**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](http://products.invitrogen.com/ivgn/product/K210010?ICID=search-product) (50) cat: K210010 (Invitrogen)

**Sample and Primer Submission**

* DNA template should be diluted in dH2O 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.
* Please include a gel photograph of 2 μl of “purified” template DNA with a molecular marker, or use Nanodrop Spectrophotometer to test your template concentration.
* 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  | 20 ng/μl  | 3.2 pmol/μl  |
| Single stranded (i.e. M13)  | 100 ng/μl  | 3.2 pmol/μl  |
| Double stranded (plasmids <15kb)  | 200 ng/μl  | 3.2 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 <500 bp  | 20 ng | 3.2 pmol |
| PCR product 500-1000 bp  | 40ng | 3.2 pmol |
| PCR product 1000-2000 bp  | 80ng | 3.2 pmol |
| Single – stranded (i.e. M13)  | 100 ng | 3.2 pmol |
| Double – stranded (plasmids <15kb)  | 300 ng | 3.2 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(-21): 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’