

DNA Sequencing Primers Concentrations and Quantities

The Sequencing Lab offers the researcher five stock primers (see below) for sequencing reactions. If you need to use a custom primer, please submit them at the required concentrations (see below). When designing your custom primers, use the standard factors for designing a primer for a PCR reaction (avoid primer dimer formations, avoid hairpin formations, avoid secondary priming sites, have a T_m between 50-65C, have a %GC of 40-60%, have a primer length of 20 to 25 bases). Also when designing your primer, have your area of interest at least 100 bases away from the 3' end of your primer. This will be helpful if your sequencing reaction produces a weak signal and our reaction purification fails to remove all of the excess BigDye Terminator nucleotides. These nucleotides will appear about 70-80 bases from the primer and in the shape of broad peaks. Depending on their intensity, you may be able to manually call the sequence bases underneath these dye blobs. Note: dye blobs are usually not observed with samples that have produced a strong sequencing signal – most of the BigDye Terminators are consumed during the reaction and the excess is removed completely during purification.

The Sequencing Core has a stock of the following primers:

-21M13 (forward):	5' TGTA AACGACGGCCAGT 3'
M13 Reverse:	5' CAGGAAACAGCTATGACC 3'
T7 (promoter):	5' TAATACGACTCACTATAGGG 3'
T3:	5' ATTAACCCTCACTAAAGGGA 3'
SP6:	5' ATTTAGGTGACACTATAG 3'

There is no additional charge for these primers.

Required Custom Primer Concentrations:

Custom primers submitted by the researcher should be at the following concentrations and amounts:

For plasmid or PCR DNA sequencing: 5 pmoles of primer per sample (**concentration of 1 pmol/ul or 1 μ M**); please provide us with at least 5 ul of primer per sample.

For BAC/Cosmids DNA sequencing: 25 pmoles of primer per sample (**concentration of 5 pmol/ul or 5 μ M**); please provide us with at least 5 ul of primer per sample.

When diluting your primers or DNA samples, please use dH₂O. Samples with high TE concentrations will give poor sequencing results.

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.