General Information about Templates

Poor template quality is the most common cause of DNA sequencing problems. Potential contaminants include: proteins, RNA, chromosomal DNA, residual salts, detergents, and organic chemicals such as phenol, chloroform, and ethanol. Also, excess PCR primers, dNTPs, and buffer components from a PCR amplification used to generate the sequencing template can interfere with the cycle sequencing reactions.

Template Purification Kit

- We recommend using following products to clean up your PCR products or plasmid before you submit them to the Genomic Core.

- Please check the concentration and purity of DNA template. DNA should give an OD260/280 of between 1.7-1.9 Low 260/280 indicates protein contamination; high OD260/280 ration indicates presence of organics contamination.

PCR DNA purification system

- QIAquick PCR Purification Kit (50) cat:28104 (Qiagen)
- EXOSAP-IT (100) cat:78200, (Affymetrix, Inc.)
- Agencourt AMpure XP kit (5ml) cat:A63880 (Beckman Coulter)

Plasmid DNA purification system

- QIAprep Spin Miniprep Kit (50) cat:27104 (QIAGEN)
- PureLink® Quick Plasmid Miniprep Kit (50) cat: K210010 (Invitrogen)

Sample and Primer Submission

- DNA template should be diluted in dH_2O not in TE buffer. Buffer components inhibit the sequencing reaction and cause failed runs.
- All templates and primers must be labeled and the concentration must be clearly written on the order form.
- If you submit a PCR product, please include a gel photograph of “purified” template DNA with the quantity of template loaded including a molecular marker.

- If you submit your template and primer separately, provide 10 µl of template and 5 µl of primer.
- If you submit your template and primer in one tube, the total volume can't exceed than 7.5µl.

Recommended Template and Primer Concentration

To submit in separated tubes:

Template Type	Template Concentration	Primer Concentration
PCR product 100 – 2000 bp	50 ng/µl	10 pmol/µl
Single stranded (i.e. M13)	100 ng/µl	10 pmol/µl
Double stranded (plasmids <15kb)	100 ng/µl	10 pmol/µl
Large DNA (Plasmid over ~20 kb, Cosmid, BCA)	500 ng/µl	25 pmol/µl
Bacterial genomic DNA	800ng/µl	25 pmol/µl

To submit in one tube (7.5µl):

Template Type	Template Quantity	Primer Quantity
PCR product 100-1000 bp	50ng	5 pmol
PCR product 1000-2000 bp	80ng	5 pmol
Single – stranded (i.e. M13)	100 ng	5 pmol
Double – stranded (plasmids <15kb)	300 ng	10 pmol
Large DNA (Plasmid over ~20 kb, Cosmid, BCA)	500 -1000 ng	25 pmol
Bacterial genomic DNA	2-3 µg	25 pmol

Sequencing universal primers supplied at Genomic Core with no extra cost

M13F:	5’-GTA AAA CGA CGG CCA G-3’
M13R:	5’-CAG GAA ACA GCT ATG AC-3’
T7:	5’-TAA TAC GAC TCA CTA TAG GG-3’
T3:	5’-ATT AAC CCT CAC TAA AGG GA-3’
SP6:	5’-GAT TTA GGT GAC ACT ATA G-3’
BGHR:	5’-TAG AAG GCA CAG TCG AGG-3’