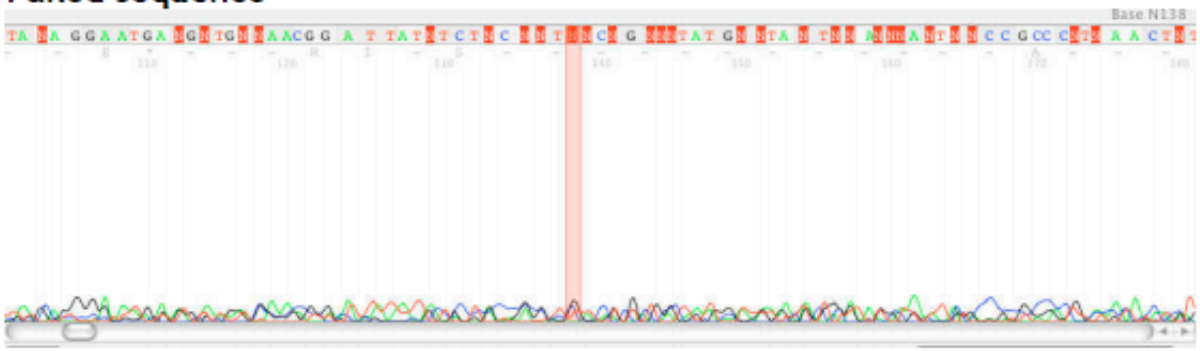
Common sequencing errors

http://genepool.bio.ed.ac.uk/sanger/Sanger_troubleshooting_guide_v1.pdf

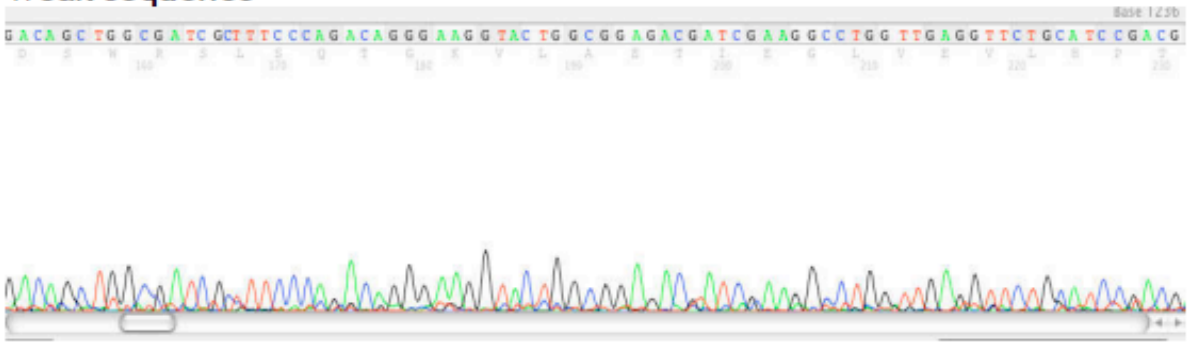
https://www.nucleics.com/DNA_sequencing_support/DNA-sequencing-troubleshooting.html

Failed sequence



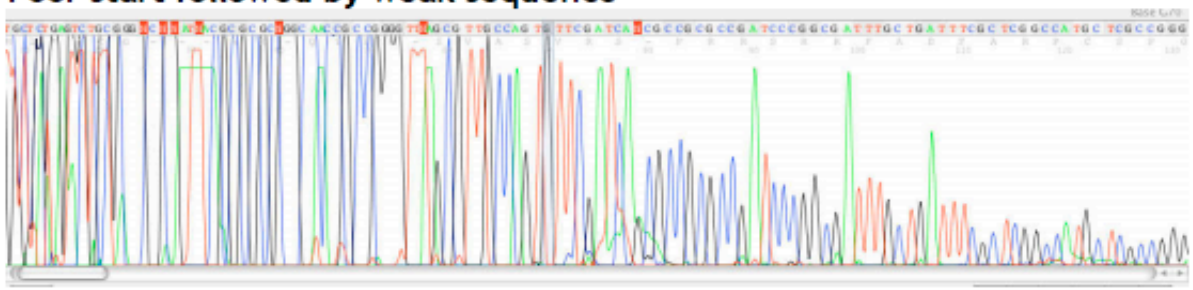
Problem	Probable cause	Solution
Lack of sequence data	No priming site present	Make sure the primer site is present in the vector you are using Redesign/ use a different primer
	Primers have degraded through freeze-thaw cycles	Make up new primer stocks
	Inefficient primer binding	Redesign primer
	Insufficient amount of DNA template	Quantify DNA Increase the amount of DNA template
	DNA has degraded	Re-extract DNA
	Inhibitory contaminant in your samples eg salts, phenol, EDTA, ethanol	Clean-up DNA template

Weak sequence



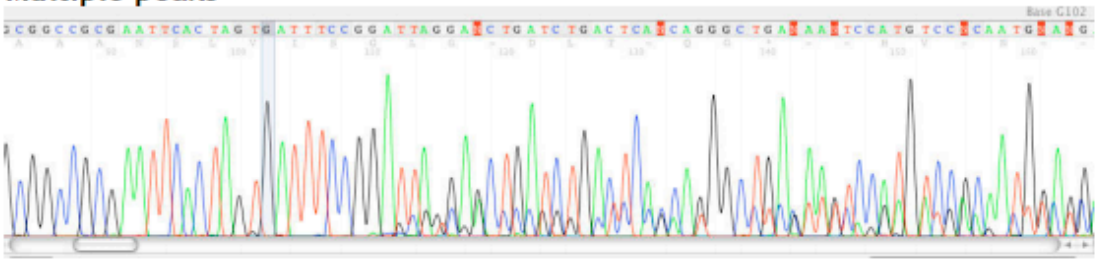
Problem	Probable cause	Solution
Low peaks throughout	Insufficient amount of DNA template	Quantitate the DNA Increase the amount of DNA template
	Inhibitory contaminant in your samples (e.g. salts, phenol, EDTA, ethanol)	Clean-up DNA template
	Insufficient amount of primer	Check primer dilution
	Inefficient primer binding	Redesign primer

Poor start followed by weak sequence



Problem	Probable cause	Solution
Poor sequence at the start followed by weak signal	Primer binding to itself	Redesign sequencing primer
	Other primers present	Check PCR clean-up has removed all other possible primers

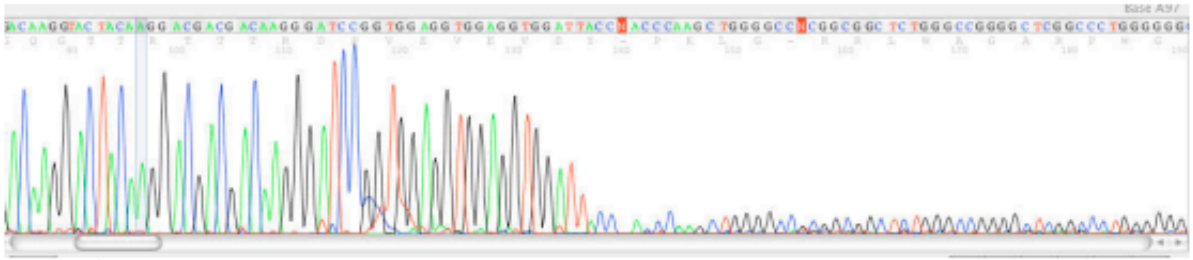
Multiple peaks



Problem	Probable cause	Solution
Overlapping peaks in the sequence data	Multiple priming sites	Use a different primer.
	Residual primers (PCR product has not been cleaned up)	Make sure all PCR primers and dNTPs have been removed
	Poor purification during primer synthesis (full-length primer is mixed in with shorter primer missing one base giving a shadow sequence one base behind the real sequence)	Order new sequencing primer, preferably HPLC purified
	Mixed plasmid prep	Contaminated template. Clean sequence at the start with mixed peaks beginning at the cloning site Ensure single colonies are picked
	INDEL in PCR product	Sequence the complementary stand Sequence from cloned PCR products

Truncated sequence

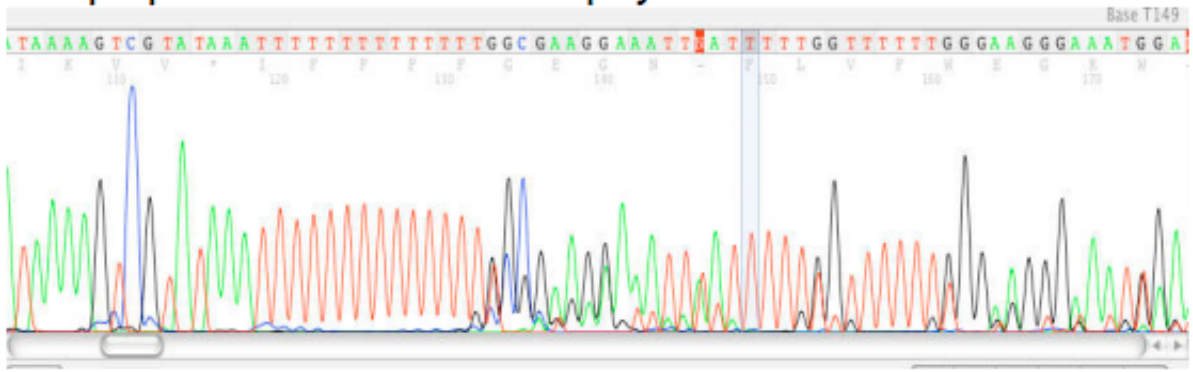
Too much template



Secondary Structure

Problem	Probable cause	Solution
Sequence starts well but signal stops abruptly	Secondary structure (GC and AT rich templates can cause the DNA to loop and form hairpins)	Add (1ul) DMSO to the sequencing reaction to help relax the structure. Design primers close to the hairpin
	Linearized DNA (restriction enzymes may have cut an internal site)	Run product out on an agarose gel to check
Sequence starts well but signal weakens gradually (ski-slope effect)	Too much DNA template (overload of DNA leads to excessive number of short fragments)	Use less DNA template
Sequence starts well but signal weakens rapidly	Repetitive region (Repeat regions, especially GC and GT repeats, can cause the signal to fade either due to depletion or slippage or secondary structure)	Add (1ul) DMSO to the sequencing reaction. Sequence the complementary strand

Multiple peaks downstream to homopolymer



Problem	Probable cause	Solution
Overlapping peaks following stretch of mononucleotide sequence	Enzyme slippage occurs giving varying lengths of the same sequence after this region (n-1, n-2 and n-3 populations)	Sequence the complementary strand

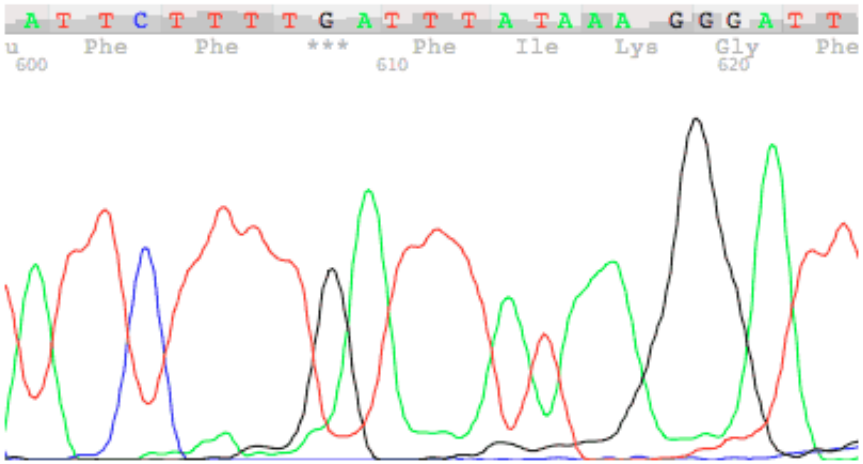


Figure 1. Example of a "blurry" or poorly resolved trace chromatogram collected using a ABI 3730 50cm array.

Causes of blurry sequencing traces

- **Capillary overload.** This is normally caused by running "dirty" samples with large amounts of template (or other) DNA, proteins or salt.
- **High sequencing run voltages.** The higher the run voltage the worse the resolution of the peaks in the trace file. High run voltages can also cause problems for the KB base caller resulting from the collection of the void peak.