

Big Dye Cycle Sequencing Protocol

Recommended DNA Quantities: (you should always quantify the amount of purified DNA. DNA should be in dH₂O and not TE.)

Template	Quantity	
PCR Products:	50-100 bp	1-3 ng
	100-200 bp	3-10 ng
	200-500 bp	5-20 ng
	500-1000 bp	10-40 ng
	1000-2000 bp	40-100 ng
	>2000 bp	100-200 ng
SS DNA (i.e., M13) DS DNA (plasmids) * Cosmid, BACs, YACs Bacterial genomic DNA		50-100 ng
		200-500 ng
		0.5-1.0 µg
		2-3 µg

*plasmids larger than 8 kb may require more template.

Preparing Reactions:

Reaction size	Big Dye Mix (µl)	Primer (pmoles)	DS (ng) Template	DMSO 5% Optional	H ₂ O to final vol.	Number of Cycles
ABI's 1X reaction	8 µl	5-10	200-500	1-2 µl	20 µl	25-30
½ reaction	4 µl	5-10	200-500	0.5-1 µl	10 µl	25-30
** ½ reaction 20 µl volume	4 µl DNA+ 4 µl 5X buffer	5-10	200-500	1-2 µl	20 µl	25-30
¼ reaction 10 µl volume	2 µl	5-10	200-500	0.5-1 µl	10 µl	25-30
1/8 reaction 10 µl volume	1 µl	5-10	200-500	1-2 µl	10 µl	25-30

**Note#1: 5X Sequencing buffer is an Applied Biosystems product.

Note#2: The 5X sequencing buffer and the diluted ABI stock should be kept on ice.

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

Thermal Cycling protocol for GeneAmp® PCR System 9600 or equivalent (Rapid Thermal ramp is very important, and should be 1°C /sec):

96°C hold for 1 min
Rapid thermal ramp to 96°C
96°C for 10 sec
Rapid thermal ramp to 50°C
50°C for 5 sec
Rapid thermal ramp to 60°C
60 for 4 min

Followed by rapid thermal ramp to 4°C and hold. Bring reactions to the Genetic Analysis Lab (DE-302) for analysis and place in refrigerator.

Preparing reactions with a dried down template:

Dry samples down at 95°C until samples are dried down

	¼ reaction	½ reaction
Water	6 µl	4 µl
Primer	2 µl	2 µl
Big Dye	2 µl	4 µl
Total Volume	10 µl	10 µl

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

For sequencing large DNA templates (e.g. Cosmids, BACs, YACs):

Reaction volume 20 µl
95°C for 5 min
Repeat the following for 50 cycles:
-Rapid thermal to 95°C
-95°C for 30 sec
-Rapid thermal ramp to 50-55°C (depending on template)
-50-55°C for 10 sec
-Rapid thermal ramp to 60°C
-60°C for 4 min
-Rapid thermal ramp to 4°C and hold.

Bring reactions to the Genetic Analysis Lab (DE-302) for analysis and place in refrigerator.

