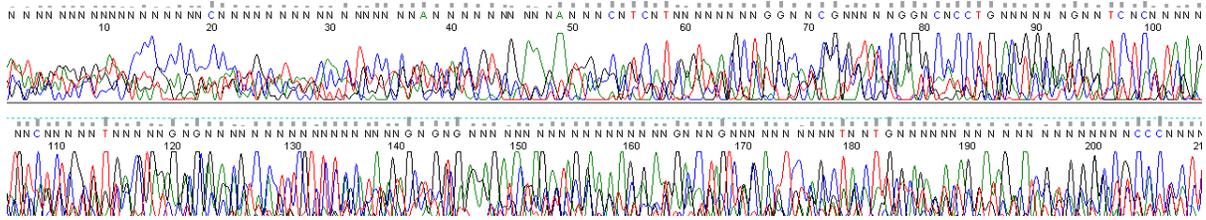
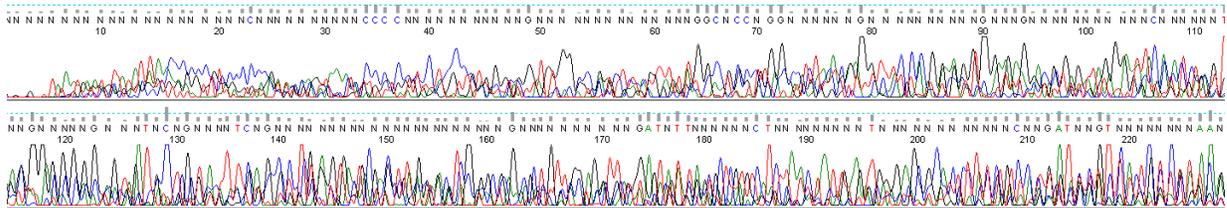


GENEWIZ Solutions Guide: DNA Sequencing No Priming Results

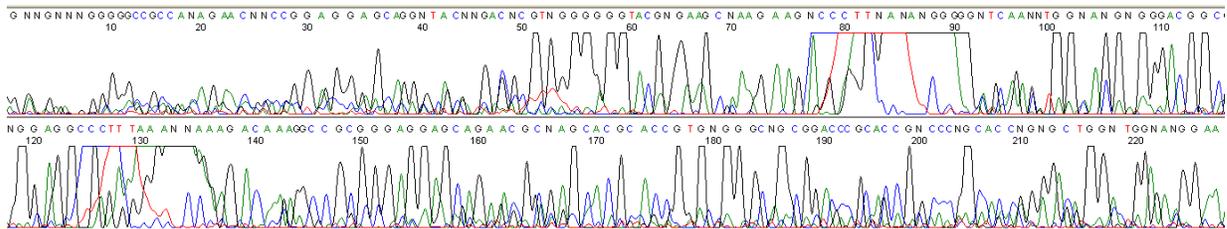
The following Examples, Possible Causes, and Solutions guidelines will help obtain your best DNA sequencing results. Please email or call GENEWIZ Technical Support for further diagnosis or assistance at dnaseq@geneviz.com or 1-877-436-3949 option 2.



Example 1: Although some peaks are visible, the overall signal intensity is only around 60 (for comparison: a good signal intensity is over 300, preferably 500-1500, and background signal intensity is 20-30). Therefore, any peaks seen in this chromatogram are not trustworthy — they are pulled up by the analysis program through normalization-rescaling, along with the background signal.



Example 2: The signal intensity is below 100 with few discernable peaks; the reaction did not work.



Example 3: One base is incorrectly favored; the reaction is still considered a failed reaction. Overall signal intensity of this reaction is 40.

Possible Causes:

- DNA concentration too high or too low
- Primer binding site not present
- Primer concentration not correct
- Primer not added
- Poorly designed primer or there is a primer dimer conformation with a 3' overhang
- Poor quality DNA template— DNA contaminated with phenol, chloroform, EDTA, ethanol, isopropanol, etc.

Solutions:

1. We recommend eluting your DNA in dH₂O and avoiding TE and EB buffers; especially for mini-preps. This removes the confounding problem of salt and EtOH contamination carry over from the purification process. For larger preparations, diluting the stock solution in water (not TE or EB buffer) is strongly recommended. NB: EDTA will inhibit part of the sequencing reaction; little or no EDTA should be present in your sample.

2. Double check that the DNA concentration is in the correct range (<http://www.genewiz.com/PrepareSample.aspx>).
3. Double check that the primer binding site is present (If using a GENEWIZ Universal primer- check the primer sequence to make sure it matches your vector (<http://www.genewiz.com/universalprimers.aspx>)).
4. Check that the primer concentration is in the correct range. (Quick reference: 5 pmol/μl is equal to 5 μM; for a 20mer, 5 pmol/μl = 33.5 ng/μl)
 - a. **Premix reaction**- add 5 μl of 5 pmol/μl (5μM) primer to your samples
 - b. **Pre-Defined reaction**- send your primer separately at 5 pmol/μl (5μM)
5. Make sure that the primer has been added (if sending a "Premix" order).
6. Use a primer design program (example: IDT's Oligo Analyzer- <http://www.idtdna.com/analyzer/Applications/OligoAnalyzer>) to verify that no self dimer is formed by your primer. (Problems with successful sequencing begin to arise at dimer conformations more negative than -10 kcal/mol)
7. If the samples still are not working, we are happy to help you with your trouble shooting efforts! Just e-mail GENEWIZ Technical Support (dnaseq@genewiz.com) or call 877-436-3949, option 2.