



Guidelines for DNA Sample Preparation

We understand that every sequencing result and turnaround time is critically important for you to proceed to the next step of your research. In order to generate quality sequencing data, please follow the instructions below to prepare your DNA samples.

Template DNA Purification Kit

The quality of the template DNA directly affects the quality of the DNA sequencing results. We recommend using one of the following methods:

Plasmid DNA purification system in a single tube:

- QIA prep spin miniprep kit from Qiagen
- RPM kit from Q-biogene
- Wizard Plus Plasmid purification from Promega
- BioRad Quantum miniprep

Plasmid DNA purification system for 96-well plate:

- QIA prep 96 Turbo Miniprep kit from Qiagen
- Plasmid 96 MiniPrep kit from Edge Biosystems

PCR DNA purification system in a single tube:

- AMPure PCR Purification from Agencourt
- PSI Clone HTS PCR purification from Princeton
- Zymo Clean Gel DNA Recovery kit from Zymo

PCR DNA purification system for 96-well plate:

- QIA prep 96 Turbo Miniprep kit from Qiagen
- Plasmid 96 MiniPrep kit from Edge Biosystems

PCR Amplification Products

It is essential that the DNA is free of contaminants, unused primers or dNTPs. PCR templates that do not undergo any kind of post PCR clean up are not suitable for sequencing and will yield unuseable sequence data. It is highly recommended that your PCR template is first checked on a gel to confirm that there is a specific product with the correct size.

The EXO/SAP protocol to clean the PCR amplicon frequently damages the PCR primer attachment sites. We recommend that internal primers are used for sequencing because it lowers the risk of poor sequencing results due to the damaged PCR primer attachment site.

Sequencing Primers

ACGT, Inc. provides 50 different free-of-charge universal primers. Specific internal primers can be ordered through ACGT, Inc. To design your own primers, please follow these considerations:

- Perfect matches
- No alternative hybridization sites in template
- No palindromic sequence present, particularly at the 3' end of primer
- Appropriate length to give Tm of ~55-62°C. Avoid low Tm (i.e. 40-45°C) If Tm is low, make the primer longer.
- GC "clamp" on the 3' end.
- Desirable [GC] = ~50-55%
- Avoid strings of four or more of the same base if possible.

DNA in General

1. It is critical that the plasmid template is free of any contaminants including buffers, salts, organics, and proteins.
2. Submit DNA in deionized water, not in TE. Buffer components inhibit the sequencing reaction and cause failed runs.
3. If possible, use of phenol or chloroform during the purification procedures should be avoided. An additional ethanol precipitation is recommended if phenol or chloroform is used.
4. DNA should give an OD260/280 of between 1.7-1.9 and an OD200/260 of about 1.1. Low 260/280 indicates protein contamination, high OD260/280 indicates possible RNA or residual organics contamination. High OD200/260 indicates contamination by organics salts.
5. If your PCR amplification procedure generates a product with a single robust band, column purification will suffice. If it generates multiple bands, gel purification of the band with the desired size is required.