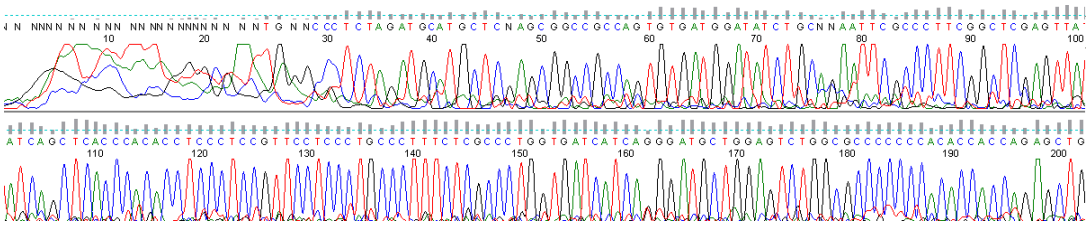
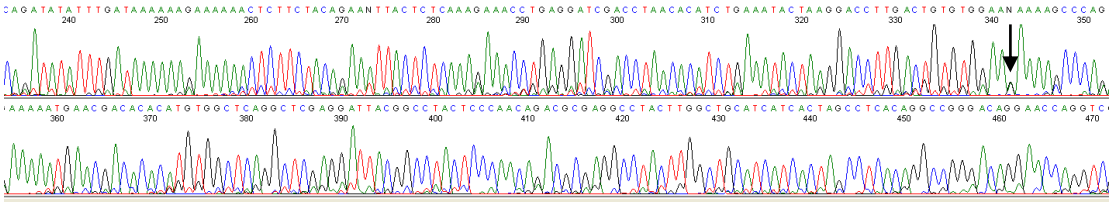
GENEWIZ Solutions Guide: DNA Sequencing
High Background Results

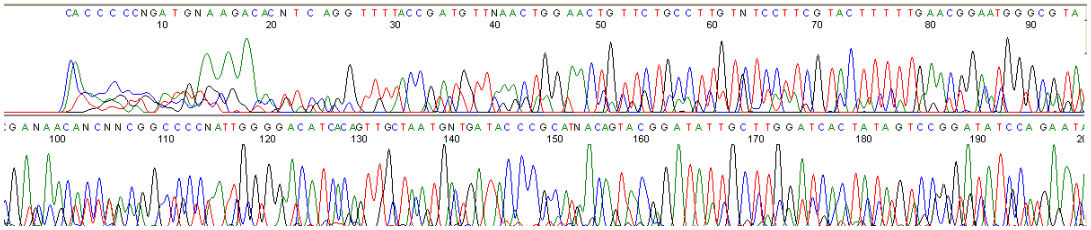
The following Examples, Possible Causes, and Solutions guidelines will help obtain your best DNA sequencing results. Please email or call GENEWIZ Technical Support for further diagnosis or assistance at dnaseq@genewiz.com or 1-877-436-3949 option 2.



Example 1: There is just a slight background signal in the chromatogram, but it does prevent the correct resolution of a few bases (76-77bp).



Example 2: High background signal throughout can interfere with the correct resolution of several bases (example at base 342 - black arrow) throughout the sequence. Note that if the contaminating sequence is the same as the correct base, you will see only one base being called at several different locations.



Example 3: This trace can almost be called “non-specific”, but the dominant sequence is still being called correctly for the majority of the sequence. Again, there are a few bases that are not able to be properly resolved and called due to the contaminating sequence.

Possible Causes:

- DNA/primer ratio is not optimal - Weak signal (inefficient reaction) may cause the analysis program to pull up the real signal as well as the background signal in order to call the bases. (Intensities would be lower than 100)
- Contaminating DNA - If you have a good intensity, the background signal could be from some contaminating plasmid or genomic DNA.
- Secondary priming site -Your primer may have a secondary site that is not a perfect match, but will still generate some sequence that interferes with your target sequence.

Solutions:

1. Check your DNA and primer concentrations to make sure they are within our guidelines (http://www.genewiz.com/PrepareSample.aspx).
2. You may need to further purify your samples to make sure that the DNA is clean enough to obtain a good sequencing reaction (extra wash step, larger DNA prep). You can also submit your samples as bacterial colonies on an agar plate. We will pick single colonies, amplify the DNA, and proceed with sequencing if you are having problems with the DNA preparation step. To do this, just pick “Custom Order” and choose “Bacterial Colony” in the drop down menu for type of DNA.

3. Re-design your primer or try a primer from the other direction.
4. If you are having trouble getting a concentration high enough to meet our guidelines, GENEWIZ can perform an amplification step on the plasmid DNA first before proceeding with your sequencing reaction. Please contact GENEWIZ Technical Support (dnaseq@genewiz.com or 1-877-436-3949 option 2) for further assistance.