GUDSF Sequencing Protocol

Template Quality and Quantity

Reference: BigDye Terminator v1.1 Cycle Sequencing Kit Protocol, Applied Biosystems 2002 (Part# 4337036A), BigDye Terminator v3.1 Cycle Sequencing Kit Protocol, Applied Biosystems 2002 (Part# 4337035A)

The success of your sequencing reaction depends upon the quality and quantity of DNA template. The capillary system is very sensitive to contaminants such as proteins, RNA and residual salts. Not only is the quality of result affected by such contaminants but also the presence of proteins and high concentrations of DNA can reduce the life of the capillary array. For detailed information on DNA template preparation, DNA template quality and quantity, primer design and quantitation, refer to Performing DNA Sequencing Reactions, Section 3 in the Applied Biosystem's Automated DNA Sequencing Chemistry Guide (Part# 4305080).

Template Quality: Potential contaminants include proteins, RNA, residual salts, excess PCR primers, dNTPs, enzyme and buffer components (from PCR amplification). The DNA should be examined by agarose gel electrophoresis and spectrophotometry in order to assess the quality of the template.
Template Quantity: Template quantitation is critical for successful sequencing reactions. The preferred method for quantitation is by gel electrophoresis with a DNA mass ladder standard.

1. Recommended amount of template and primer to use in a cycle sequencing reaction:

PRIMER	3.2 pmol
TEMPLATE:	**QUANTITY**
PCR product:	
100-200 bp	1-3 ng
200-500 bp	3-10 ng
500-1000 bp	5-20 ng
1000-2000 bp	10-40 ng
>2000 bp	20-50 ng
Plasmid, single-standed	25-50 ng
Plasmid, double-standed	150-300 ng
Cosmid, BAC	0.5-1.0 µg
Bacterial Genomic DNA	2-3 µg
Reference: Page 2-6 of the Applied Biosystems Sequencing Protocol	

Reaction Set-Up

2. Recommended Reaction Set-ups using BDTv1.1/BDTv3.1 (BDT = BigDye Terminator reagent):

Reaction	1X	1/2 X	1/4 X	1/8 X
BDT (µL)	8	4	2	1
5X SEQ Buffer (µL)	0	2	3	3.5
Primer (µL)	3.2 pmol	3.2 pmol	3.2 pmol	3.2 pmol
Template	As required*	As required*	As required*	As required*
MilliQ Water	**To 20 µL**	**To 20 µL**	**To 20 µL**	**To 20 µL**

*See table above for recommended amounts of template.

Mix well and spin briefly.

3. Cycling Conditions

1. 96°C for 1 min
2. 96°C for 10 sec
3. 50°C for 5 sec
4. 60°C for 4 min

Repeat steps 2-4 for 25-30 times and then hold at 4°C until ready to purify.