ski Slope in STR Profile (Amelogenin Unaffected)

Light to Medium Degraded DNA



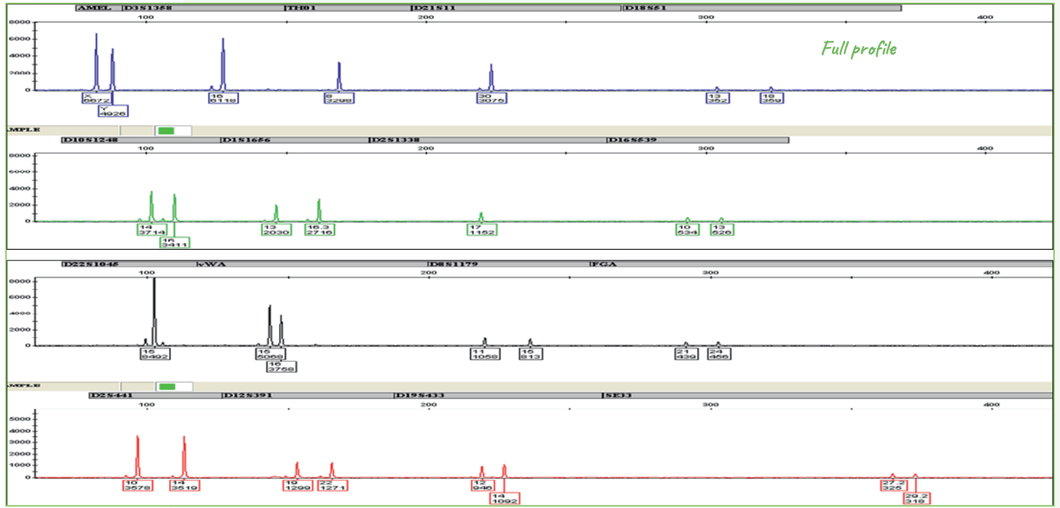
PowerPlex® Fusion System. Amplified product was separated using an Applied Biosystems® 3500 Genetic Analyzer (1.2 kV, 24-second injection).

What can you do, if the quantification result indicates light to medium DNA degradation?

Add more DNA template into PCR than usual.

Light to Medium Degraded DNA

Addition of More Template into PCR



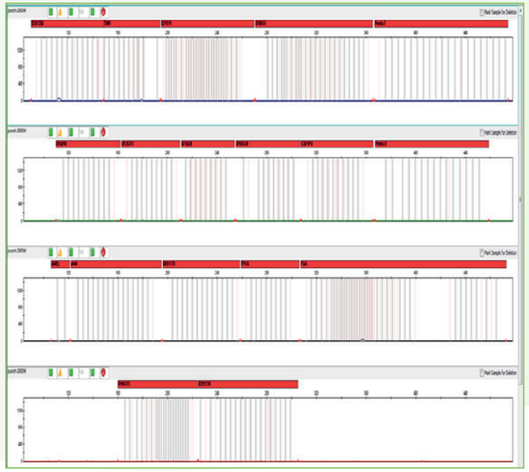
PowerPlex® ESX 17 System. The reaction was performed with 1.5 ng DNA instead of 0.5 ng DNA. Amplified product was separated using an Applied Biosystems® 3130xl Genetic Analyzer (3 kV, 5-second injection).

Data Courtesy of Antonio Alonso, Instituto Nacional de Toxicología y Ciencias Forenses, Department of Biology, Madrid, Spain

Light to Medium Inhibitor Presence

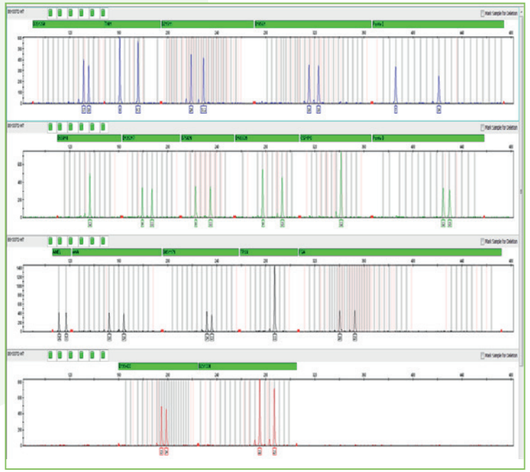
Total PCR Inhibition

Undiluted DNA sample



PowerPlex® 18D System

1:10 Dilution of DNA sample

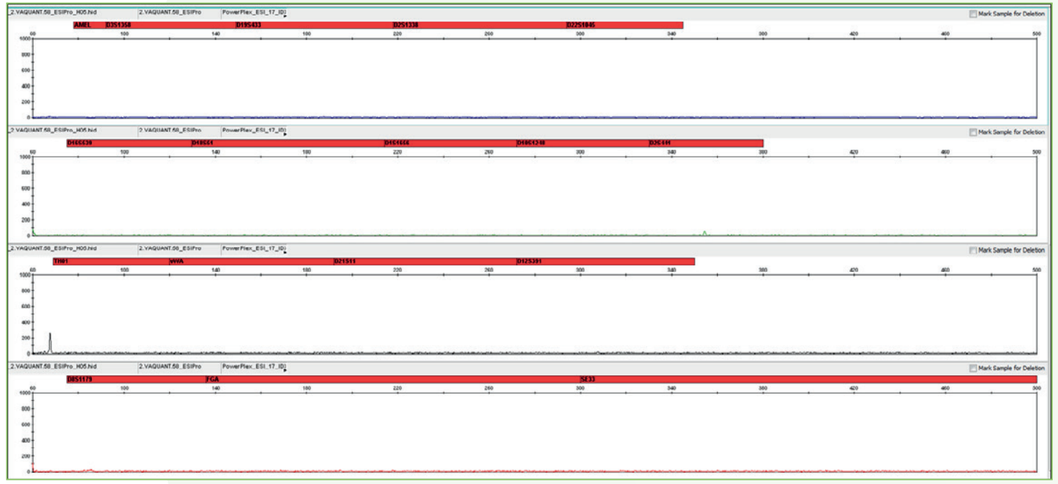


PowerPlex® 18D System. 1:10 diluted same sample

Amplified products were separated using an Applied Biosystems® 3130xl Genetic Analyzer (3 kV, 5-second injection).

No STR Profile

No DNA in the sample



PowerPlex® ESI 17 Pro System. Amplified product was separated using an Applied Biosystems® 3130xl Genetic Analyzer (3 kV, 5-second injection).

Trust Zero Quant result.

Auto Quant (ng/µl)	Y Quant (ng/µl)
0.000	0.000

STR

DNA

AMPLIFICATION

STR Analysis Troubleshooting

DNA Amplification

- Use the recommended thermal cycler and cycling protocol
- Use the recommended plasticware
- Vortex thorougly
- Pay attention to accurate pipetting
- Do not prepare amplification mix in advance

PowerPlex® Amplification

Recommended Cycling Conditions

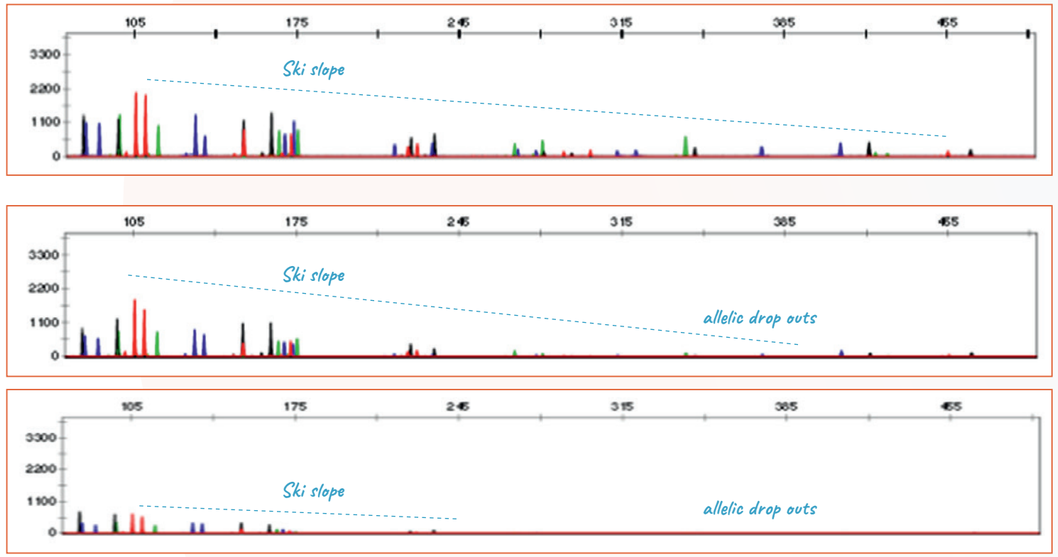
PowerPlex® System	16 HS	ESX & ESI	ESX Fast & ESI Fast	18D	21	Y23	Fusion	Fusion 6C
Recommended Thermal Cycler	9700 9600	9700 Veriti	9700 Veriti	9700 Veriti	9700 Veriti	9700 Veriti	9700 Veriti	9700 Veriti
Ramp Rate given in "Modi"	9600 Mode *	9600 Mode	Max Mode	9600 Mode	Max Mode	Max Mode	Max Mode	Max Mode 100% (Veriti)

*additionally, modified ramp rates for each PCR step required

9600 Mode = 1°C/Sec
Max Mode = 4°C/Sec

Ski Slope in STR Profile (Amelogenin Not Affected)

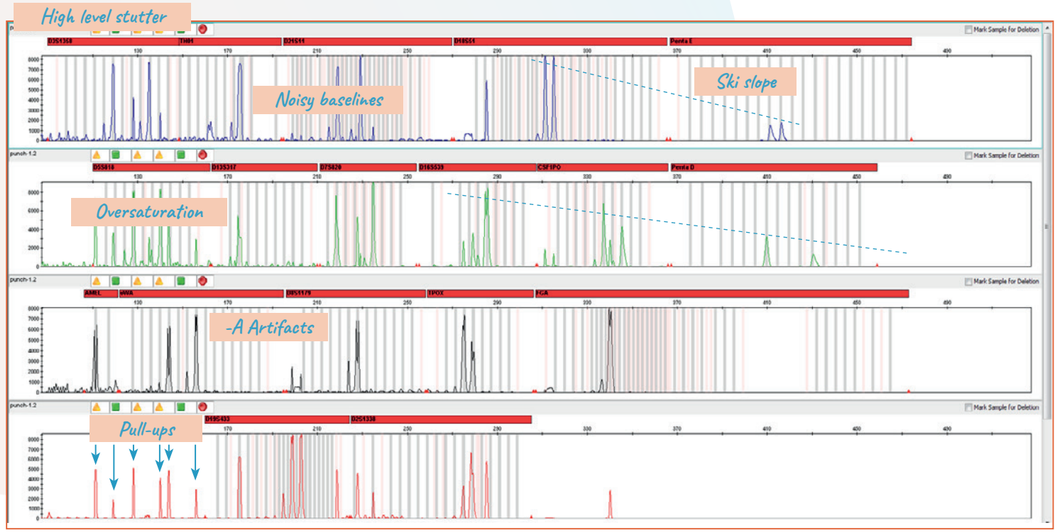
Degraded DNA



PowerPlex® Fusion System. Amplified products were separated using an Applied Biosystems® 3500 Genetic Analyzer (1.2 kV, 24-second injection).

Noisy STR Profile

Too Much Template DNA Added into PCR

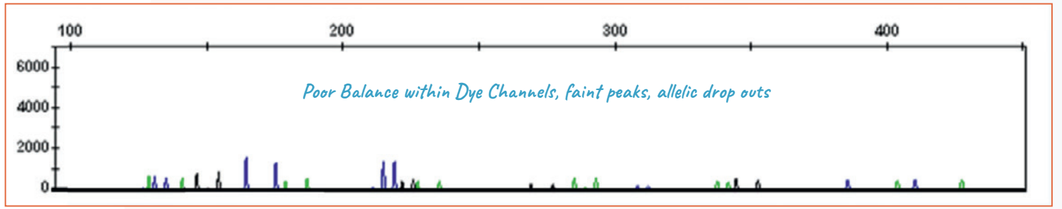


PowerPlex® 18D System. Amplified product was separated using an Applied Biosystems® 3130xl Genetic Analyzer (3 kV, 5-second injection).

Once you know the DNA concentration of your sample, you can avoid such a poor PCR result.

Low Peak Heights in Imbalanced STR Profile

Insufficient Template DNA Added into PCR



PowerPlex® 21 System. Amplified products were separated using an Applied Biosystems® 3500 Genetic Analyzer (1.2 kV, 24-second injection).

Once you know the DNA concentration of your sample, you can avoid such a poor PCR result.

PowerPlex Amplification

Recommended Thermal Cyclers

- **GeneAmp® PCR System 9700 thermal cycler** with silver or gold-plated silver sample block (Applied Biosystems)
- **Veriti® 96-Well Thermal Cycler** (Applied Biosystems)
- **ProFlex PCR System** (Applied Biosystems)

When using other than recommended thermal cyclers, please adjust the ramp rates:
9600 Mode = 1°C/sec
Max Mode = 4°C/sec

Please note: We do **NOT** recommend the use of thermal cyclers with aluminum heating blocks with PowerPlex® Systems!

Recommended Plasticware

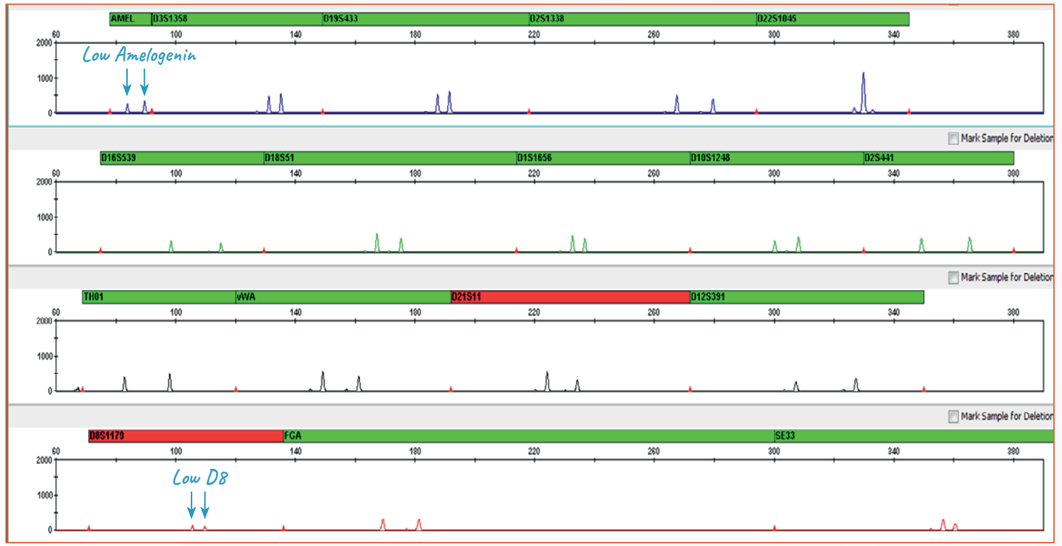
- **MicroAmp® Optical 96-Well Reaction Plates** (Applied Biosystems)
- **0.2 ml MicroAmp® Reaction Tubes** (Applied Biosystems)

Plates other than these, may deliver **different heat transfer rates** because of differences in plastic material, thickness and well shapes.

Effects of Reduced Heat Transfer Rate

Alternative Cycler and Plasticware Used

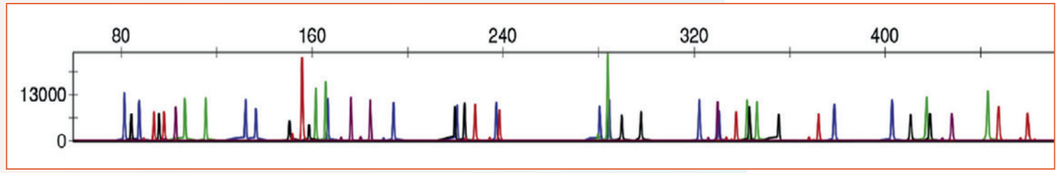
Particularly poor amplification of Amelogenin and D8S1179



PowerPlex® ESI 17 Fast System. Amplified product was separated using an Applied Biosystems® 3500 Genetic Analyzer (1.2 kV, 24-second injection). PCR was performed using a thermal cycler with an aluminium heating block and Framestar 96 semi skirted plates from 4titude®.

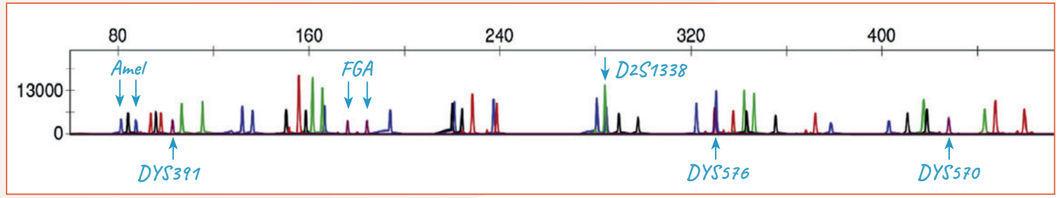
Effects of Reduced Heat Transfer Rate

Alternative Recommended Plasticware Used



MicroAmp® Optical 96-Well Reaction Plate

Poor amplification of some loci due to the use of alternative PCR plates

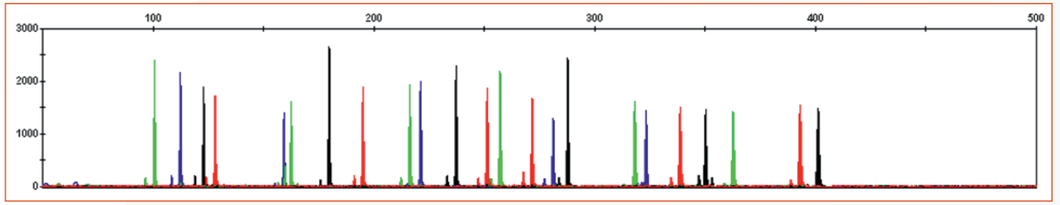


Axygen® Scientific, 96 Well PCR Plate

PowerPlex® Fusion 6C System on GeneAmp® 9700 Cycler in Max Mode. Amplified products were separated using an Applied Biosystems® 3500 Genetic Analyzer (1.2kV, 24-second injection).

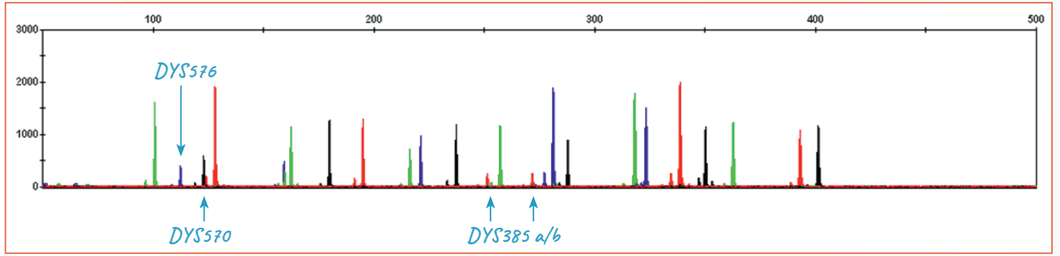
Effects of Reduced Heat Transfer Rate

Alternative Recommended Plasticware Used



MicroAmp® Optical 96-Well Reaction Plate

Poor amplification of some loci due to the use of alternative PCR plates



Axygen® Scientific 96 Well PCR Plate

PowerPlex® Y23 System on GeneAmp® 9700 Cycler in Max Mode. Amplified products were separated using an Applied Biosystems® 3500 Genetic Analyzer (1.2 kV, 24-second injection).

Imbalanced Profile

Primer Pair Mix Not Vortexed Thoroughly

Things to know about Primer Pair Mix

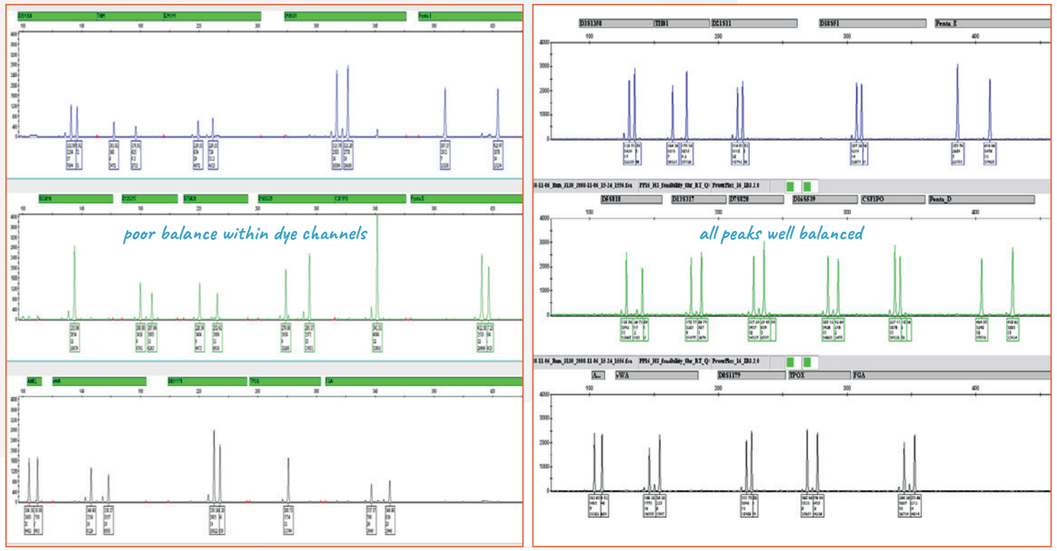
Storage at -20°C leads to the formation of a gradient within the Primer Pair Mix!

Therefore the following steps are necessary:

- Thaw completely
- Vortex thoroughly (10–20 seconds!)
- Do not centrifuge after vortexing!

No vortexing of primer pair mix

15-second-vortexing of primer pair mix

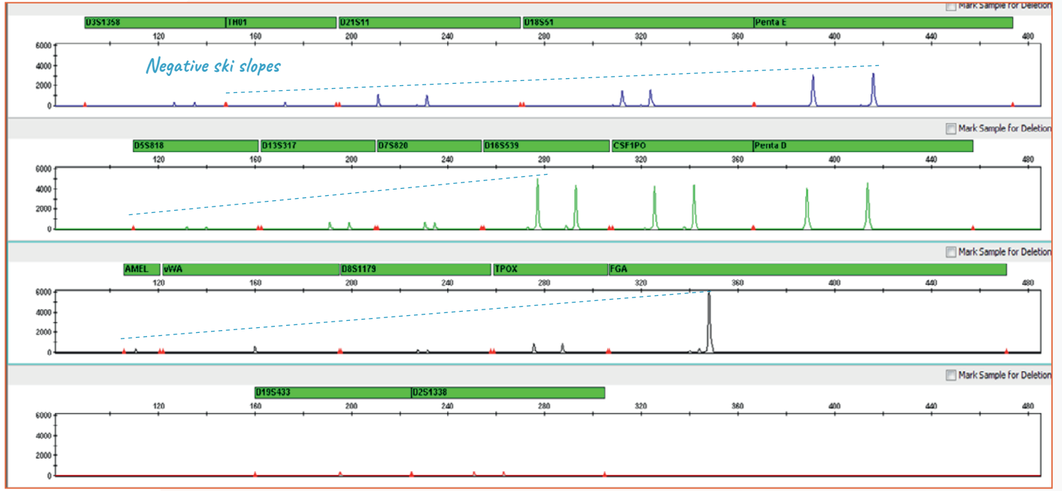


PowerPlex® 16 HS System. Amplified products were separated using an Applied Biosystems® 3130xl Genetic Analyzer (3 kV, 5-second injection).

Vortex thoroughly!

Effects of Inaccurate Pipetting

Primer Pair Concentration Too Low



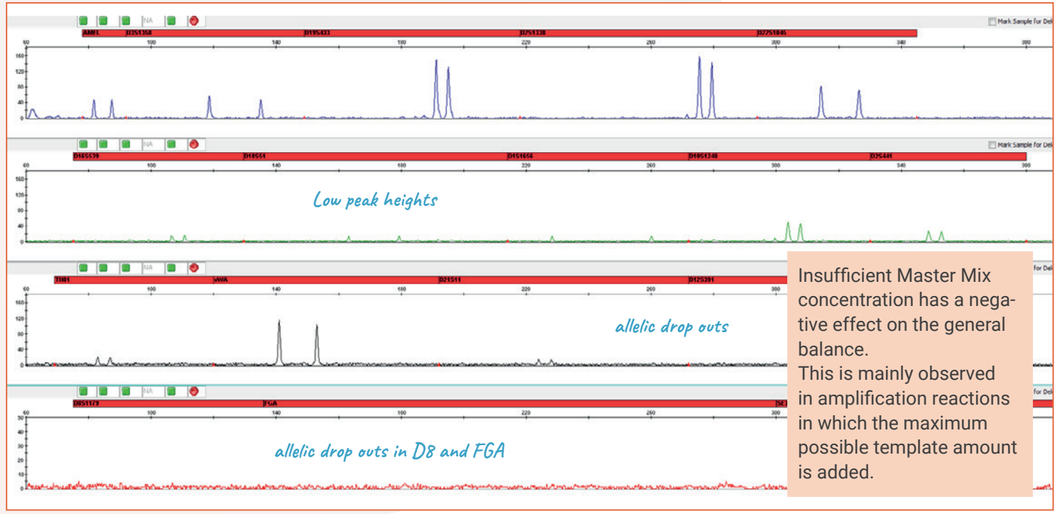
PowerPlex® 18D System with half concentration of primer pair mix (0.5 X instead of 1 X). Amplified product was separated using an Applied Biosystems® 3500 Genetic Analyzer (1.2 kV, 24-second injection).

Insufficient primer pair concentration has a negative effect on the amplification of low molecular weight markers.

Pay attention to accurate pipetting!

Effects of Inaccurate Pipetting

Master Mix Volume Too Low



Insufficient Master Mix concentration has a negative effect on the general balance.
This is mainly observed in amplification reactions in which the maximum possible template amount is added.

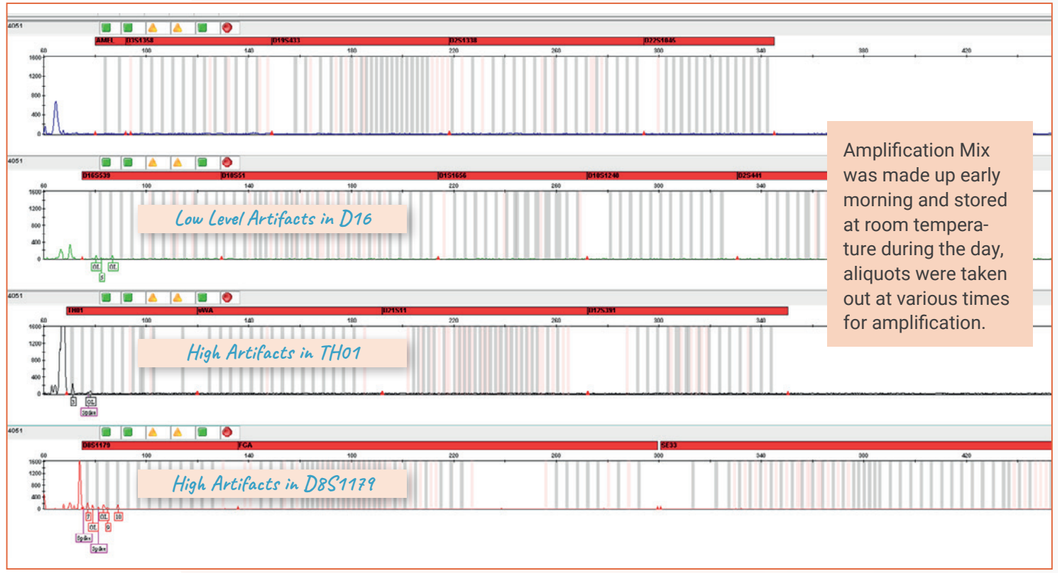
PowerPlex® ESI 17 System. Amplified product was separated using an Applied Biosystems® 3500 Genetic Analyzer (1.2 kV, 24-second injection).

Pay attention to accurate pipetting! Master Mix is more viscous than primer pair mix. Pipette slowly!

Effects of Pre-Preparation of Amplification Mix

Artifacts in STR Profile

Negative amplification control with artifacts



Amplification Mix was made up early morning and stored at room temperature during the day, aliquots were taken out at various times for amplification.

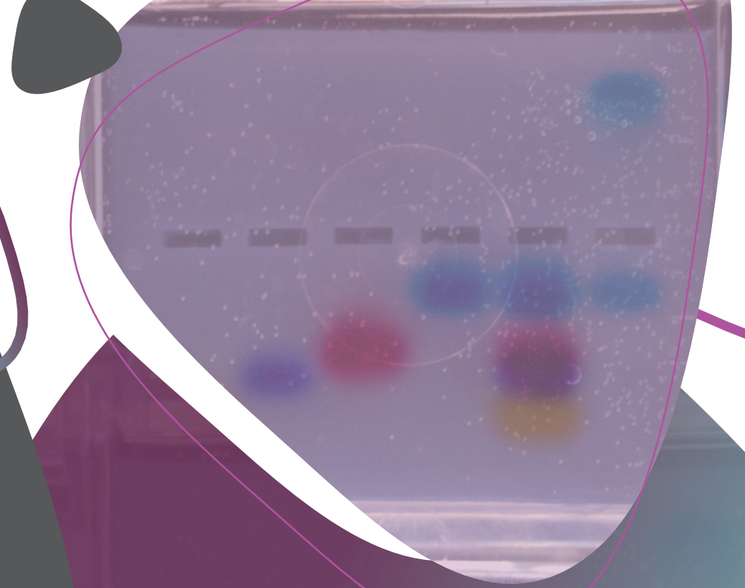
PowerPlex® ESI 17 Fast System, negative amplification control. Amplified product was separated using an Applied Biosystems® 3500 Genetic Analyzer (1.2 kV, 24-second injection).

Do **NOT** store the PCR amplification mix for a prolonged period.

STR

DNA

SEPARATION & DETECTION



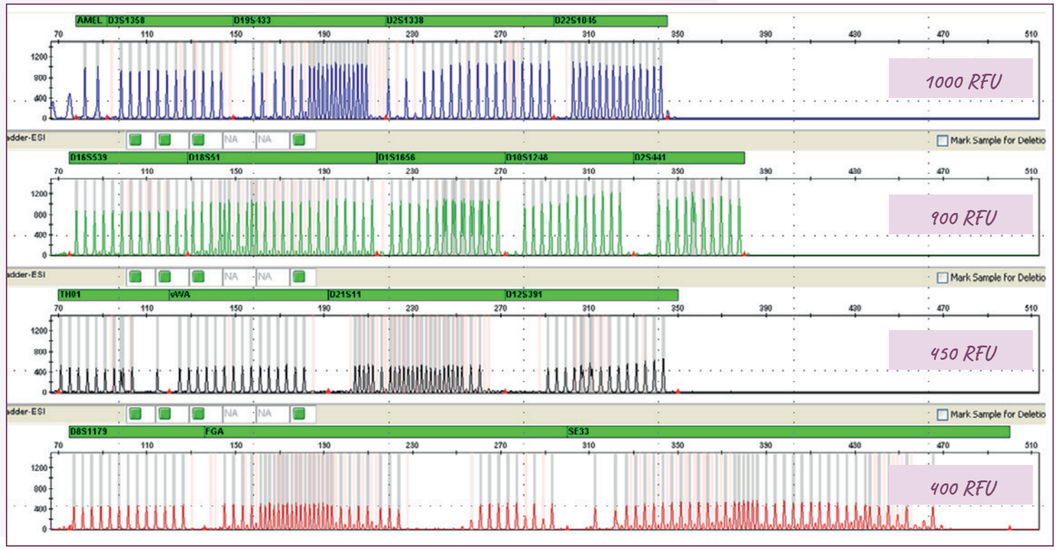
STR Analysis Troubleshooting

DNA Separation and Detection

- Use the recommended dye set
- Use the recommended polymer type
- Use deionized formamide
- Do not prepare the loading cocktail in advance.
- Do not store dilutions of the 2800M DNA
- Follow the vendor-recommended maintenance procedures of your CE instrument

Imbalance between Dye Channels

Incorrect Dye Set



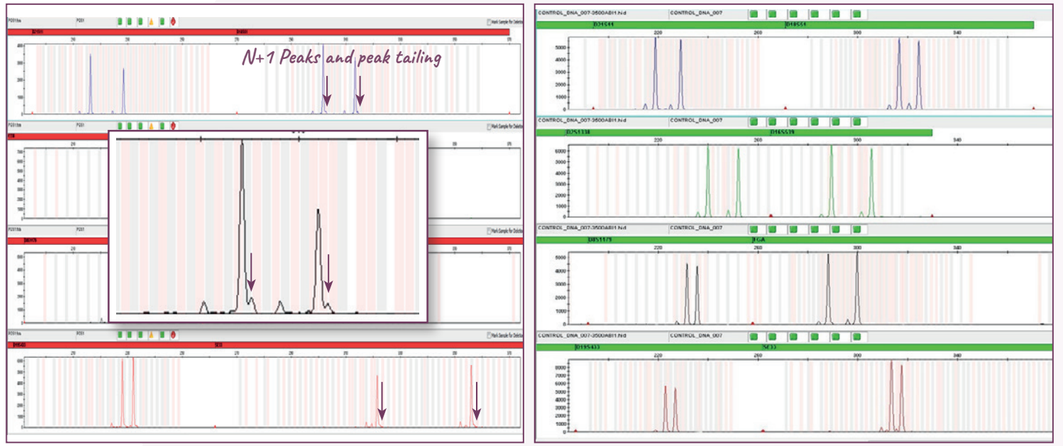
PowerPlex® ESI 17 System Allelic Ladder on Any5Dye. Amplified product was separated using an Applied Biosystems® 3500 Genetic Analyzer (1.2 kV, 24-second injection).

Use the recommended dye sets for each chemistry!

Overview of recommended dye sets	
PowerPlex® Dye Chemistry	**Dye Set**
4 C	F
5 C	G5
6 C	J6

N+1 Artifacts in STR Profile

Using Other than Recommended Polymer and Capillary Length



007 DNA
POP6
50 cm capillary

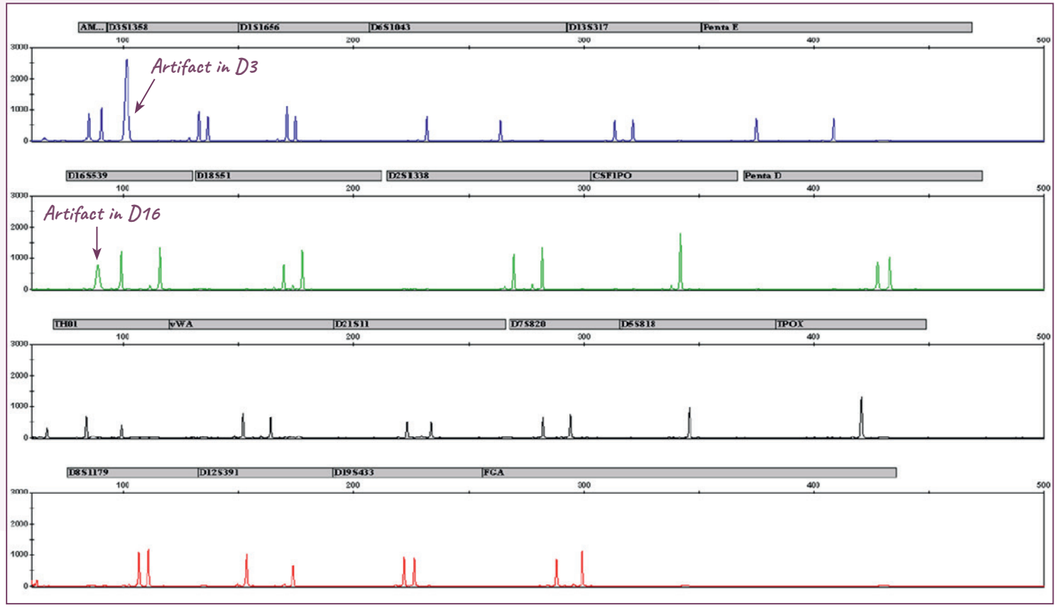
2800M DNA
POP4
36 cm capillary

PowerPlex® ESX 17 Fast System. Amplified products were separated using an Applied Biosystems® 3500 Genetic Analyzer (1.2 kV, 24-second injection).

Use POP4 and 36 cm capillaries with PowerPlex® STR Systems

Artifacts in STR Profile

Using Other than Recommended Polymer



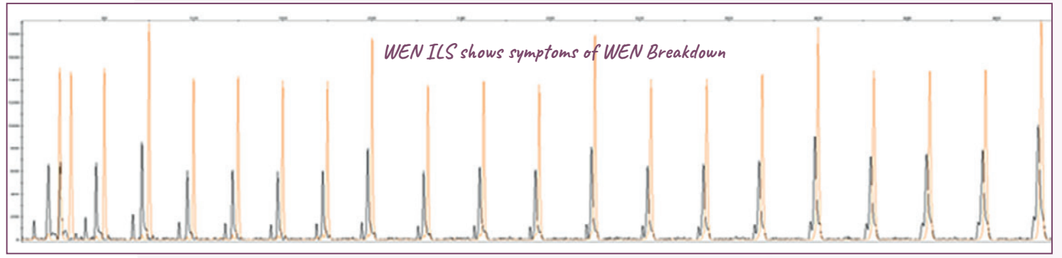
PowerPlex® 21 System on POP7. Amplified product was separated using an Applied Biosystems® 3500 Genetic Analyzer (1.2 kV, 24-second injection).

With POP7, artifacts migrate into D3 and D16

We recommend the use of POP4 for PowerPlex® STR Systems.

Effects of Pre-Preparation of Loading Cocktail

Degradation of WEN ILS500



WEN ILS 500 was separated using an Applied Biosystems® 3500 Genetic Analyzer (1.2 kV, 24-second injection).

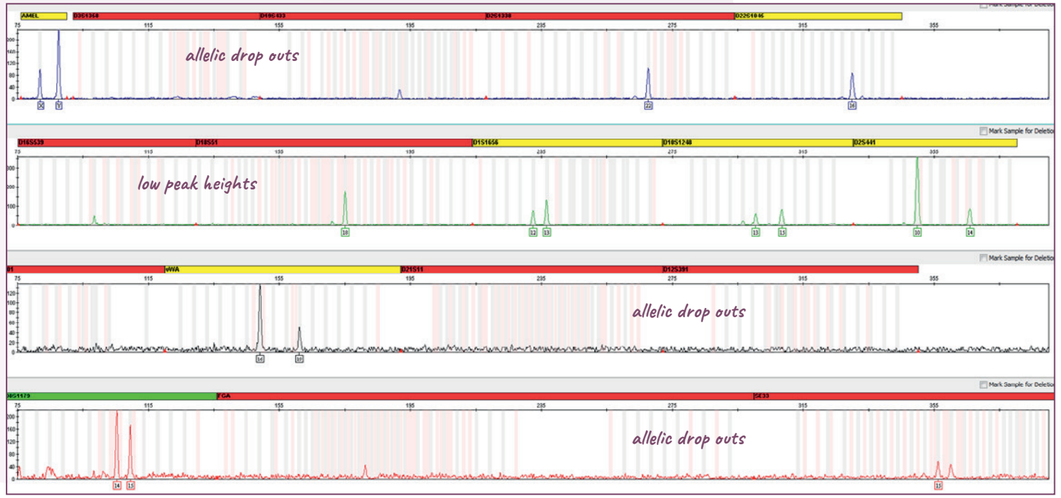
Formamide and WEN ILS500 were premixed and kept at 4°C

Do **NOT** store the loading cocktail for a prolonged period.

Effects of Diluting and Storing 2800M DNA

Degradation of Control DNA

Positive amplification control showing signs of degradation



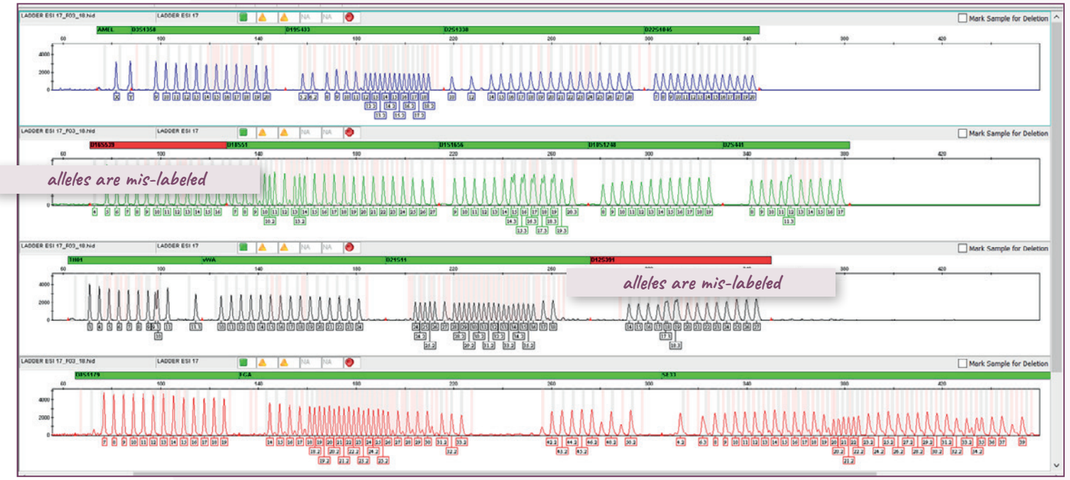
PowerPlex® ESI 17 Fast System. Amplified product was separated using an Applied Biosystems® 3130xl Genetic Analyzer (3 kV, 5-second injection).

Dilution of 2800M DNA was stored at 4°C. 2800M DNA Dilution shows symptoms of degradation

Please note: control DNA, when diluted in water, is unstable!

STR Profile with Mis-Labeled Alleles

Poor Resolution because of Old Consumables or Capillary Array

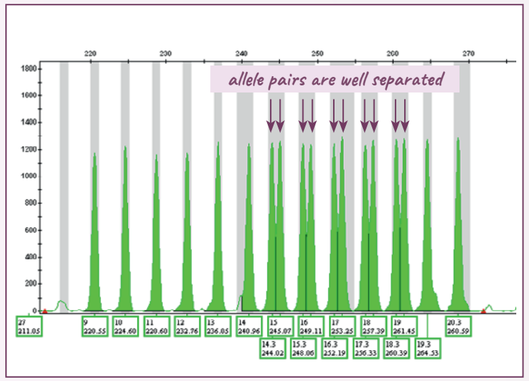


PowerPlex® ESI 17 Pro System. Allelic Ladder was separated using an Applied Biosystems® 3500 Genetic Analyzer (1.2 kV, 24-second injection).

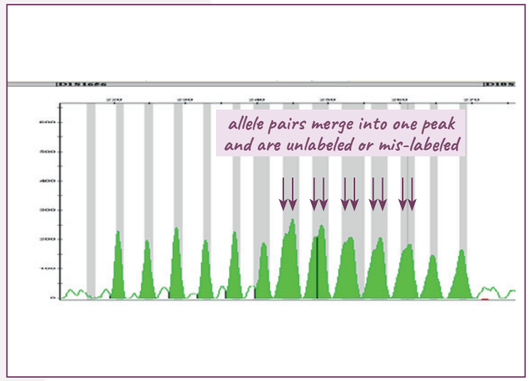
STR Profile with Mis-Labeled Alleles

Poor Resolution Because of Old Consumables or Capillary Array

Good resolution



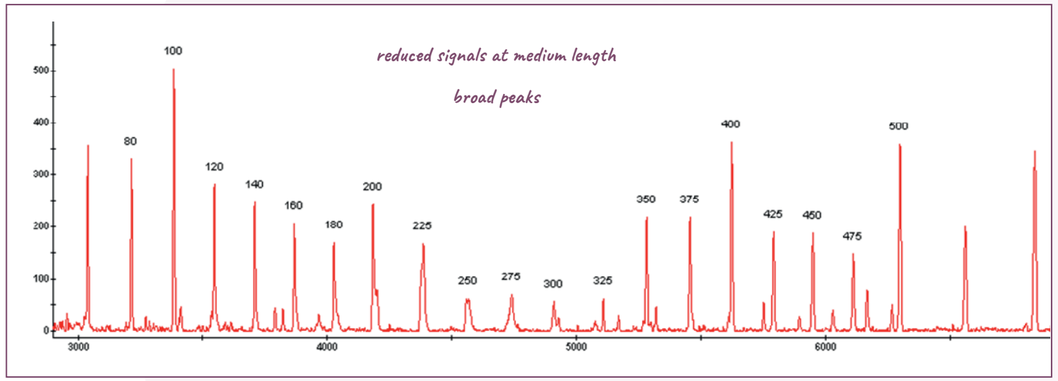
Poor resolution



D1S1656 Locus in **PowerPlex® ESI Systems**

Golden Gate Effect

Poor Formamide Quality

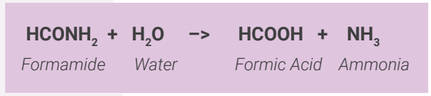


ILS600 was separated using an Applied Biosystems® 3130xl Genetic Analyzer (3 kV, 5-second injection).

Golden Gate Effect is attributed to poor formamide quality.

Things to Know about Formamide

- Formamide keeps the DNA samples denatured.
- Formamide is hygroscopic, i.e. absorbs humidity from the air. Over time it degrades into formic acid and ammonia and the conductivity increases.
- Formamide with conductivity > 100 µS/cm competes with DNA fragments during injection.



If you are not sure whether the formamide quality is poor, make a comparison run in which you replace the formamide in the loading cocktail with amplification grade water.

If the peak heights are higher with the use of water than with formamide, you know that the conductivity of your formamide is too high.

- Use deionized Formamide
- Minimize exposure to air
- Store in aliquots at -20°C
- Use each aliquot only once
- Do not re-freeze aliquots

Maintain your CE Instrument

Instrument Mainenance Schedule

CE	Replace Buffer	Replace polymer	Run Water wash, Rinse Pump Block	Flush water trap	Replace Capillary array
Applied Biosystems® 3130, 3130xl Genetic Analyzers	Every 48 hours	weekly	weekly	monthly	After 100 injections
Applied Biosystems® 3500 Genetic Analyzers	weekly	weekly	weekly	monthly	After 160 injections
Spectrum Compact CE System	after 14 days or 80 injections	after 14 days or 80 injections	–	–	After 200 injections

Follow the maintenance schedule thoroughly.

STR ANALYSIS

TROUBLESHOOTING

Promega GmbH
Gutenbergring 10
69190 Walldorf, Germany
www.promega.com

Germany/Austria
Phone +49 6227 6906-290
de_techserv@promega.com

Poland
Phone +48 22 531 06 68
pl_techserv@promega.com

Central/Eastern Europe
Phone +49 6227 6906-0
de_ceesales@promega.com