DNA SEQUENCING RESULTS GUIDE

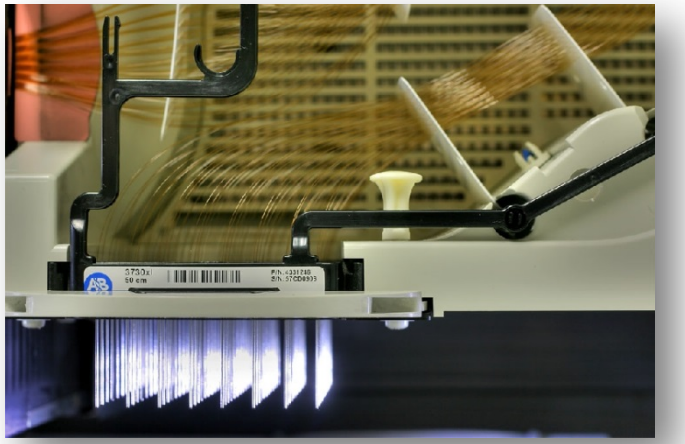
Examining Your Results

This **illustrative guide** briefly explains some of the most frequently encountered sequencing data irregularities in your electropherograms.

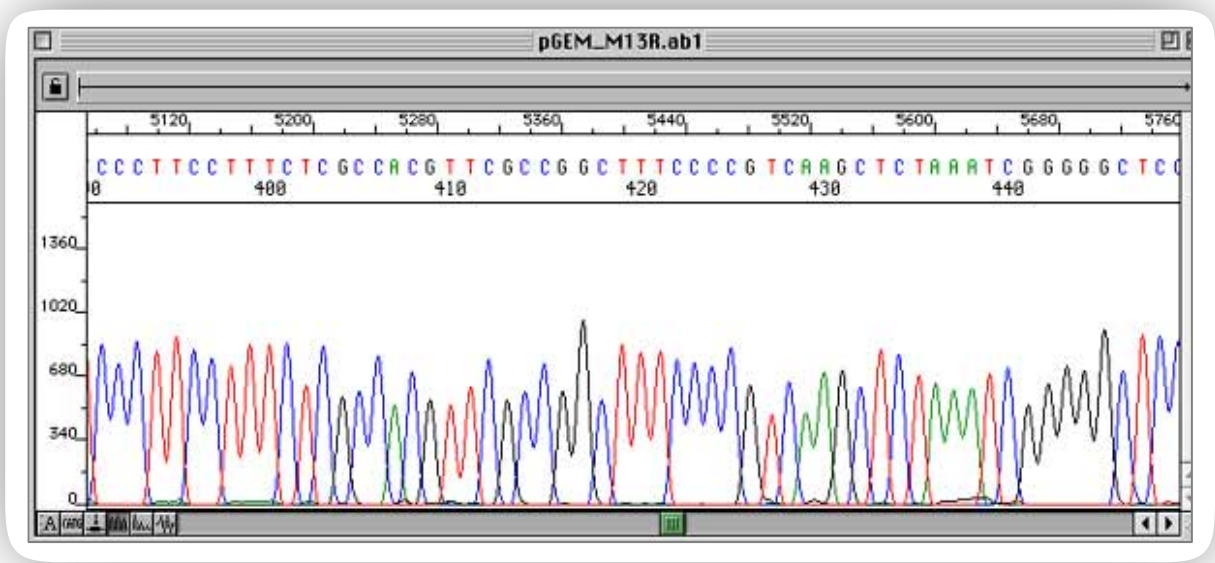
A good starting point to correctly evaluate sequencing data is the electropherograms of raw and analysed data files.

Using the **original *.ab1 file** gives you the best chance to precisely compare the raw and analysed data traces.

Several excellent freewares such as Applied Biosystems Sequence Scanner exist to easily examine the electropherograms in your sequence data. For instance, the “**Annotation**” **tab** in the Sequence Scanner software displays signal strength, signal to noise ratio, and even reflects the quality of a sequence in an electropherogram



Successful Sequencing Reactions



Electropherogram of a normal DNA sequencing read

Chromatogram Characteristics

- Well-formed, distinctive single coloured peaks
- Peaks evenly separated
- Absence of background signals

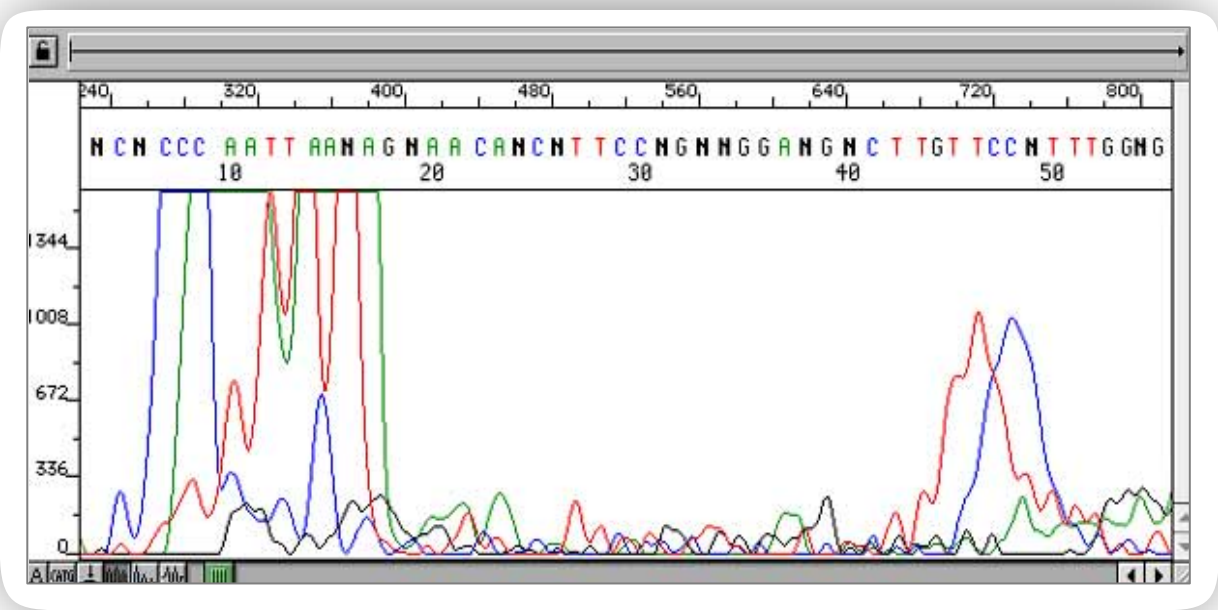
Reasons

- Appropriate template and primer concentrations
- Excellent purity of DNA and template
- Optimum primer design

Tip!

Use our free primer design tool to achieve optimum primer design

Failed Sequencing Reactions



Electropherogram of a failed sequencing results

Chromatogram Characteristics

- Absence of clearly defined peaks in raw and analysed data
- Occurrence of excess dye peaks
- Very low signal-to-noise ratios (S/N A/C/G/T: <15)

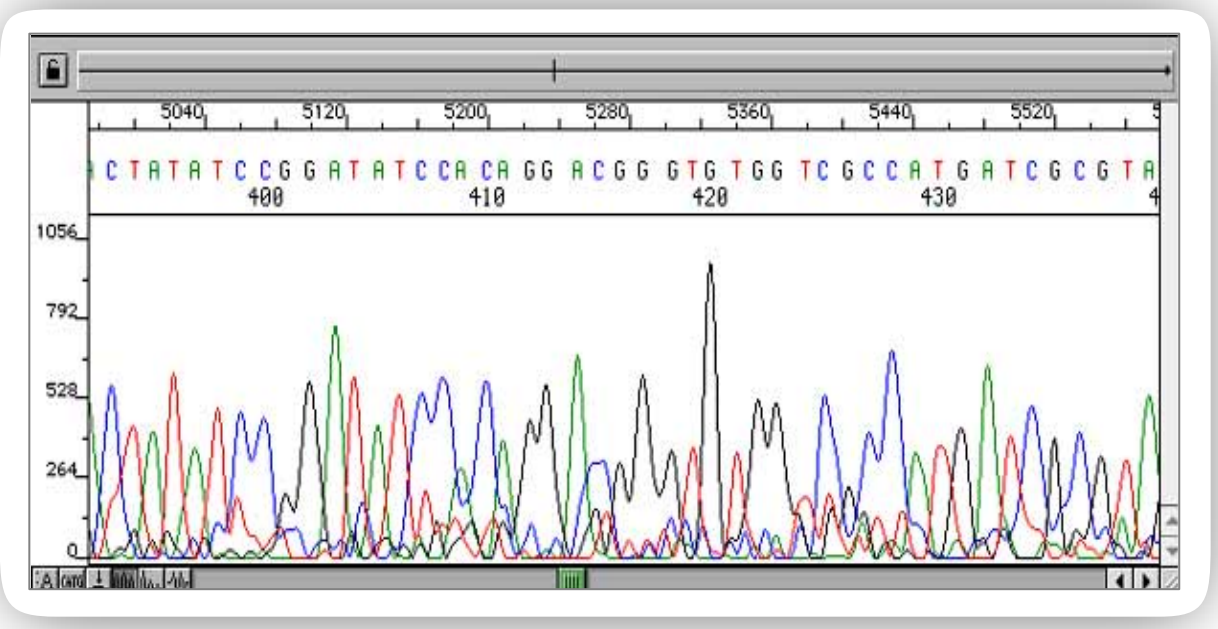
Reasons

- Insufficient or poor quality template and /or primer
- Primer binding site absent, deleted or mutated

Tip!

Don’t despair. Give us a call to get some expert advice

Multiple Sequence Signals



Electropherogram of a multiple sequence signals

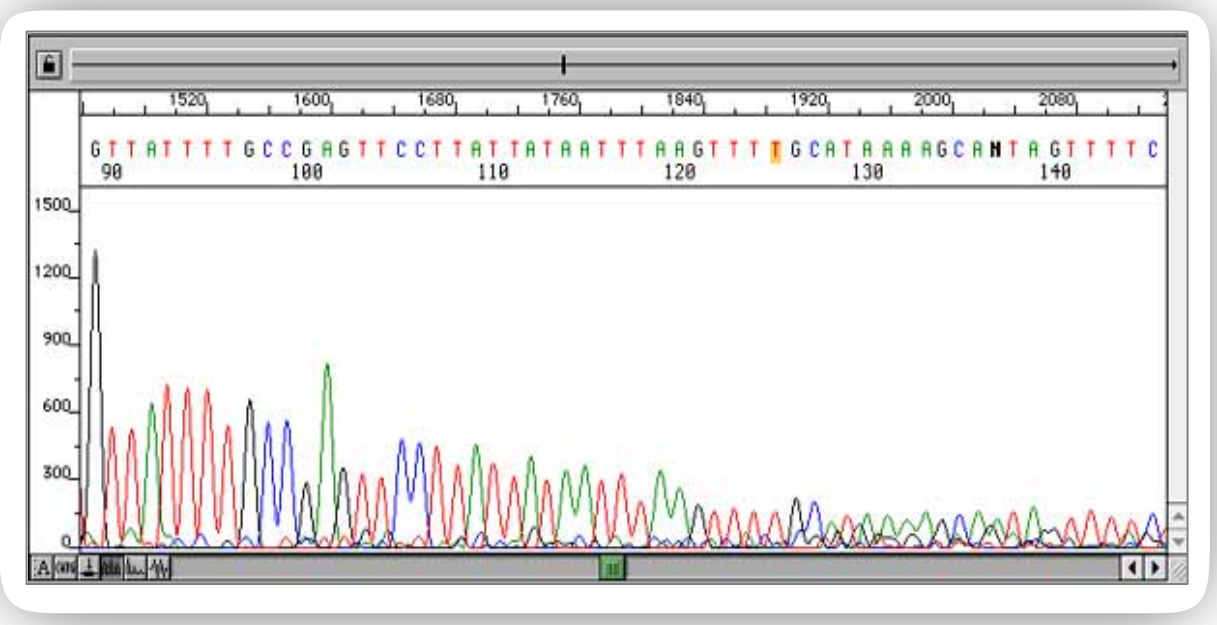
Chromatogram Characteristics

- Peaks unevenly spaced
- Overlapping of peaks in raw and analysed data
- Presence of artifacts beneath peaks

Reasons

- Contaminated template or primer
- Poor quality template and /or primer
- Multiple priming

Low Signal Strength



Electropherogram with low signal strength and signal die out

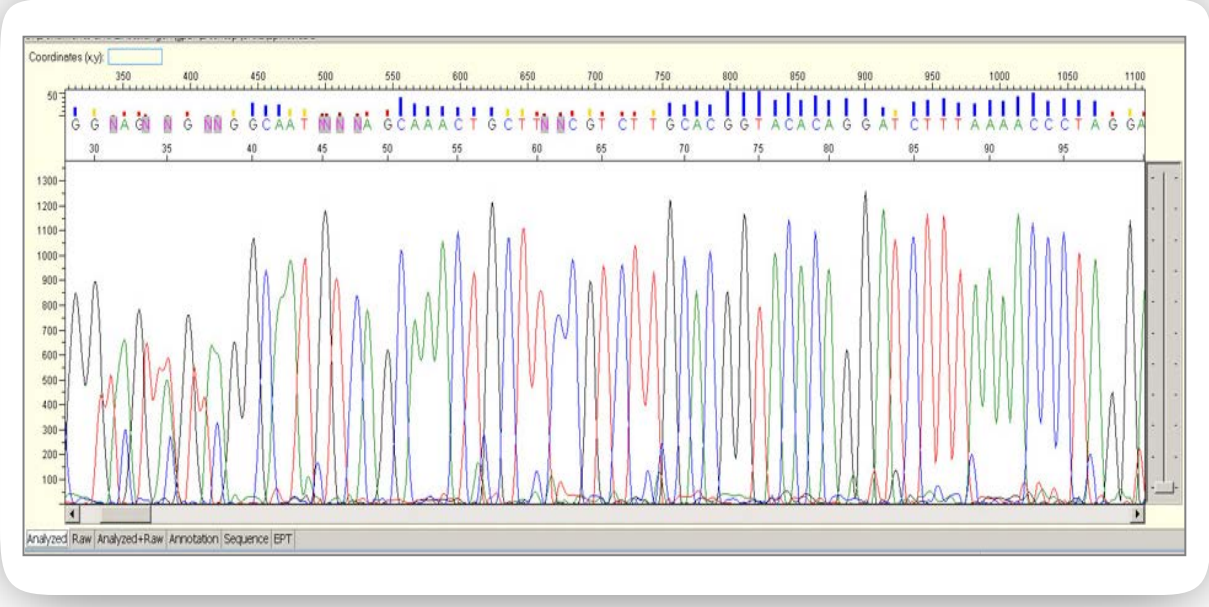
Chromatogram Characteristics

- Base calls fade off before the end of the read
- Very low peak heights in raw data trace
- Low signal-to-noise ratios (S/N A/C/G/T: <50)

Reasons

- Suboptimal DNA and primer concentrations
- Impure DNA templates
- Primer mutated
- Poor primer design

Very Strong Signals and Pull-Up Peaks



Electropherogram with very strong signals and Pull-up peaks

Chromatogram Characteristics

- Very high peaks in the raw data trace
- High peaks in the analysed data with pull-up secondary peaks and poor base calls
- Very high signal-to-noise ratios S/N A/C/G/T: > 750

Reason

- Excessive template during cycling sequencing

Tip!
Reduce the amount of template

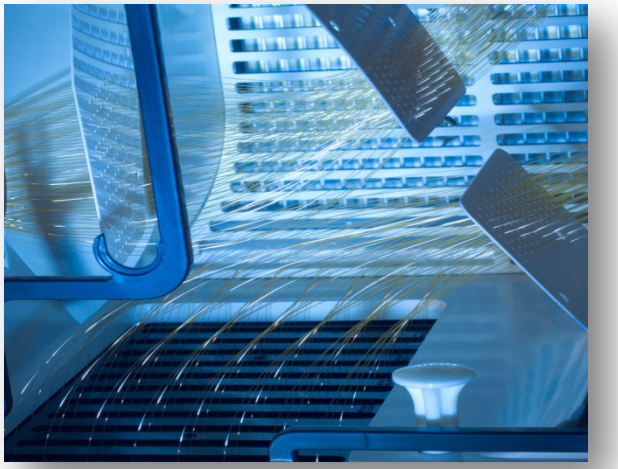
Mixed Sequenced Reactions

Characteristic of mixed sequence reactions

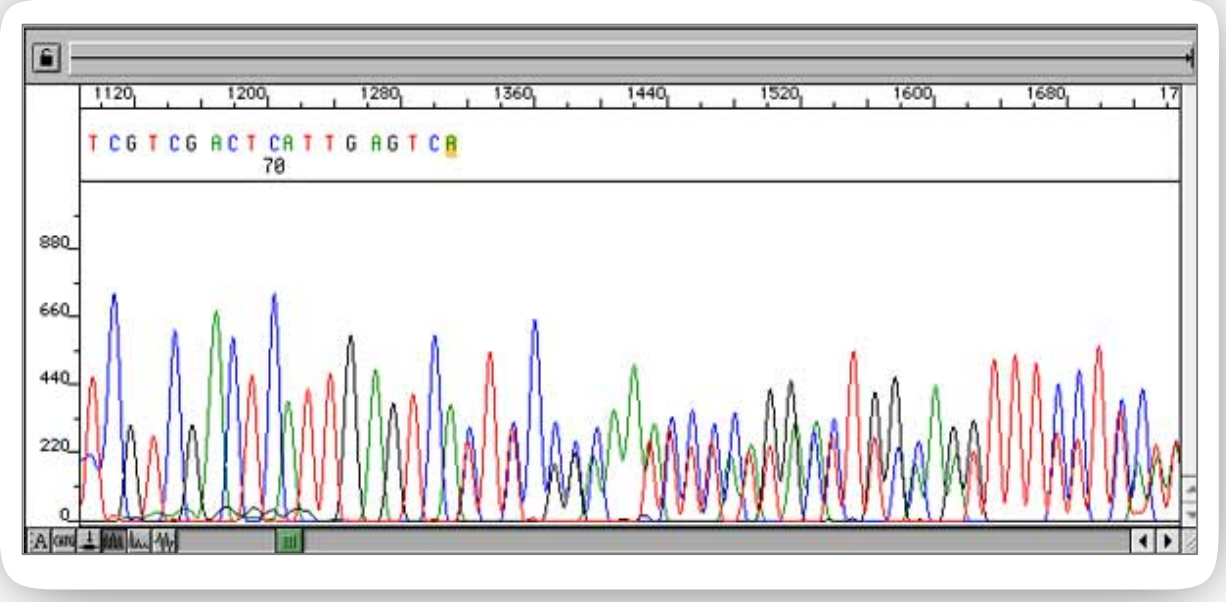
- More than one sequence in the analysed trace data
- More than one sequence starting after base 30 to 100 (multiple cloning site)
- Lower raw data peaks

Reasons for mixed sequence reactions

- Mixed plasmid preparations
- Multiple PCR products
- Multiple priming sites
- Multiple primers in reaction mix e.g. due to incomplete removal of PCR primers
- Primer-dimer contamination
- Frame shift mutation
- Primer with n-1 contamination or degrading primer
- Slippage due to homopolymer or repeat regions in the template



Mixed Sequences Starting From One Cloning Site



Electropherogram with mixed sequencing starting at a cloning site

Chromatogram Characteristics

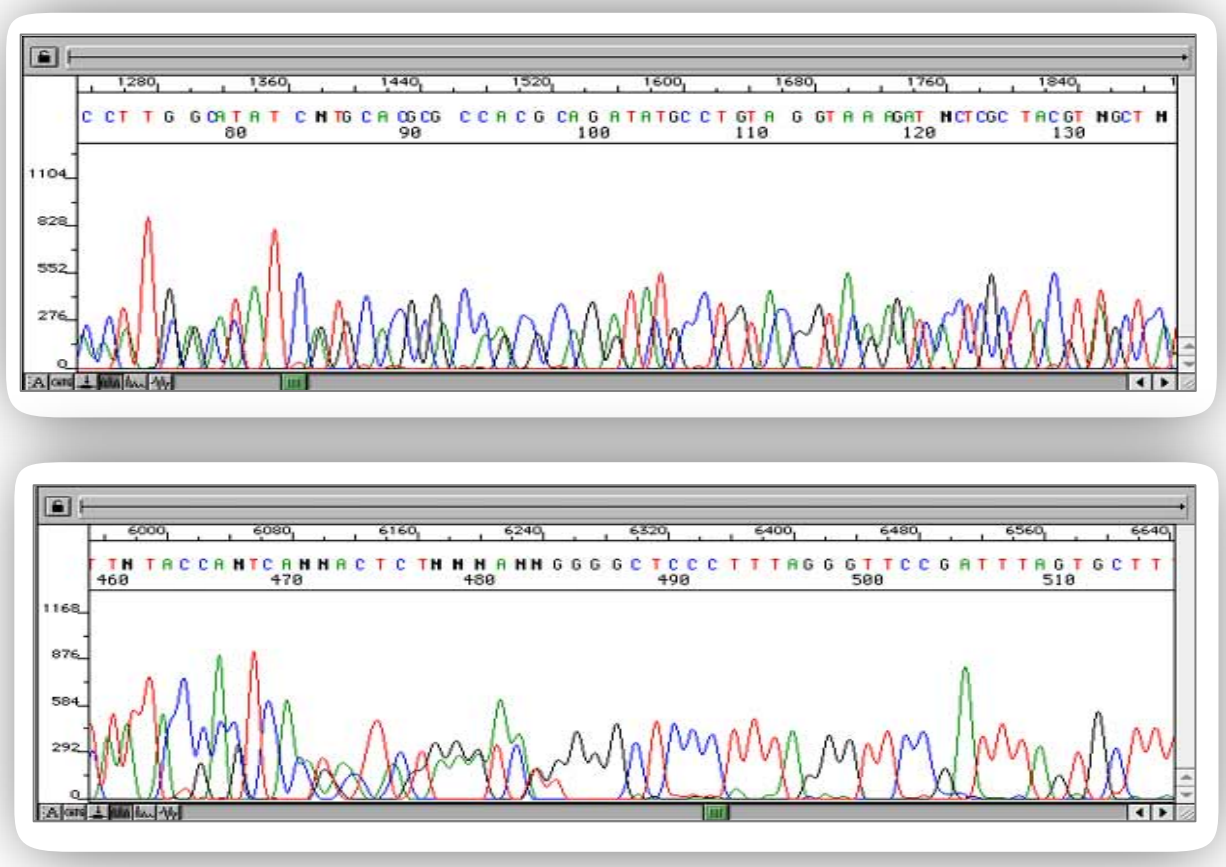
- Overlapping peaks

Reason

- Use of more than one colony for plasmid growth and preparation

Tip!
Streak out single colonies at least on a selected medium

Mixed Sequences Starting From The Beginning



Electropherogram with mixed sequencing starting from the beginning

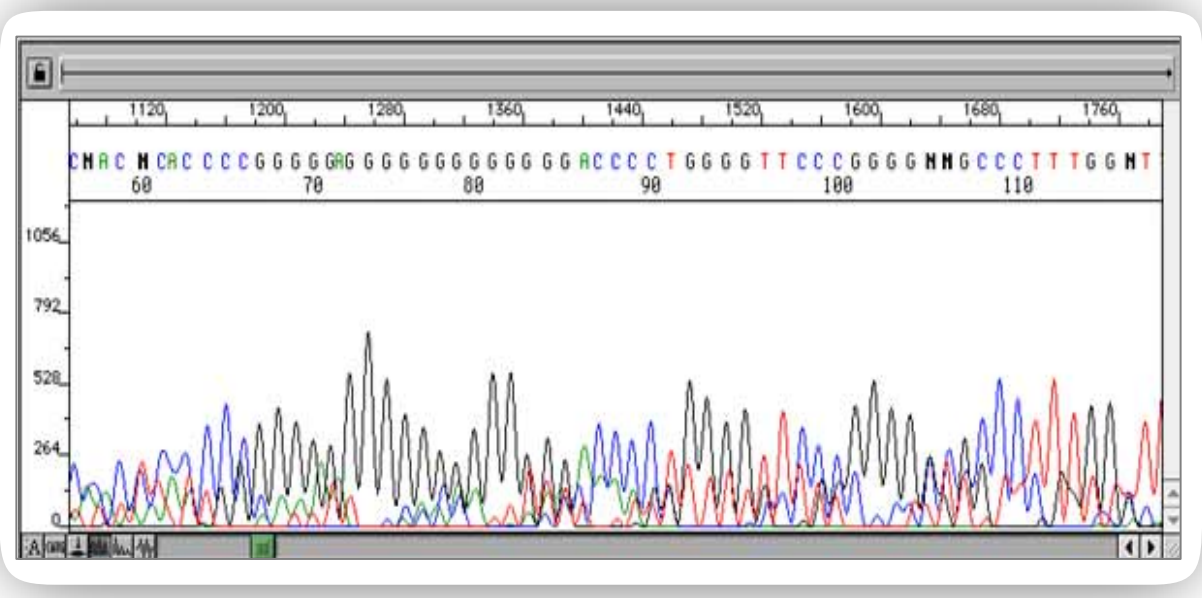
Chromatogram Characteristics

- Overlying sequences throughout the entire read or up a certain region
- Several sequences in the analysed trace data
- More than one sequence starting after base 30 to 100 (multiple cloning site)

Reasons

- Presence of several templates in a sequencing reaction
- Primer dimer contamination

Sequencing Result Comment – Degraded Primer



Electropherogram from degraded primers

Chromatogram Characteristics

- Mixture of different peaks
- Low raw data peaks

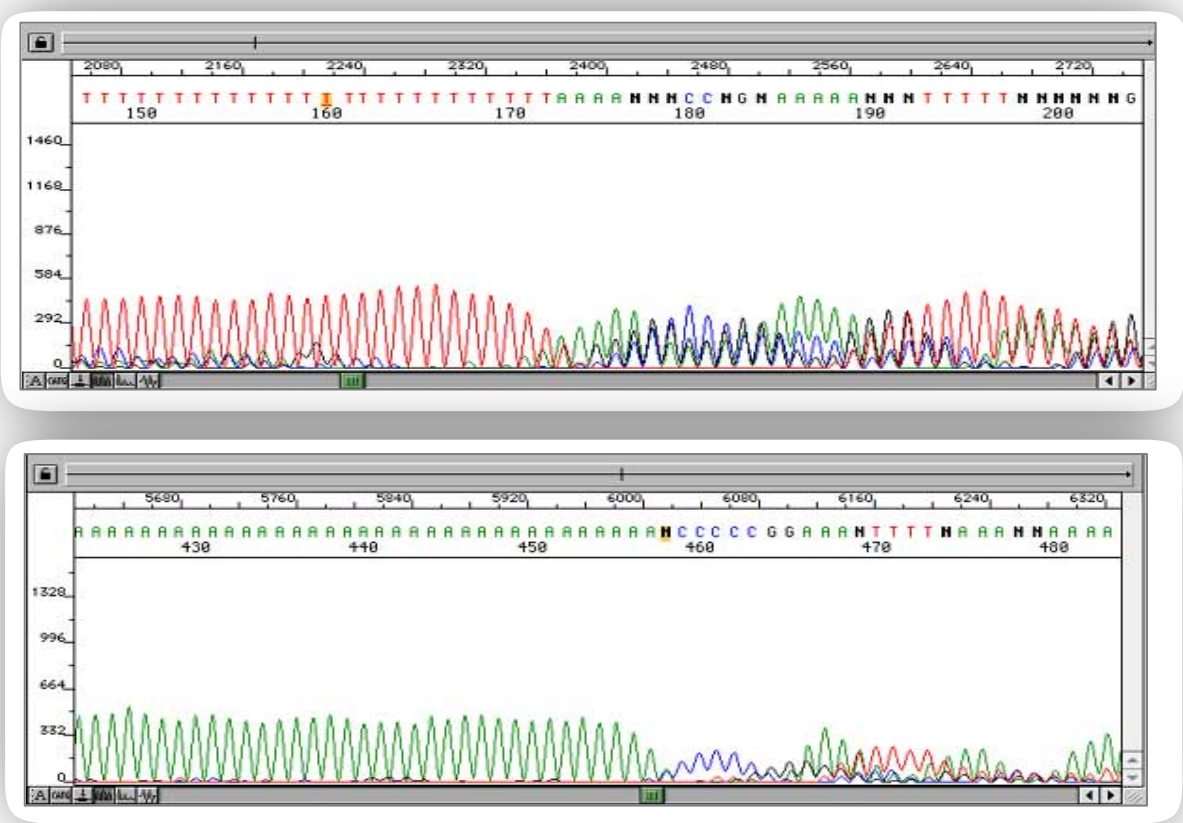
Reason

- Unspecific binding due to a mixture of various primer products with differing lengths

Tip!

- Isolate new DNA from a pure single colony and re-sequence (re-streak the clone if necessary)
- Optimize PCR conditions if multiple bands appear after checking PCR template on an agarose gel
- Gel purify your PCR product
- Ensure primer has one binding site
- Ensure that only one primer is used for sequencing
- Check for remaining primers after PCR clean-up on an agarose gel
- Re-design primer and/or optimise PCR amplification

Long Homopolymer Regions



Electropherogram with homopolymer regions

Chromatogram Characteristics

- Mixed sequences downstream of long homopolymer T or A regions

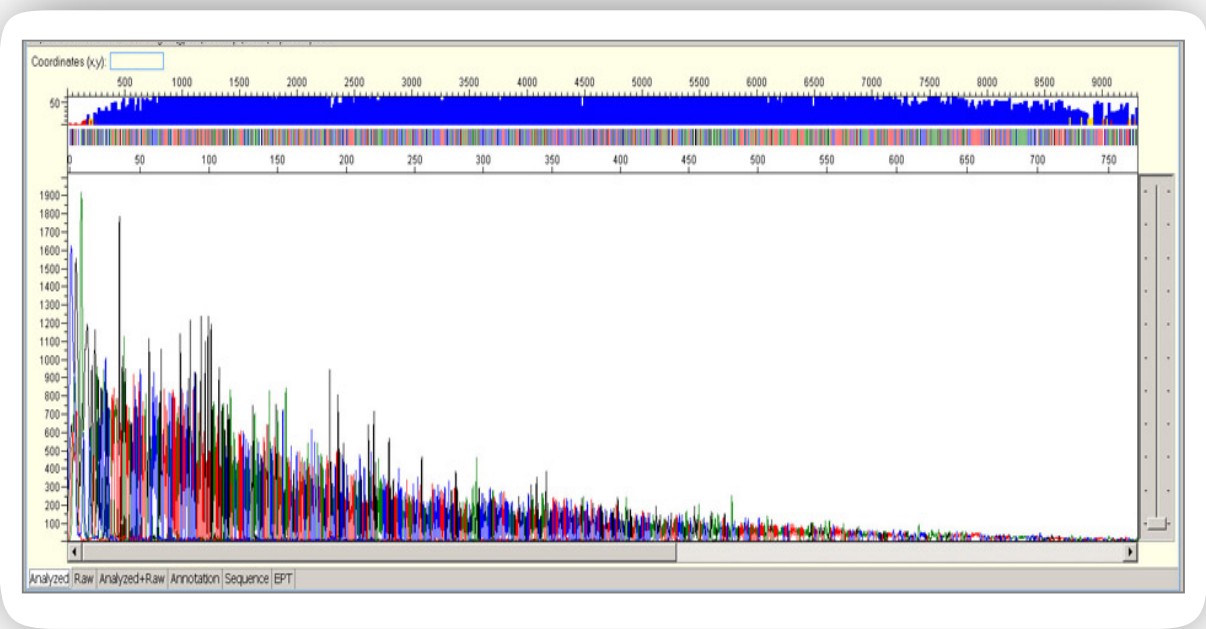
Reason

- Slippage of the enzyme: incorporation of 20 T bases or 21 or 22 T in a 20 T region. Effect more pronounced in PCR templates than plasmids

Tip!

- Sequence the complementary strand
- Try using an anchored primer: e.g. polyT sequencing primer with an A, C or G base at the 3' end of the polyT to anchor the primer at the end of the homopolymer region
- Special sequencing conditions (low annealing temperatures) necessary with anchored primers. Try our Sequencing Service à la Carte

Top Heavy Sequences



Electropherogram with heavy sequences

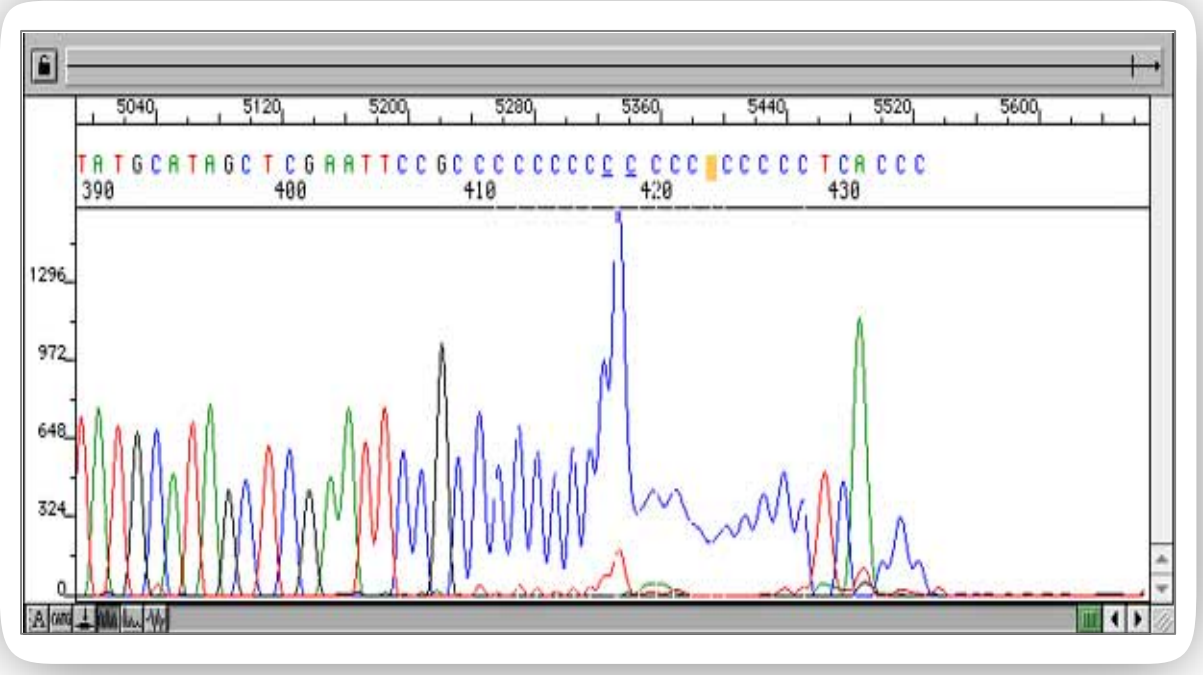
Chromatogram Characteristics

- Very high peaks at the beginning followed by a sharp decrease in signal intensity
- Short fragments are generated in excess and preferentially injected into the capillary

Reasons

- Template or primers are depleted in the early rounds of cycle sequencing creating an excess of short fragments
- Short fragments are preferentially injected into the capillary leading to blockage which inhibits the rapid flow of reaction products
- Excessive use of template or primer in the sequencing reaction

Abrupt Signal Loss



Electropherogram with abrupt peak loss

Chromatogram Characteristics

- High peaks that stop abruptly at a certain position
- Base calls may stop before the end of a read

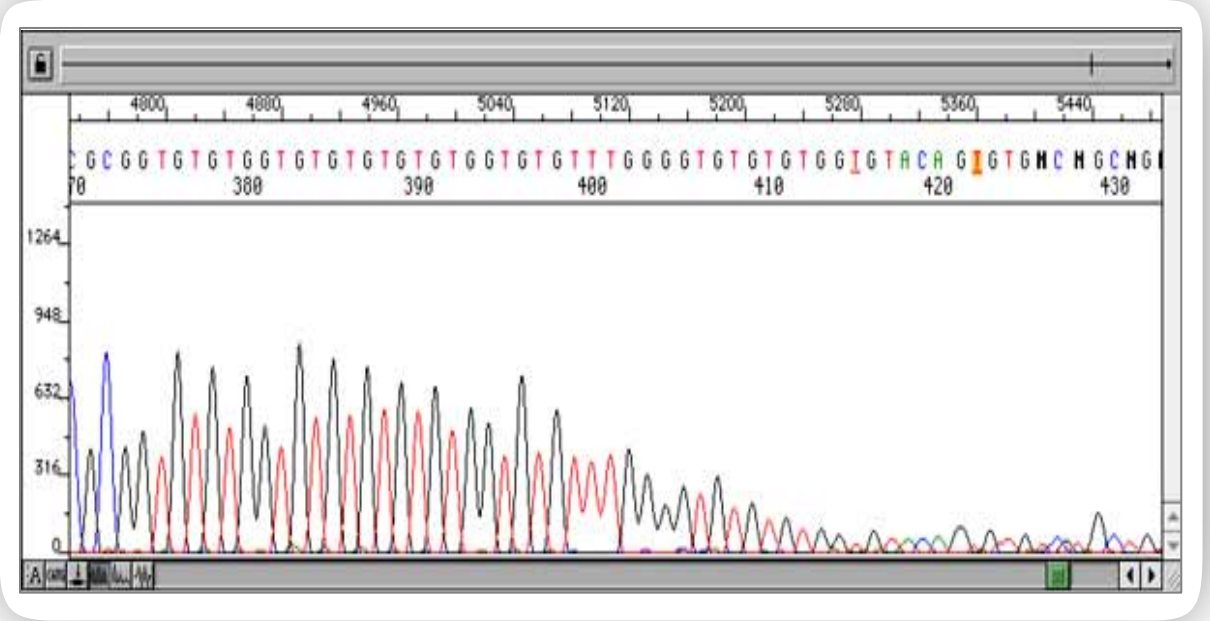
Reasons

- Template has a secondary structure
- High GC content

Tip!

- Sequencing the template from the opposite direction
- Use a primer at a different position
- Apply 7-deaza-dGTP at the PCR amplification stage to relax the hairpin structure (75% of the dGTP in the PCR)
- Try our Sequencing Service à la Carte in combination with the additional service “Sequencing with special conditions

Repetitive Regions



Electropherogram with mixed sequencing starting at a cloning site

Chromatogram Characteristics

- Gradually decreasing peak height starting from within the repeat
- Base calls are fading off after the repeat region)

Reason

- Use of more than one colony for plasmid growth and preparation

Tip!

- Sequencing the template from the opposite direction
- Use a primer at a different position