



COLLEGE OF VETERINARY MEDICINE

Veterinary Pathobiology



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Instructions for sequencing samples using BigDye XTerminator

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

Reagent	Amount
*Big Dye v1.1 (ABI, cat# 4337451)	2.0 μ L
Half Big Dye (Genetix, cat# K1104)	2.0 μ L
*5X sequencing buffer (ABI, cat# 4339843)	2.0 μ L
Template	
PCR product DNA 100-200bp	0.5-3 ng
PCR product DNA 200-500bp	1-10 ng
PCR product DNA 500-1000bp	2-20 ng
PCR product DNA 1000-2000bp	5-40 ng
PCR product DNA >2000bp	10-50 ng
Single-Stranded DNA	10-50 ng
Double-Stranded DNA (plasmid)	50-300 ng
Primer	2.5 pmol
Deionized Water	q.s.
Total Reaction Volume	10 μ L

*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. Sequence reactions are prepared according to the table above, and amplified using the following thermal profile:
 1 cycle at 96°C for 2 minutes (1 cycle at 98 °C for 5 minutes if high GC content)
 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
2. Excess dye-terminators are removed using BigDye XTerminator (ABI, cat# 4376486), following the protocol recommended by the manufacturer
(http://www3.appliedbiosystems.com/cms/groups/mcb_marketing/documents/generaldocuments/cms_042775.pdf).
3. The samples are stored at 4-20°C until they are run on the 3130xl Genetic Analyzer.
4. Results are posted daily at <http://www.cvm.tamu.edu/dnacore> under the link labeled "Link to FTP site"

5. 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.