

BIG-DYE SEQUENCING

Big Dye Version 1.1 Terminator RR mix	Aliquots:	
Applied Biosystems (cat# 4337450) 100rxns	concentrated	to read up to 1100bp
	dilute 1:1	to read ~500-600bp
	dilute 1:4	to read ~250 bp

2.5X Big Dye Buffer (2.5X BD buffer)

Dye is diluted in buffer:

Final concentration:

200mM Tris, pH 8.0

5mM MgCl₂

in dH₂O

2.5X BD buffer	10ml
Tris, pH 8.0	2 ml
5mM MgCl ₂	50 µl
dH ₂ O	8 ml
filter sterilize	

1. Sequence Reaction

Template (0.1µg -1µg / rxn)

X µl

Primer (3.2 pmol / rxn)

X µl

Big Dye

4 µl

2.5X BD buffer

4 µl

q.s. to 20 µl with dH₂O

2. Sequence Precipitation

1. Add the following to a 1.5ml microfuge tube:

2 µl 3M NaOAc

50 µl 95% EtOH, RT

2. Add the sequencing reaction to the NaOAc/EtOH mixture and vortex.
3. Place on ice for 10min (no longer or unincorporated dye will precipitate).
4. Spin in a microcentrifuge at maximum speed for 15-30min.
5. Wash with 70% EtOH (room temp).
6. Vortex and spin for 5min.
7. Dry in the speed-vac 10-15min. without heat. Do not overdry.
8. Resuspend in 10 µl formamide or store pellet at -20°C.

Comments:

1. Residual ethanol and salt will interfere with the sequencing reaction.
2. DNA should not be stored in formamide for long periods of time prior to sequencing (eg. the evening before a morning sequencing is ok but not a couple of days). If the pellets prepared are not to be sequenced immediately, it is best to store them dry at -20C.

Reference: Applied Biosystems User Manual