

FHCRC Genomic Resources Lab

Big Dye Cycle Sequencing Protocol:

(From PE-Biosystems)

Recommended DNA Quantities: (you should always quantitate the amount of purified DNA by measuring A-260 or by running on gels a comparing to a know standard. DNA should be in ddH2O, not TE, as the EDTA may interfere with Taq polymerase incorporating the Dyes

Template	Quantity	
PCR product:	50-100 bp	1-3ng
	100-200 bp	3-10ng
	200-500 bp	5-20ng
	500-1000 bp	10-40ng
	1000-2000 bp	40-100ng
	>2000 bp	100-200ng
SS DNA (I.e.,M13) DS DNA (plasmid)--5kb Cosmid, BAC Bacterial genomic DNA		50-100ng
		200-500ng
		0.5-1.0ug
		2-3ug

Reaction Mixes:

Reaction Mix:

Reaction size	Big Dye Mix (uL)	Primer (pmoles)	DS (ng) Template	DMSO 5% Optional	H2O to final Vol.	Number of Cycles
ABI's 1x reaction	8 uL	5-10	200-500	1-2 uL	20 uL	25-30
1/2 Reaction	4 uL	5-10	200-500	.05-1 uL	10 uL	25-30
**1/2 reaction 20uL volume	4uL+4uL 5 X buffer	5-10	200-500	1-2 uL	20 uL	25-30
1/4 reaction 10uL volume	2 uL	5-10	200-500	.05-1 uL	10 uL	25-30
1/8 reaction + 3730 Buffer	1:1 ratio 2uL	5-10	200-500	1-2 uL	10 uL	25-30

**** Note #1: 5X CSA buffer is PE Applied Biosystems product**

Note #2: Both the 5X CSA buffer and the diluted ABI stock should be kept on ice

Note #3: DMSO is optional, but will not hurt the reaction. DMSO is for GC rich reactions.

Thermal Cycling protocol, for PE 9600 or equivalent (ramping time is very important, and should not be > 1 degree/sec):

95degC hold for 5 min
Rapid thermal ramp to 95degC
95degC for 10sec

Rapid thermal ramp to 50degC
50degC for 5sec
Rapid thermal ramp to 60degC
60degC for 4min

followed by rapid thermal ramp to 4 degC and hold.
Bring reactions to Genomic Resources Lab for analysis and place in small fridge,
and turn in your sample sheet.

Add your DNA with the appropriate concentration

Dry samples down at 95degC until samples are dried down

	1/4 reaction		1/2 reaction	
Water	6ul		4ul	
Primer	2ul	{5-10pmole} final	2ul	{5-10pmole} final
Big Dye	2ul		4ul	
Total Vol.	10ul		10ul	

Run your samples for 25-30 cycles.
See above for the Thermal Cycler protocol.