

Elongase Protocol (From Nickerson Lab)

Elongase Cocktail 1.5 mM MgCl₂ for 2mM MgCl₂ solution use 234 µL of Buffer B

345µL H₂O
117µL Buffer A
117µL Buffer B
29.25µL dNTPs 10 mmol
46.8µL Elongase enzyme

Elongase PCR recipe 1 X

4.4 µL Cocktail
1.2 µL each Primer/tube 10 µg/λ
2.2 µL Water
1 µL Template

Sequencing Rxn (From Nickerson Lab)

1. Dilute PCR plate 5X (1:4)
2. Make one Master Mix (MM) per primer
Master Mix Recipe per each 10 µL rxn (at 1/8 dilution of BigDye)
5 X diln. Buffer 1.7 µL
H₂O dd 1.5 µL
Primer @ 10 µM µL
BigDye 1 µL
3. Aliquot MM to plate
4. Add PCR diln.
5 µL
Total volume per sequencing rxn = 10 µL

Sequencing Rxn: (From Collen, Phil Green Lab)

Rxn	1 X	106 X for 96 well plate
PCR 1:20	2.0 µL	
Primer 1 uM	2.0 µL	212.0 µL
BGDye 3.1	0.4 µL	42.4 µL
5X Dilution Buffer	2.6 µL	275.6 µL
dH ₂ O	3.0 µL	318.0 µL

8 µL of cocktail / tube + 2 µL primer dilution

5X Sequencing Buffer: (From Collen, Phil Green Lab)

400 mM Tris HCl

10 mM MgCl₂

pH 9

Ethanol Precipitation

1. Add to each well 26 μ L of 95 % ethanol
2. Mix and put at -20° C for 15 minutes
3. Centrifuge 30 minutes at 3,000 G (program 35)

4. Remove supernatant by inverting plate or ask him to leave
5. Centrifuge inverted 1 minute at 700 G (program 36)

6. Add to each well 125 μ L of 70 % ethanol
7. Centrifuge 10 minutes at 3,000 G (NOT inverted) (program 37)

8. Remove supernatant by inverting plate
9. Centrifuge inverted for 1 minute at 700 G (program 36)

10. Wells now contain precipitated DNA. Let dry on bench top (cover)
11. Store at -20° C

Running DNA Controls/Standards BigDye v3.0 for 3100

1. Add 170 μ L of HiDi formamide to one tube
2. Heat at 95° for 2 minutes
3. Put on ice
4. Dispense 10 μ L to each well A1 – H2 (first two rows)
5. Run in sequencer

Dilution of 1 kb DNA Ladder (Fermentas)

To one vial of concentrated ladder (.5mg/ml), add:

250 μ L sterile water

50 μ L 6x loading dye

Load 1-1.5 μ L diluted ladder per lane on a gel.

6x Loading Buffer

0.25% (w/v) bromophenol blue

0.25% (w/v) xylene cyanol FF

40% (w/v) sucrose in H₂O