FHCRC Genomics June 2011

READY TO GO DNA SEQUENCING GUIDELINES

Tube/Plate requirements: Samples should be submitted numerically in strip tubes with strip lids. We recommend strip tubes from Fisher cat#14-230-210.
Plates must be loaded A-H with no spaces. V-bottom plates only, half or non-skirted.

Recommended DNA Quantities: DNA should be in dH2O or Tris buffer. Please ensure DNA templates are free of salts, proteins, organics, and florescent tags. PCR products should be free of extra primers and dNTP's.

Template Type	DNA Quantity/Reaction (ng)
PCR products	
100 to 200 bp	0.5 to 3
200 to 500 bp	1 to 10
500 to 1000 bp	2 to 20
1000 to 2000 bp	5 to 40
>2000 bp	10 to 50
Plasmids	
<6 kb	50 to 300
6-10 kb	300 to 500
>10 kb	500 to 1,000
Other types of template	
single-stranded	10 to 50
Cosmid or BAC DNA	200 to 1,000
Bacterial genomic DNA	1,000 to 3,000

Preparing Reactions:

Reaction size	BigDye mix	Primer (pmoles)	Template	DMSO**(5-10% final volume)	H2O to final volume	Number of cycles
1/2 reaction in 10ul	4ul	3-5	See above	0.5-1ul	10ul	25
1/2 reaction in 20ul	4ul BD + 4ul 5X buffer	3-5	See above	1-2ul	20ul	25
1/4 reaction in 10ul	2ul	3-5	see above	0.5-1ul	10ul	25
1/8† reaction in 10ul	1ul BD + 1.5ul 5X buffer*	3-5	see above	0.5-1ul	10ul	25
1/8† reaction in 5ul	1.0ul BD + 0.5ul 5X buffer*	3-5	see above	0.25-0.5ul	5ul	25

Note: Applied Biosystems full reaction is 8ul.
*5x sequencing buffer is an Applied Biosystems product and is available in DE-302 at no additional charge
**DMSO is an optional reagent and will not hurt the reactions. It is useful for GC-rich sequences.
†1/8 reactions should be tried only after success at 1/4 reaction

Thermal Cycling Conditions: These thermal cycling conditions were optimized using GeneAmp PCR system 9700, Applied Biosystems 9800 Fast Thermal Cycler, the Veriti 96-Well Thermal Cycler, and the Veriti FAST 96-Well Thermal Cycler. If you choose other thermal cyclers, you may need to adjust the conditions.

For Double-stranded DNA, Single-stranded DNA, and PCR Products:

Stage	Description	Temp (°C)	Time
1	Denaturation	96	1 min
2	Amplification: 25 cycles	96	10 sec
		50	5 sec
		60	4 min
3	Hold	4	Indefinite hold

For BAC DNA:

Stage	Description	Temp (°C)	Time
1	Denaturation	95	5 min
2	Amplification: 50 cycles*	95	30 sec
		50-55**	10 sec
		60	4 min
3	Hold	4	Indefinite hold

*Some laboratories have found increasing number of cycles gives better results.
**Set the annealing temperature according to the template.

Additional information:

-For templates with secondary structure we recommend using dGTP BigDye. Aliquots are available in the DE-302 freezer.
-For small templates and reading close to the primer we suggest decreasing both BigDye and template.
-In-House primers are M13 Forward, M13 Reverse, T7, and SP6. These are in the DE-302 refrigerator at 3uM concentration and are no additional charge.

Note from Hahn lab: For sequencing double stranded plasmids, use the conditions: 1/8 reactions with 10 microliters total volume. This works well for nearly everything and saves on Big dye reagent – Steve Hahn.