



DNA Technologies Core Laboratory

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Instructions for submitting samples for sequence analysis

For samples sequenced by the DNA Core Laboratory, start at step 1
For ready-to-run sequence samples, start at step 5

Recommended protocol for 1/2 sequencing reaction (10 µL total volume)

Table with 2 columns: Reagent and Amount. Rows include Big Dye v1.1, Half Big Dye, 5X sequencing buffer, Template (Single-Stranded DNA, Double-Stranded DNA, PCR Product DNA), Primer, Deionized Water, and Total Reaction Volume.

*Big Dye and Sequencing Buffer available for purchase through our lab; sequencing buffer should be used at 1X concentration in Big Dye v3 sequencing reactions; sequencing buffer is not necessary for Big Dye v1.1 reactions and can be substituted for extra volumes of template or primer as needed.

- 1. Drop off your template and your primer in the freezer in our laboratory. WE MUST KNOW THE CONCENTRATIONS! The amount of template and primer that we use are dependant upon the concentrations. Results may vary if concentrations are not reported.
- Prior to submission, template DNA quality should be checked on an agarose gel.
- For sequencing fragments <100 bp, it is best to subclone your products into a plasmid and then sequence across the insert by flanking primers.
- It is critical to submit CLEAN template DNA. For plasmid DNA, most mini-prep kits will yield sufficiently clean DNA. For PCR products, we recommend using one of the commercially-available purification kits (e.g. QIAGEN cat# 28104) to ensure complete removal of primer dimers.
- For PCR products, sequencing primers internal to the original PCR primers are recommended.
2. Sequence reactions are prepared according to the table above, and amplified using the following thermal profile:
1 cycle at 96°C for 2 minutes
35 cycles of:
96°C for 30 seconds
50°C for 15 seconds
60°C for 4 minutes
1 cycle at 60°C for 5 minutes
4°C hold

3. Excess dye-terminators are removed using Spin-50 mini-columns (BioMax, cat# 97-161L), following the protocol recommended by the manufacturer (copies available upon request).
4. Samples are completely dried. We have a SpeedVac available for customer use which holds 1.5 mL eppendorf tubes and takes approximately 25 minutes to dry a 25 μ L sample.
5. Samples are resuspended in 10 μ L HiDi formamide and placed in a 96-well format.
6. The sample mixture is denatured at 96°C for 4 minutes and then stored at -20°C until it is run on the 3130xl Genetic Analyzer.
7. Results are posted daily at <http://www.cvm.tamu.edu/dnacore> under the link labeled “*Link to FTP site*”
8. You can download free analysis software at http://www.appliedbiosystems.com/support/software_community/free_ab_software.cfm. For sequencing, download the Sequence Scanner Software.

IMPORTANT NOTES:

- Samples are processed in the order in which they are received, and we make every attempt to process samples as quickly as possible.
- The preceding text is a general protocol. There are situations in which alternate protocols must be used. For example, a full Big Dye reaction (4 μ L Big Dye in 10 μ L) sample is recommended for “difficult” samples, such as those with poly-N regions.
- Any extra template or primer submitted (step 1 above) will be kept in our freezers for 2 weeks, after which time they will be discarded unless otherwise requested.