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 $MgCl_2$
in dH_2O

2.5X BD buffer	10ml
Tris, pH 8.0	2 ml
5mM $MgCl_2$	50 µl
dH_2O	8 ml
filter sterilize	

1. Sequence Reaction

Template (0.1ug -1ug / rxn)	X□l
Primer (3.2 pmol / rxn)	X□l
Big Dye	4 □l
2.5X BD buffer	4 □l
	q.s. to 20□l with dH_2O

2. Sequence Precipitation

1. Add the following to a 1.5ml microfuge tube:
   2□l 3M NaOAC
   50□l 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□l 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