DNA SEQUENCING SAMPLE PREP AND SUBMISSION

DNA SEQUENCING FACILITY

MCLM 852
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Sequencing Sample Information is entered into the web-based dnaLIMS system (http://seqcore.uab.edu) prior to sample submission to the sequencing facility. New users may also setup an account at this site.

Template Preparation: The *amount* and *quantity* of the template DNA are some of the more critical factors affecting the quality of the DNA sequencing reaction. DNAs are purified preferably using a silica-based kit (Qiagen, Invitrogen, Sigma, etc.) and eluted using ONLY high quality dH_2O. DNA should be quantified (A_{260}), and should be visualized for integrity and purity by agarose gel electrophoresis. DNA quantity per reaction is according to the following guidelines:

Plasmid DNA -		500 ng in 5 μL H_2O
PCR DNA -	100 - 500 bp	30ng in 5 μL H_2O
	500 - 1000 bp	100ng in 5 μL H_2O
	> 1000 bp	150ng in 5 μL H_2O

Primer Design and Concentration: [NOTE – a selection of primers (below) is available free of charge.] Primers should be 18 to 25 bases long, with AT rich sequences longer and GC rich sequences shorter. Primers are ideally 50-55% GC, with a melting temperature (Tm) of 55-60° C. Avoid internal secondary structure and tandem repeats of more than 4 identical bases. Two of the last three bases at the 3' end should be G or C while also being careful to avoid self-complementarity at the 3' end. Working stocks of primer should be at 5 pmole/μL in high quality dH_2O, with 1 μL added to each template-reaction. When using your own primer, samples should be submitted with template and primer pre-mixed.

Primers Offered Free of Charge:

M13F(-20)	CGTTGTAAAACGACGGCCAG
T7-pro	TAATACGACTCACTATAGGG
pMSCV-3'	GAGACGTGCTACTTCCATTTGTC
pMSCV-5'	CCCTTGAACCTCCTCGTTCGACC
T7-term	GCTAGTTATTGCTCAGCGG
T3	AATTAACCCTCACTAAAGGG
SP6	CATACGATTTAGGTGACACTATAG
BGH-Rev	TAGAAGGCACAGTCGAGG
pET-For	TAATACGACTCACTATAGGG
pET-Rev	GCTAGTTATTGCTCAGCGG
pGEX-3'	CCGGGAGCTGCATGTGTCAGAGG
pGEX-5'	GGGCTGGCAAGCCACGTTTGGTG
pCMV-Fwd	CGCAAATGGGCGGTAGGCGTG
M13R(-20)	TCACACAGGAAACAGCTATGAC