

Sequencing Reaction

~50 ng Template

PROGRAM

Primer 2 uM- 1 uL

1. 96°C for 1 minute

5X BFF- 1.5 uL

2. 94°C for 30 seconds

*Big Dye- 1 uL

3. 50°C for 15 seconds

HPLC H₂O- up to 10 uL

4. 60°C for 4 minutes

*B.D. is light sensitive

Go to Step 2 for 35 cycles.

Cleaning the Sequencing Reaction

1. Transfer Sequencing RX mixture to 1.5 mL eppendorf tube.
2. Add 64 uL of 100% EtOH and 16 uL of HPLC H₂O.
3. Incubate at R.T. between 5 and 10 minutes.
4. Spin for 10 minutes at 13,000 rpms.
5. Pour off the supernatant.
6. Wash pellet with 200 uL of 70% EtOH.
7. Spin for 5 minutes at 13,000 rpms.
8. Pour off supernatant.
9. Let pellet dry for 5 minutes.
10. Add 10-20 uL of HPLC H₂O.
11. Store at -20°C.