DNA Sequencing Sample Concentrations and Quantities

The quality and concentration of the DNA are two of the most important factors in obtaining a positive result from sequencing. DNA samples that are of poor quality will typically produce a weak sequencing signal or none at all. Samples with high concentrations of sodium acetate will show lower signal strength and a shorter read length in the base calling. No sequencing signal will be detected when the concentration is too high (greater than 20 mM). Potassium acetate has similar effects, but at higher concentrations. Ethanol can also cause low signal strength and thus a shorter read length in the base calling. A concentration of ethanol greater than 10% usually results in no sequencing signal being detected. Phenol will also produce poor sequencing signals. At concentrations of 0.7% (v/v), the signal intensity, accuracy and read length are decreased. At concentrations greater than 1.5%, no signal will be detected.

For PCR products, it is very important to remove all of the PCR primers and the dNTPs from the DNA sample. If the PCR primers are still in the sample, the sequencing reaction will occur on both strands of the DNA and thus produce a sequence that is a mixture of both strands. The dNTPs will alter the sequencing kit ratio of dNTP to ddNTP terminators and thus will cause a weaker signal (shorter read length) or no signal at all (if the dNTPs concentration is too high).

Too much EDTA can also cause problems – the EDTA will chelate the necessary Mg++ ion for the Taq enzyme and thus decrease the signal strength and read length. If your stock samples are in EDTA and they need to be diluted to our required concentrations (see below), please dilute with dH2O.

As for the DNA concentration, it is important to be within an appropriate range for the various types of samples. If too much DNA is used in the sequencing reaction, a large amount of shorter sequence fragments will be generated and the base calling read length will be less. If too little of DNA is used, the signal will be weaker than normal. If the signal is too weak, the sequence data will not be accurate and the read length will be shorter than expected.

Required DNA concentrations:

Please submit your DNA samples using the following concentrations and amounts:

Plasmid DNA: 500 ng of DNA per primer (**concentration of 100 ng/ul**); please provide us with at least 5 ul for each primer.

PCR Products: < 500 bp = 25 ng of DNA per primer (**concentration of 5 ng/ul)**; please provide us with at least 5 ul for each primer.

500 - 1000bp = 50 ng of DNA per primer (**concentration of 10 ng/ul**); please provide us with at least 5 ul for each primer.

1000 -2000bp = 75 ng of DNA per primer (**concentration of 15 ng/u**l); please provide us with at least 5 ul for each primer.

> 2000bp = 100 ng of DNA per primer (**concentration of 20 ng/u**l); please provide us with at least 5 ul for each primer.

BAC/Cosmid DNA: 1000 ng of DNA per primer (**concentration of 200 ng/ul)**; please provide us with at least 5 ul for each primer.

When submitting DNA samples and primers to the lab for sequencing reactions, they should be submitted in 500 ul microcentrifuge tubes (unless you are submitting them in a 96-well PCR plate). If you have chosen the option of performing your own BigDye Terminator reactions, they should be submitted in 200 ul PCR tubes (unless you are submitting them in a 96-well PCR plate). The tubes should be clearly labeled with your sample or primer name. Please print as clearly as possible. It is highly recommended to label the tubes so that the Sequencing Lab can identify the sample/primer as yours (we receive many tubes). It is best to avoid simple numbers (1, 2, 3 etc.) or letters (A, B, C etc.) and to place your initials or the submission ID # on your tubes.

If you have any questions or comments, please email us at weseqdna@wayne.edu or call us at (313) 577-0024.