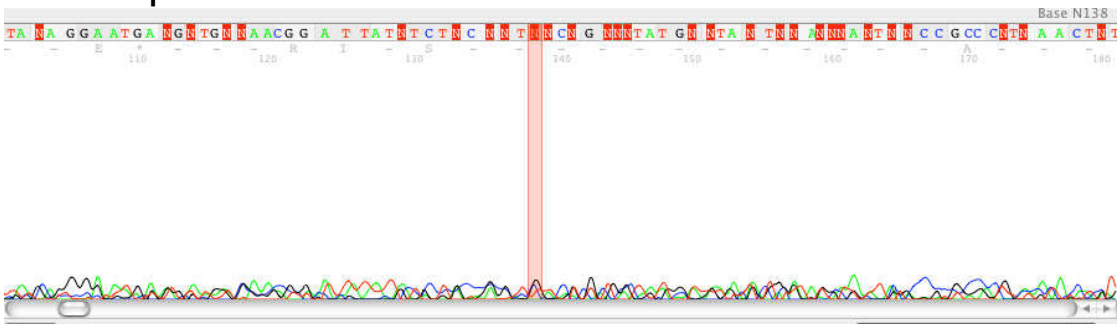
# Sanger Sequencing

# Troubleshooting Guide

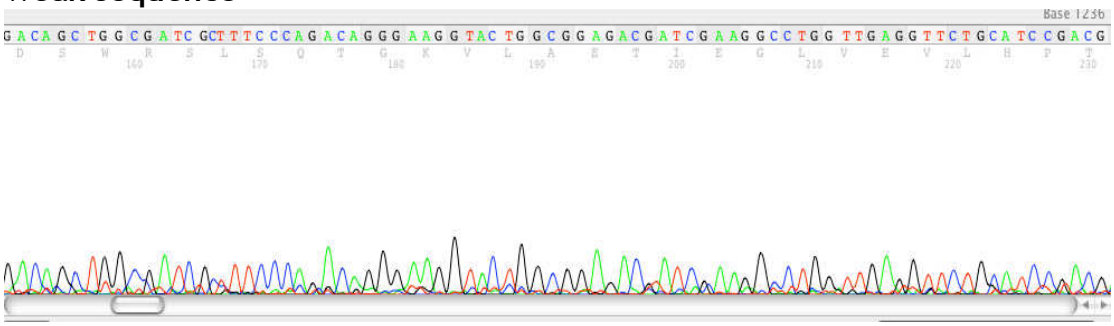
Below are examples of the main problems experienced in ABI Sanger sequencing. Possible causes for failure and their solutions are listed below each example. The list is not exhaustive so please contact us at genepool-sanger@ed.ac.uk if you have any other solutions to add.

## Failed sequence



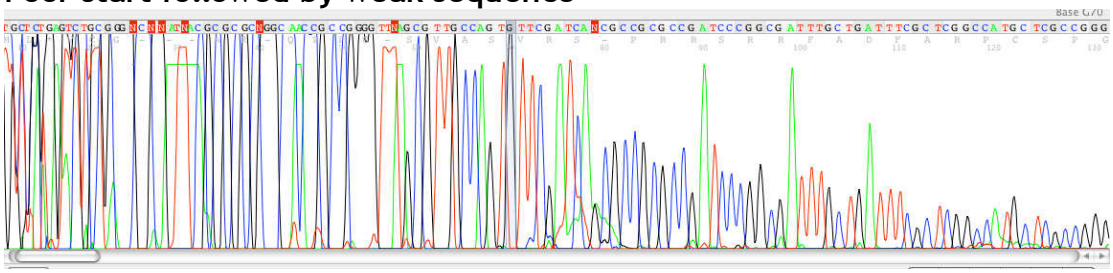

| Problem | Probable cause | Solution |
|---|---|---|
| Lack of sequence data | No priming site present | Make sure the primer site is present in the vector you are using<br>Redesign/ use a different primer |
| | Primers have degraded through freeze-thaw cycles | Make up new primer stocks |
| | Inefficient primer binding | Redesign primer |
| | Insufficient amount of DNA template | Quantify DNA<br>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



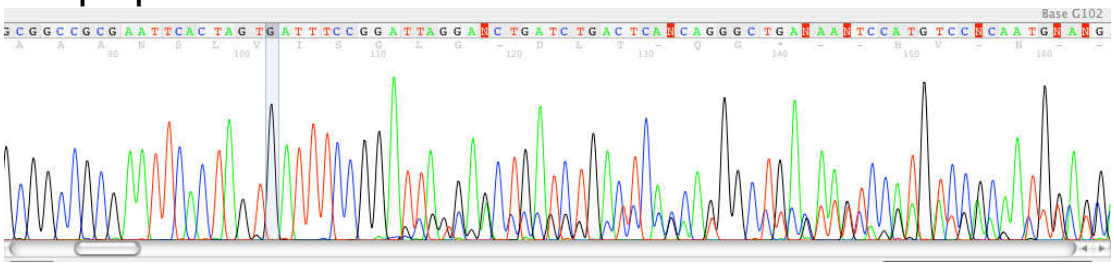
| Problem | Probable cause | Solution |
|---|---|---|
| Low peaks throughout | Insufficient amount of DNA template | Quantitate the DNA<br>Increase the amount of DNA template |
| | Inhibitory contaminant in your samples (e.g. salts, phenol, EDTA, ethanol) | Clean-up DNA template |
| | Insufficient amount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

## Poor start followed by weak sequence



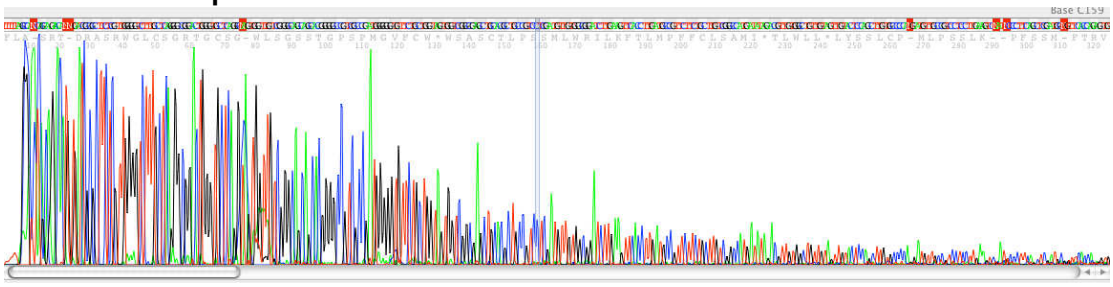
| Problem | Probable cause | Solution |
|---|---|---|
| Poor sequence at the start followed by weak signal | Primer binding to itself | Redesign sequencing primer |
| | Other primers present | Check PCR clean-up has removed all other possible primers |

## Multiple peaks

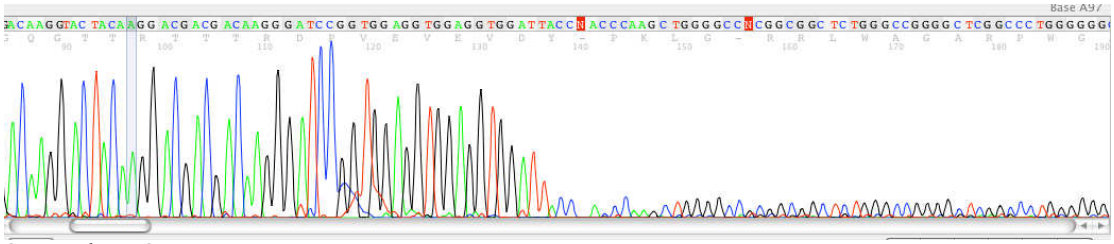


| Problem | Probable cause | Solution |
|---|---|---|
| Overlapping peaks in the sequence data | Multiple priming sites | Use a different primer. |
| | Residual primers (PCR product has not been cleaned up) | Make sure all PCR primers and dNTPs have been removed |
| | Poor purification during primer 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<br>Ensure single colonies are picked |
| | INDEL in PCR product | Sequence the complementary stand<br>Sequence from cloned PCR products |

## Truncated sequence



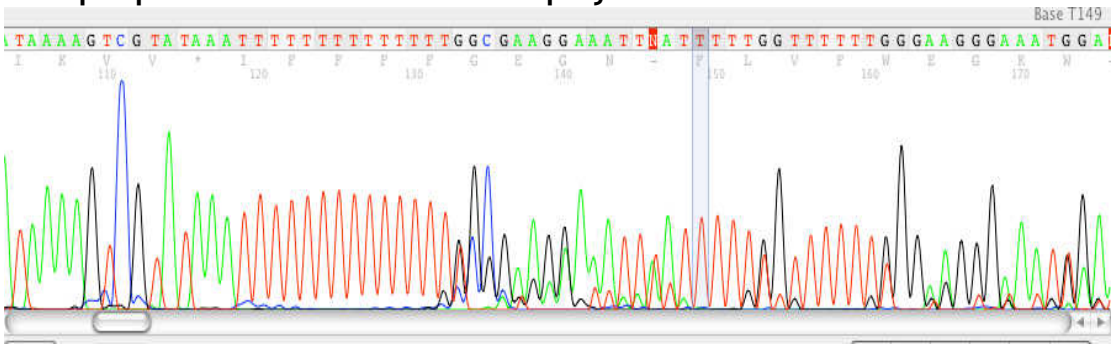
**Too much template**



**Secondary Structure**

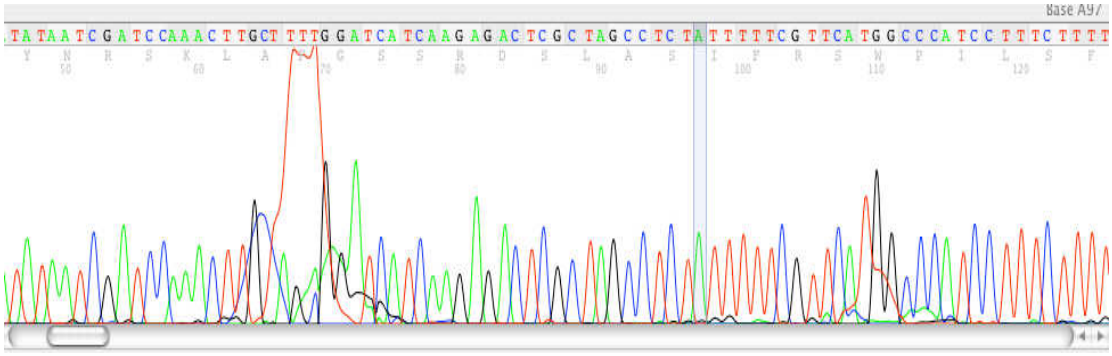
| Problem | Probable cause | Solution |
|---|---|---|
| Sequence starts well but signal stops abruptly | Secondary structure (GC and AT rich templates can cause the DNA to loop and form hairpins) | Add (1ul) DMSO to the sequencing reaction to help relax the structure.<br>Design primers close to the hairpin |
| | 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.<br>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 | Small air bubble of dried polymer within the capillary | Contact us and sample can be re-run |
| Sample peaks become lumpy and increasingly unreadable early in the sequence (before 500bp) | 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 |