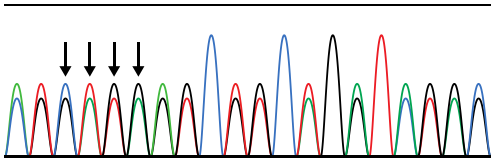
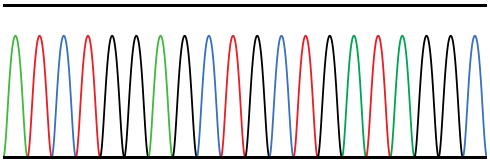
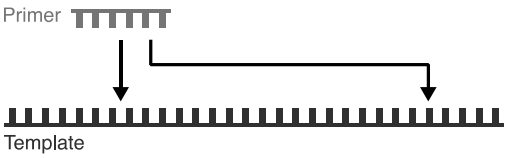
Description

Non-specific results have good signal intensity but overlapping or mixed peaks for all or some of the chromatogram. The data represents a mixed population of sequencing products. Non-specific and high background results are sometimes difficult to differentiate; chromatograms are considered non-specific if the competing traces have similar intensities.

NON-SPECIFIC	**GOOD RESULT**
Overlapping peaks of roughly equal intensity 	Well defined peaks with low background for at least 500 bp 

Troubleshooting

There are several possible causes for a non-specific result. The list below is not exhaustive but represents the most common issues. For more assistance, please contact Technical Support at 877-436-3949 ext. 2 or dnaseq@genewiz.com.

POSSIBLE CAUSE	**SOLUTION**
Primer binds to multiple sites. 	• Redesign your primer or try a primer from the other direction. • Check your expected sequence for possible secondary priming sites. • When cloning from one vector to another, ensure that universal primer binding sites are not duplicated in the final construct.

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POSSIBLE CAUSE	SOLUTION

The sample contains a mixed population of templates. Below are some examples.
• Two plasmids with different inserts. When using a primer that binds to the vector backbone, the trace will start cleanly and become non-specific at the cloning site.
• PCR products amplified from a heterozygous individual. If the two alleles differ by an insertion/deletion (indel), the trace would start cleanly but become non-specific when the polymerase encounters the indel. The non-specificity will continue until the polymerase reaches the end of the shorter PCR product.
• Hypervariable regions
	• For plasmids or BACs, re-isolate the clone to ensure the prep has only one species of plasmid.
	• For bacterial samples and phage, re-isolate the clone or sequence another sample.
	• For agar plates with a high density of colonies, expect a higher rate of non-specific results (as colonies are more likely to contain multiple clones). We recommend submitting no more than 200 colonies per plate with uniform density.
	• For PCR products, run your PCR reaction on a gel to ensure only one band is visible. For multiple banding, perform one of the following approaches:
	  ○ Gel purification
	  ○ Band-stab PCR. As with gel purification, run the nonspecific PCR products on an agarose gel. Instead of excising the entire band of interest, you can use a pipette tip or needle to stab a small portion of the band. Next, set up another PCR with either the original or nested (internal) primers. Release the template DNA into the PCR mix by swirling the pipette or needle. Prepare and run a sample of the second reaction on an agarose gel to confirm the presence of a single band. The PCR mixture can then be added to a DNA-binding matrix for purification or cleaned up by the addition of enzymes that degrade leftover primers and dNTPs.
	  ○ Nested primers. To increase the specificity of PCR, a second round of amplification can be performed using a nested primer set. In practice, a highly diluted sample of the original PCR is used as the template in a second PCR containing primers designed to anneal at sites internal to the original primer set.
	  ○ Clone the product into a vector. After inserting the linear product into a vector and transforming bacteria cells, select a random set of colonies and either sequence them directly (via GENEWIZ's colony sequencing service) or prepare mini-preps and submit the purified plasmid for sequencing.
	• For additional tips on how to optimize PCR for sequencing, see our Sanger Tips & Tricks series.

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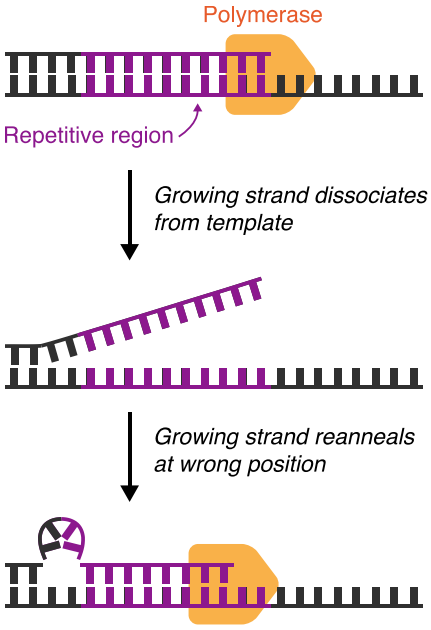
POSSIBLE CAUSE	SOLUTION
Repetitive sequence. The polymerase "slips" within the repetitive region, producing multiple populations of sequencing products that differ slightly in length. Usually this failure cause would be scored as "homopolymeric or repetitive region". However, if the repetitive sequence is not obvious, it may be classified more generally as "non-specific".  	• Unfortunately, with currently available technologies there is no reliable method to make the enzyme faithfully reproduce homopolymeric or repetitive regions. • To obtain the sequence on the other side of the repetitive region, you will need to sequence from the other direction and then align the two sequences. • Alternatively, you can use an anchored primer that binds to the repetitive region and one or more nucleotides flanking the region. • Please be aware that the exact number of bases in the repetitive region may not be able to be deduced. If the specific number of repetitive bases is the ultimate goal, you will need to use a method other than sequencing (e.g. fragment analysis). • Repetitive regions are especially problematic in PCR products since slippage may occur during PCR, sequencing, or both. It may be necessary to clone the fragment into a vector and sequence the resulting plasmid to reduce slippage.