

### **Sample sequencing reaction set up if using Big Dye Terminator Mix v3.1:**

*Life Technologies, [www.lifetechnologies.com](http://www.lifetechnologies.com), Catalog# 4337457, \$321/24 reactions*

4µl Big Dye Terminator Mix  
2µl ddH<sub>2</sub>O  
1µl primer (15pmol/µl)  
3µl template

General rule of thumb on DNA quantity per reaction:

For PCR product: 1ng/100bp per reaction

For plasmid: 150ng per reaction

Typical cycling profile is as follows:

25 cycles: 96°C 30 seconds  
50°C 15 seconds  
60°C 4 minutes

If the T<sub>m</sub> of your primer is 60°C or higher, you can do 2-step cycles of 96°C for 30 seconds and 60°C for 4 minutes

The sequencing reaction must be purified before it is run on the Genetic Analyzer to get rid of any unincorporated dye terminators.

Some labs do this with home made sephadex columns in reusable or disposable spin columns but it is more time consuming with less quality control though some labs have good luck with it.

Using G-50 w/spin column

1. rinse old spin column under faucet
2. add ~300 uL H<sub>2</sub>O to spin column, quick spin in microfuge
3. add 600 uL Sephadex G-50 slurry (1g Sephadex G-50/15mL in ddH<sub>2</sub>O made before hand and hydrated at least over night) to spin column
4. microfuge @ 4900 RPM exactly 60 seconds
5. load 20 uL of sequencing reaction (would add 10µl ddH<sub>2</sub>O to above seq reaction) to the center of the top surface of the Sephadex
6. microfuge @ 4900 RPM exactly 60 seconds into fresh 1.5 mL eppendorf tube

The best columns to purchase are Performa DTR gel filtration cartridges by Edge Bio.

*Edge Bio, [www.edgebio.com](http://www.edgebio.com), Catalog# 98780, \$85/Qty=36 or Catalog#42453, \$190/Qty=108*

- Spin column at 850 x g for 3 minutes.
- When the microcentrifuge is done, retrieve your column, dispose of the bottom waste tube and put your column in a fresh labeled eppie tube (be careful not to jostle column, it is just resin beads lightly packed down).
- load your entire sequencing reaction onto the top center of the column without touching the column.
- Place column back in centrifuge for another spin at 850 x g for 3 minutes.
- Remove the column from the tube and dispose. Close the eppie, make sure it is labeled properly, and it is ready to run on the genetic analyzer.