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

- 4.5 µl template/primer mix
- 1.0 µl BigDye (version 3.1)
- 0.5 µl 5M Betaine (0.5 µl DMSO may be substituted)
- 2.5 µl 5X sequencing buffer
- 3.5 µl ddH2O

Total volume of 12 µl

* Amount of PCR product required for sequencing reaction: ng needed =
  (length of PCR product in bp)/5
  Ex: 250 bp product, amount needed is 250/5 = 50 ng ÷ 4 = 12.5 ng in reaction

* The above concentration of 50 ng calculation is used for full services
  sequencing submission of an 18µl template primer mix in which 4.5µl is added to
  the above master mix.

* Primer concentration needed is 25 pmoles in 18ul
  25/18 = 1.39 pmole x 4.5ul = 6.25 pmole per reaction

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

http://www.biotech.cornell.edu/node/556

2. Follow the standard ABI thermocycling program:

- 96 C 4 min
- Followed by 30 cycles of:
  - 96 C 10 sec
  - 50 C 5 sec
  - 60 C 3 min
- 4 C hold

3. To preform dye terminator removal by desalting, the BRC first adds 5 µl ddH2O to each 12 µl
reaction before loading onto Edge Biosystems 96-well dye terminator removal columns (Edge
part number 60122). We then follow the manufacturer’s protocol. These plates are available
from the BRC. The Genomics Core can do the cleanup step using these plates. Please inquire for
pricing. Reaction products are eluted in water into 96-well plates that fit the 3730xl instruments.

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