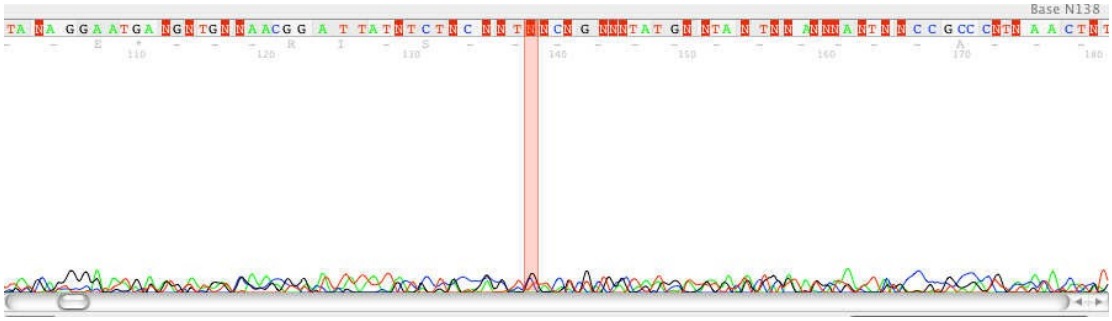
Sanger Sequencing

Troubleshooting Guide

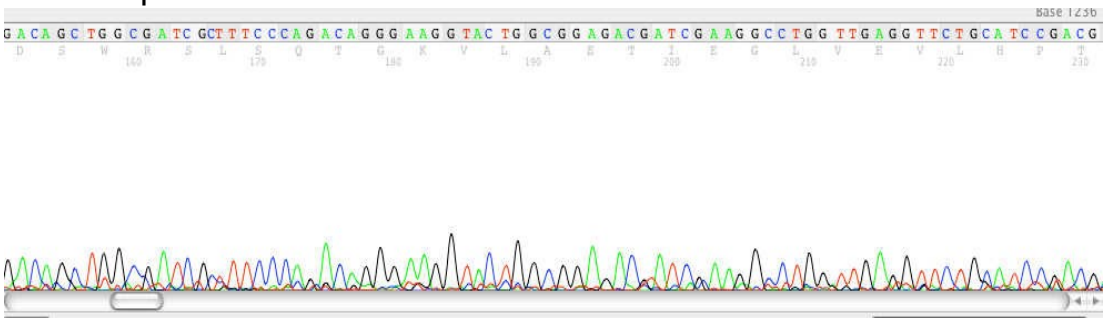
Below are examples of the main problems experienced in ABI Sanger Sequencing. Possible causes for failure and their solutions are listed below each example. The list is not exhaustive so please contact us at 'edgenomics-sanger@ed.ac.uk' if you have any othersolutions to add.

Failed sequence



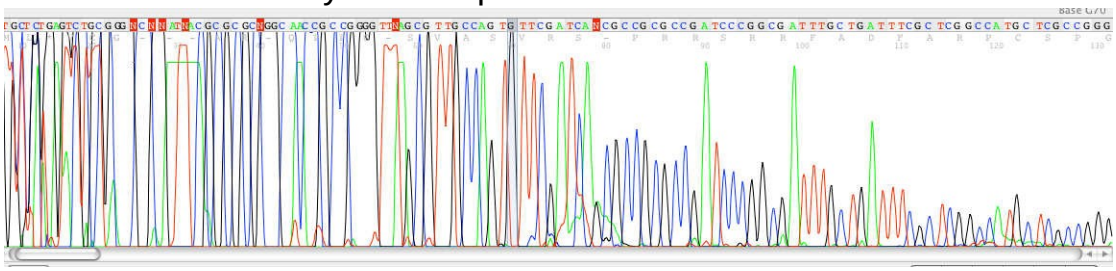
Problem	Probable cause	Solution
Lack of sequence data	No priming site present	Make sure the primer site is present in the vector you are using Redesign/ use a different primer
	Primers have degraded through freeze-thaw cycles	Make up new primer stocks
	Inefficient primer binding	Redesign primer
	Insufficient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

Weak sequence



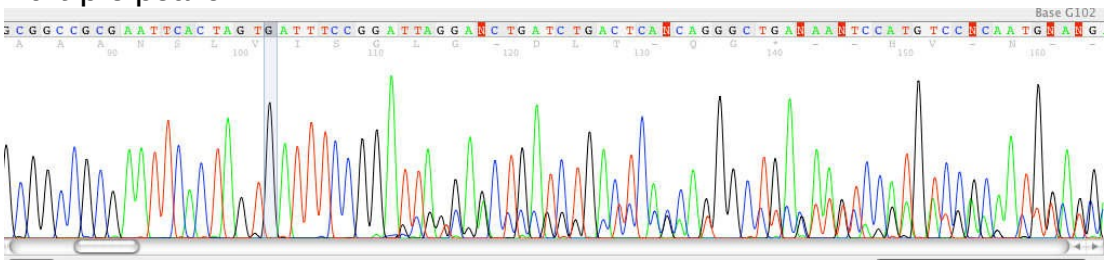
Problem	Probable cause	Solution
Low peaks throughout	Insufficient amount of DNA template	Quantitate the DNA Increase the amount of DNA template
	Inhibitory contaminant in your samples (e.g. salts, phenol, EDTA, ethanol)	Clean-up DNA template
	Insufficient amount of primer	Check primer dilution
	Inefficient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

Poor start followed by weak sequence



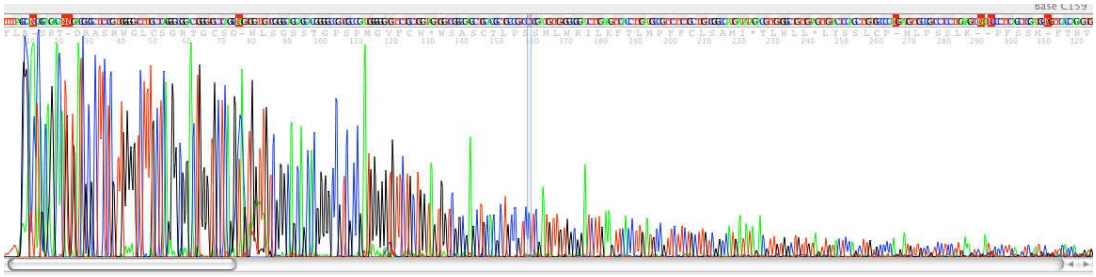
Problem	Probable cause	Solution
Poor sequence at the start followed by weak signal	Primer binding to itself	Redesign sequencing primer
	Other primers present	Check PCR clean-up has removed all other possible primers

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 onebase 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 strand Sequence from cloned PCR products

Truncated sequence

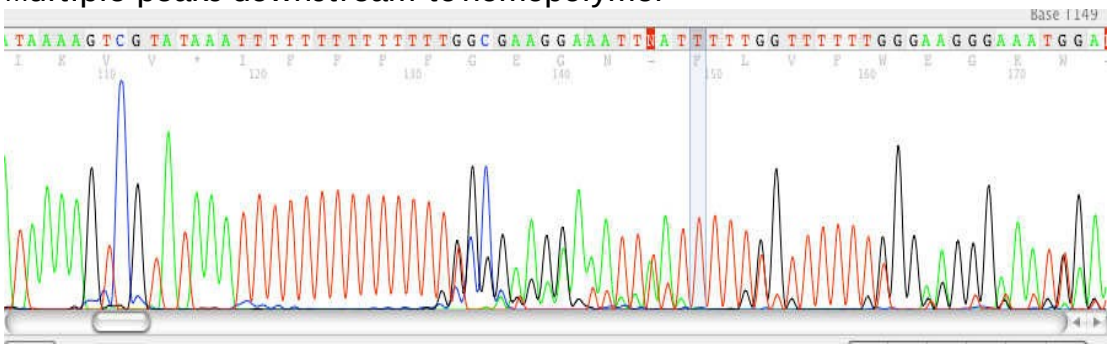


Too much template

Secondary Structure

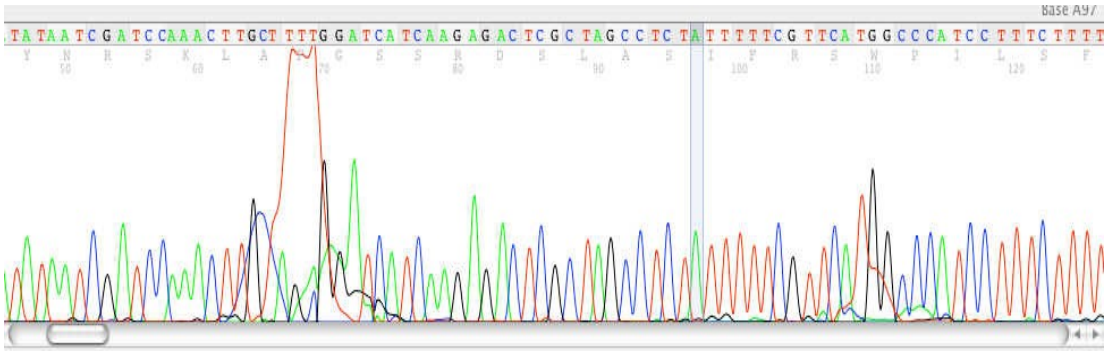
Problem	Probable cause	Solution
Sequence starts well but signal stops abruptly	Secondary structure (GC and AT rich templates can cause the DNA to loop and formhairpins)	Add (1ul) DMSO to the sequencing reaction to help relax the structure. Design primers close to the hairpin
	Linearized DNA (restriction 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 todepletion or slippage orsecondary 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

Artifacts



Problem	Probable cause	Solution
Large peaks obscuring the real sequence	Dye blobs caused by unincorporated BigDye and typically seen at 70bp and 120bp. Usually seen in failed or weak sequences. Real sequence can still be read underneath these blobs	Add more DNA template or less BigDye to sequencing reaction
Sudden large multicoloured peak covering 1-2 bases Sample peaks become lumpy and increasingly unreadable early in the sequence (before 500bp)	Small air bubble of dried polymer within the capillary	Contact us and sample can be re-run
	If related to individual samples this is due to a contaminant in the sample	Clean up template DNA
	Degradation of polymer or capillary array	Inform us if loss of resolution continues