How Much DNA to Use in Preparing a Successful Sequencing Reaction

At the Nevada Genomics Center we offer DNA sequencing using dye-terminator Sanger sequencing with analysis on an Applied Biosystems Prism 3730 DNA Analyzer. A Sanger sequencing reaction is run with a single primer, unlike polymerase chain reaction (PCR) which is run with two primers. In a PCR there is exponential amplification of the amplicon; however in a sequencing reaction there is only one primer so there is no exponential amplification. Consequently, the amount of template DNA and primer used to set-up a sequencing reaction is vital to the success of the sequencing.

In considering the amount of template DNA added to a sequencing reaction you must first consider the size, in base pairs, of the template molecule. Just as you must consider the molecular weight of a chemical when calculating the grams necessary for preparing a solution; you must consider the length of the entire template DNA molecule. As the template length increases more DNA is needed to be within the optimal range. The table below lists how much template DNA to use in a sequencing reaction. A few things to keep in mind:

- Look carefully at the method you use to quantify your DNA. Is it accurate?
- Even if you use the optimal amount of template, if the DNA is of poor quality (sheered DNA or contaminated DNA) your sequencing will fail.
- Using too little OR using too much template DNA can cause the sequencing to fail. The old adage "if one is good than ten must be better" does not hold true when setting up a sequencing reaction.

Template	Amount of Template	Amount of Primer
Plasmid DNA		
less than 5000bp	250ng	2 picomoles = 1ul of 2uM primer
5000bp to 10,000bp	500ng	10 picomoles = 1ul of 10uM primer
10,000bp to 20,000bp	1ug	20 picomoles = 1ul of 20uM primer
20,000bp and larger	2 to 3ug	20 picomoles = 1ul of 20uM primer
BACs, Cosmids, Fosmids	2 to 3 ug	20 picomoles = 1ul of 20uM primer
PCR Amplicons		
less than 300bp	8ng	2 picomoles = 1ul of 2uM primer
300bp to 800bp	40ng	2 picomoles = 1ul of 2uM primer
800bp to 1200bp	80ng	2 picomoles = 1ul of 2uM primer
greater than 1200bp	divide size by 10	10 picomoles = 1ul of 10uM primer
	eg: 1500bp = 150ng	

The next important facet to successful sequencing is the ratio of the primer to template molecules. The table above recommends quantities of primer for an optimal ratio. As with calculating the template DNA, there are a few important facets to keep in mind:

- Having the correct amount is not going to help if the priming efficiency of your primer to your template is poor. Test your primers!
- The primer should be suspended in water.
- Use a SINGLE primer in a sequencing reaction.