

PCR SEQUENCING REACTION

Clean up PCR

Measure concentration (need 150 ng to 1200 ng)

Big Dye Terminator Enzyme	4 ul
Buffer	4 ul
Primer (0.8 pmol/ul)	1 ul
DNA Template	11 ul

File 43

96C 30 seconds

Annealing temp 15 seconds

60C 4 minutes

30 cycles

Link to file 11 5 minutes

Line to file 10

Clean up with G50 Column (Amersham Pharmacia Biotech)

Loosen cap

Spin column 1min at 5000g

Add 300 ul H₂O

Spin 1 min at 5000g

Add sample directly onto beads

Spin 1 min 5000g

Collect flow through

Dry sample in speed vacuum

Take to biotech