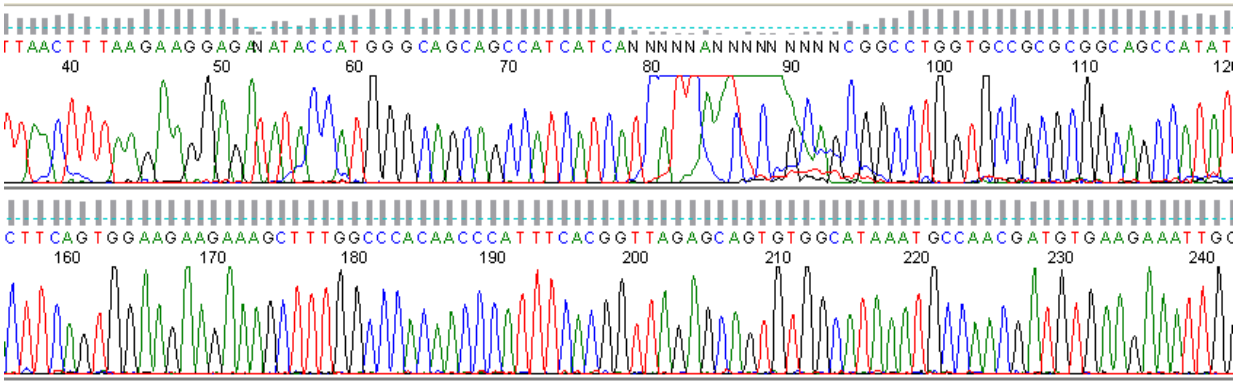
When sequence data is uploaded to iLAb, an email is sent notifying the user that data is ready. The staff of the DNA facility has the ability to edit this message to include specific remarks about how your samples ran, so please look at this message!

This document will provide examples and explanations for the most common remarks that we add about your sequence samples. Here is a brief description about the process to familiarize you with some terms that are used in our messages. Cycle sequence reactions are set up using default protocols from the information provided on your order form. These reactions are cleaned with a sephadex plate to remove excess unincorporated dyes and the samples are loaded on our capillary electrophoresis sequencing instrument. The samples are electrophoretically injected and the data collected and analyzed. If there has been a problem with the sequence run the sample that is already set up and cleaned can go through another injection on the sequencing instrument. This is what we refer to as a reinjection. If this is not possible and more sequence data is required, the process needs to be repeated from the beginning with a new cycle sequence reaction. In this case you will be told to resubmit your sequence order. The format for the sequence data files is: Well Location_template-primer, (A01_pGem-M13For, i.e.). In these messages, we often refer to your individual samples by the injection number (A01), not the full name.

Dye-Blob message:

"Your sequence sample, injection number (A01) has a dye leak which interferes with the basecalling in that region. These cannot be corrected with a reinjection of the sample. It is our policy to repeat the cycle sequence reaction and sequence run once for free to eliminate this problem. If the dye leak was not a problem for you we will not repeat the sample. If it is a problem, resubmit the sample on iLab and put "re-run for free due to a dye leak" in the comment section of the order form. Please make sure that we have enough template DNA to repeat the reaction."

Example:

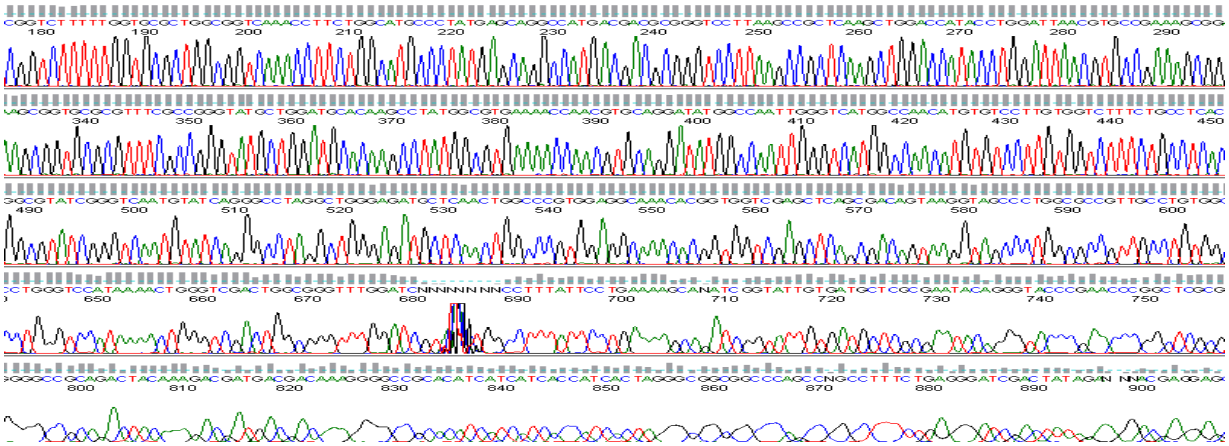


Causes	Solutions
Generally, this is due to incomplete excess dye removal of the cycle sequence reaction.	If the DNA facility cleaned the reaction, we consider this an error on our part and we will repeat the cycle sequence reaction and the sequence run for free. If the clean up was done by the user, we charge for the sample, but we will be happy to help with troubleshooting if the problem continues.
Dye blobs can sometimes occur when the cycle sequence reaction has a very low signal.	Optimize the template and primer for a successful cycle sequence reaction.

Spike message:

"One of your samples had spikes. It is being reinjected at no charge and we will send the data to you when it is ready."

Example:

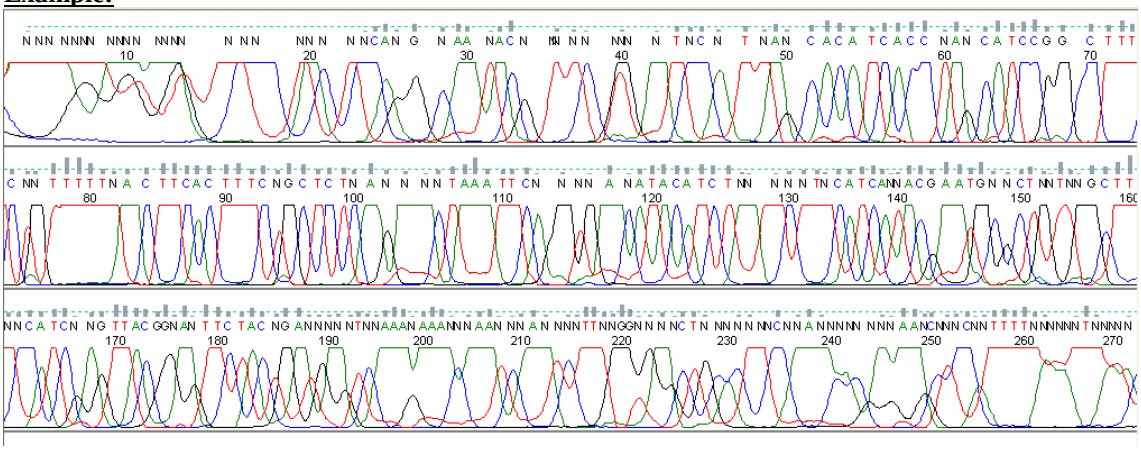


Causes	Solutions
This is a sequencing instrument artifact. It is caused when a small bubble or crystals in the polymer migrate through the capillary with the sample and cause a sharp peak of multicolors. These can mask the true data peaks.	Reinjection of the sample usually gets rid on the spike.

Bad Injection message:

"One of your samples (A01) had a bad injection. It is being reinjected at no charge and we will send the data to you when it is ready."

Example:



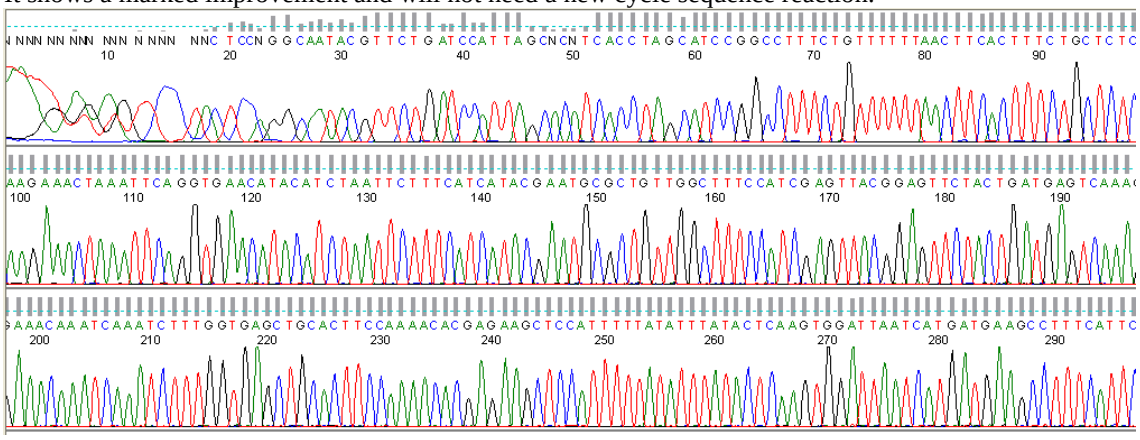
Causes	Solutions
This is often another sequencing instrument artifact. It can be caused by air bubbles in the polymer, old polymer, or a capillary array that has had a high number of injections.	Reinjection of these samples is often successful so we routinely reinject them. Samples that were run on a Friday, however, have degraded during the weekend are not good candidates for re-injects. In this case, you will be notified to resubmit your order for free.
At times, we see bad injections that seem to be sample related. In this case it is thought to be caused by an unknown contaminate in the sample.	Reinjection of these samples does not always result in a better sequence. We recommend that you clean the template DNA and resubmit your order.

Reinjection message:

"This is reinjection of your sample, if it was no better (or worse), and you did not get enough information, resubmit the sample and put "for free, bad injection" in the comments section of the order form."

Example:

This sequence is from a sample that was reinjected. (Same sample as the previous Bad Injection example). It shows a marked improvement and will not need a new cycle sequence reaction.

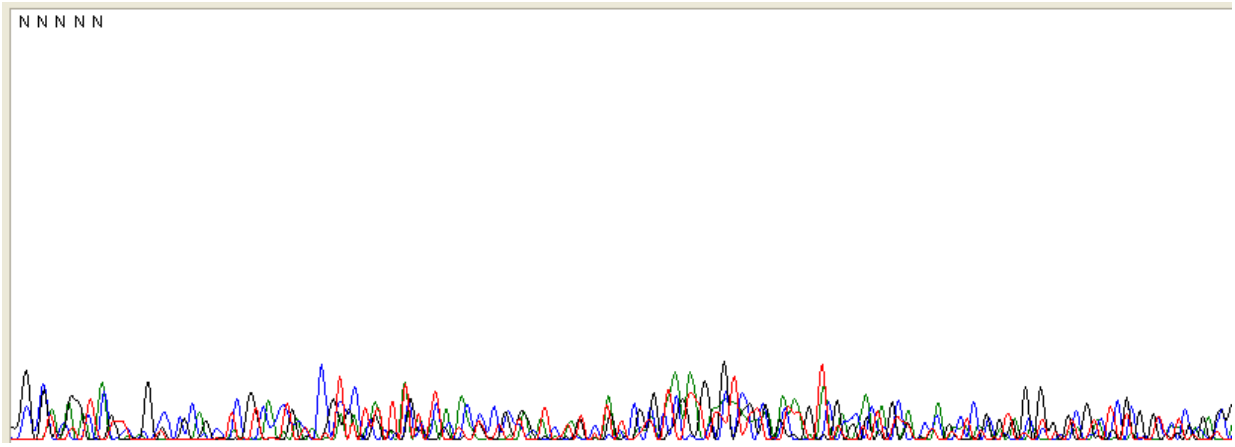


Causes	Solutions
Sequence is improved after renjection.	The sample does not need to be repeated.
Reinjected sample has very low signal or still has bad peaks.	The cycle sequence reaction will have to be repeated. Resubmit the sample with a new order form and put "for free, bad injection" in the comment section of the order form.

No Signal message:

"All of your samples were no signals. Please contact the staff at the facility if you have any questions about why this might have occurred."

Example:



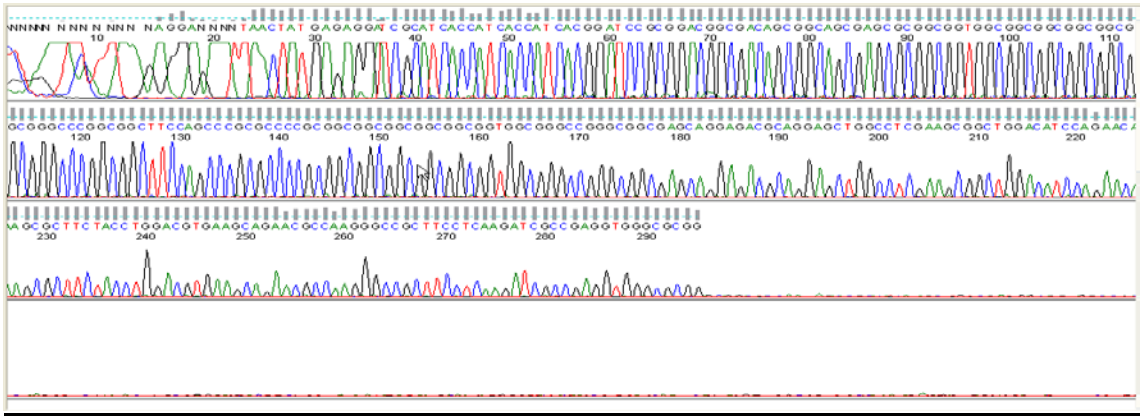
Causes	Solutions
No fluorescently labeled products formed.	
Template does not contain primer sequence.	Resubmit your order with a new primer. Verify the primer site by trying a PCR reaction with the template, primer in questions and another known primer.
Template or primer concentration incorrect.	Check your concentrations and resubmit the order with proper dilutions.
Template contains a contaminate that inhibits cycle sequence reaction.	Clean the template and resubmit your order.
Poor primer efficiency or bad synthesis.	Redesign or reorder primer.
One component left out of the cycle sequence reaction.	DNA Facility error, re-submit the order for free.
Sequencing chemistry is bad, thermal cycler is bad, capillary is blocked.	DNA Facility error, re-submit the order for free.

Sometimes we cannot determine the cause of a no signal until a second cycle sequence reaction is done. If you believe your sample should have worked, you can re-submit the order and in the comment section put "For free if it works, no signal on first run". If a second cycle sequence reaction generates a good sequence, we consider the first no signal to be an error on our part and we won't charge you for the second run. If, however, the second cycle sequence reaction fails, we will assume the problem is on your end and you will be charged.

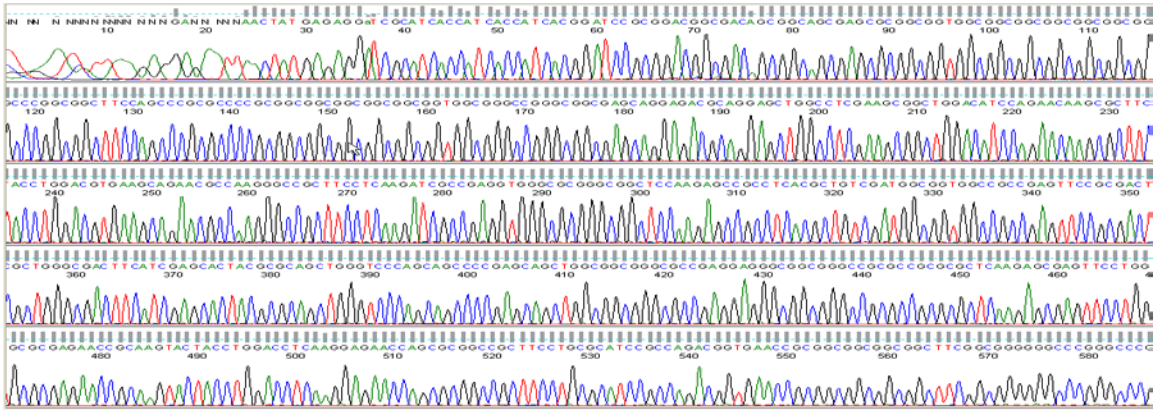
Possible secondary structure message:

"One (or more) of your samples look like they might have secondary structure interfering with the sequencing reaction. You will see this as a sudden drop or stop in sequence. If the data you got wasn't sufficient, you can resubmit your order and put "use GC rich protocol" in the comments section of your order form. These conditions can sometimes improve the chance of sequencing through secondary structure."

Example: The peaks of this very GC rich template get short and the sequence dies early.



This is an example of the same template and primer using our GC Rich conditions.



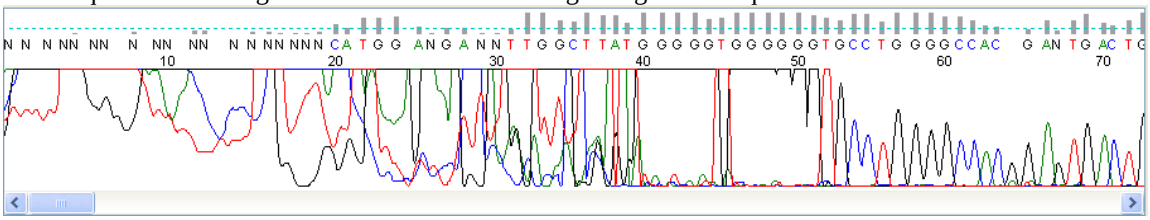
Causes	Solutions
The sequence context of some templates can form hairpin structures that are difficult to sequence through. These can be observed as a gradual decline and then a stop in sequence or an abrupt stop in sequence.	GCRich Protocol: We add DMSO (or possibly other agents) to the cycle sequence reaction to help melt the hairpin structures. We also change our cycle sequencing parameters to include a preincubation cycling step of 95ºC for 5 minutes and increase the denaturation temperature (98ºC vs 96ºC).
	Try sequencing the reverse strand.
	Moving the location of the primer can sometimes improve the results.

Run of G's message:

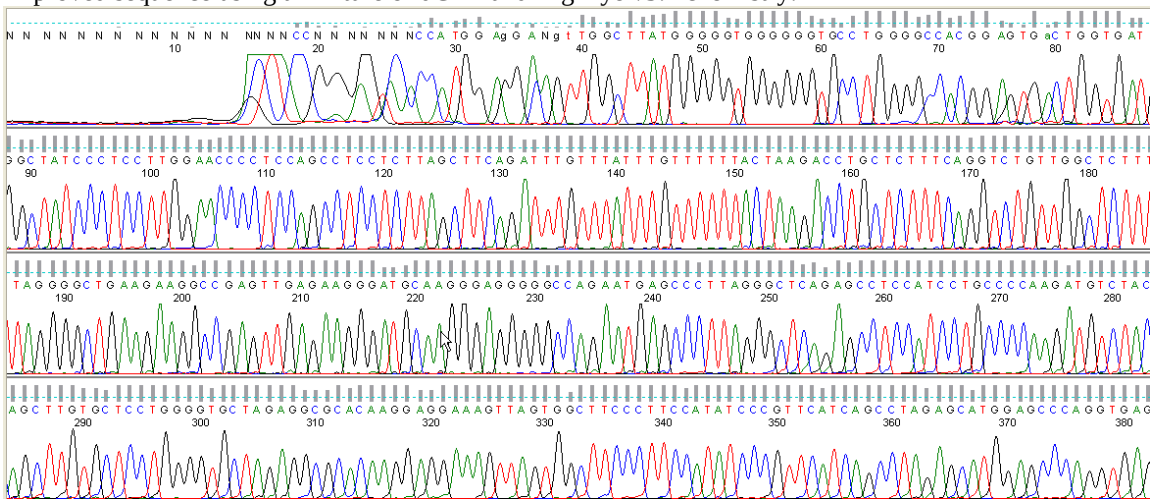
"One (or more) of your samples look like they might have a run of G's interfering with the sequencing reaction. You will see this as a sudden drop or stop in sequence. If the data you got wasn't sufficient, you can resubmit your order and put "use GC rich protocol with dGTP chemistry" in the comment section of your order form. This can sometimes improve the chance of sequencing through a run of G's."

Example:

Poor sequence following two runs of G's near the beginning of the sequence:



Improved sequence using a mixture of dGTP and Big Dye v3.1 chemistry:

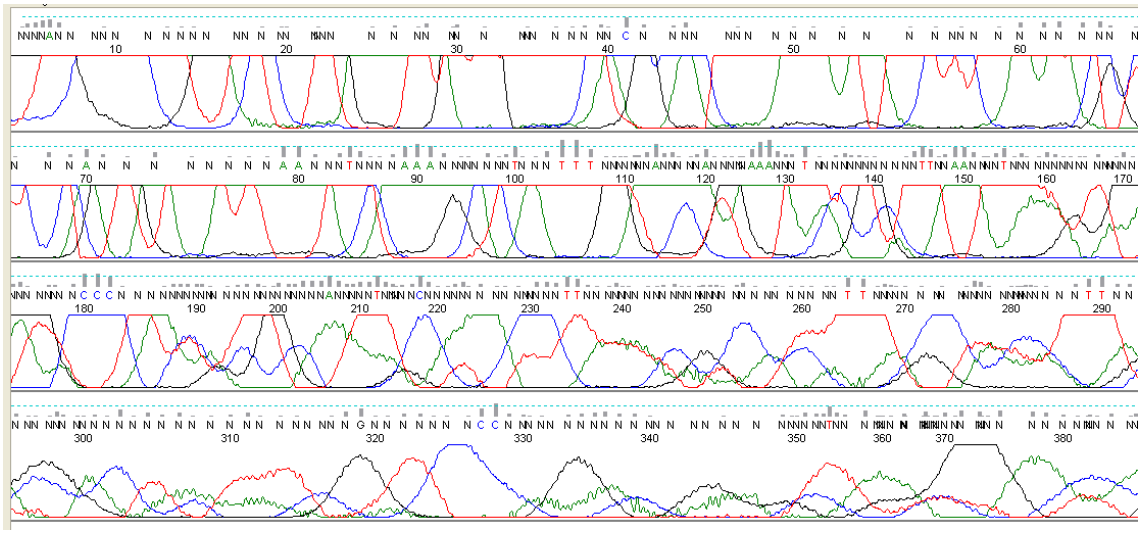


Causes	Solutions
Runs of G's in the sequence context cause a decline or abrupt stop in peak heights.	We use our GC Rich protocol and also substitute our BigDye v3.1 chemistry with a mixture of BigDye v3.1 and dGTP BigDye. The dGTP chemistry replaces dITP with dGTP. This has been shown to be better for sequencing through runs of G's and GT rich templates.

Correct template concentration is important!

Example:

This is a sequence generated when the template was submitted at 350ng/uL, not the required 50ng/uL:



This is the same sample done after the template was diluted to the correct concentration: