Ready To Load Sequencing Appendix I

1. Assemble reaction in 96 or 384 well PCR Plate:

4.5 ul template/primer mix (containing 250 ng plasmid DNA or PCR product* of appropriate mass + pmoles primer)
0.5 ul BigDye (version 3.1)
0.5 ul 5M betaine (0.5 ul DMSO may be substituted)
2.5 ul 5x sequencing buffer
4 ul ddH2O
Total volume of 12 ul

* Amount of PCR product required for sequencing reaction: ng needed = (length of PCR product in bp)/5 For example: for 250 bp product, amount needed is 250/5 = 50 ng.

The Sequencing Handbook and ABI protocols contain calculations and recommendations for PCR products and large constructs plus additional information on purity considerations.

2. Follow the standard ABI thermocycling program:

96 C 4 min
Followed by 25 cycles of:
   96 C 10 sec
   50 C 5 sec
   60 C 3 min
4 C hold

3. To perform dye terminator removal by desalting, the BRC first adds 10 ul ddH2O to each 12 ul reaction before loading onto Edge Biosystems 96-well dye terminator removal columns (Edge part number 88415). We then follow the manufacturer's protocol. These plates are available from the BRC at a cost of $65 per plate. Reaction products are eluted in water into 96-well plates that fit the 3730xl instruments.