SANGER SEQUENCING TROUBLESHOOTING

1. NO REACTION

- No DNA o less than needed.
- EDTA or other polymerase inhibitors, sample contamination.
- Sequencing *primer* with secondary structures.
- No *primer* or concentration below 3.2 pmoles.
- Sequencing *primer* does not hybridize.
- Tm *primer* below 50ºC.

2. NOISY ELECTROPHEROGRAM

- Weak sequencing signal due to low DNA amount, contaminants, low primer concentration…

3. SEQUENCING SIGNAL GOES DOWN

- Sample contaminants: EDTA, salts…
- DNA and primer quantities are not compensated.

4. DOBLE SEQUENCE ELECTROPHEROGRAM

- More than one DNA template.
- More than one *primer* priming sites.
- *Primer* contamination or unproper purification.
- PCR product unproperly purified

5. SEQUENCING SIGNAL STOPS ABRUPTLY

- DNA is broken or digested at that level.
- Strong DNA secondary structure.

6. DOUBLE SEQUENCE AFTER POLI-A OR POLI-T

- DNA polymerase slippage.