Setting Up Big Dye Terminator Reactions for a 96-Well Format

Preparing the Reactions

Template Quantity

The table.1 shows the template quantities for the Big Dye terminator chemistry for a 1X cycle sequencing run.

Template	**Template Quantity**
PCR product:	
100-200 bp	1-3 ng
200-500 bp	5-30 ng
500-1000 bp	30-50 ng
1000-2000 bp	50-80 ng
>2000 bp	80-120 ng
Single stranded plasmid	50-100 ng
Double stranded plasmid	250-400 ng
Cosmid, BAC	0.5-1.0 µg
Bacterial genomic DNA	2-3 µg

Table.1

Preparing 1X Reactions

Reagent	**Quantity**
Terminator Ready Reaction mix	2.2µl
Template single-stranded DNA double stranded DNA PCR product DNA	- 50-100 ng 250-400 ng 1-120 ng (depending on size, see table.1)
Primer	3.2 pmols
Deionized water	q.s
Total volume	10µl

Performing Cycle Sequencing

To perform cycle sequencing under standard conditions:

Step 1: 95°C for 5 minutes
Step 2: 95°C for 15 seconds
Step 3: 50°C for 15 seconds
Step 4: 60°C for 4 minutes

Go to step 2, 49 more cycles

4°C, forever.

Preparing Extension Products for Electrophoresis

To perform EDTA-Ethanol precipitation:

1. Add 10µl of deionized water and 5µl of 125mM EDTA for 10µl reactions.
2. Add 60 µl of 200 proof ethanol.
3. Seal the plate with adhesive-backed aluminium foil tape. Press the foil onto the plate to prevent any leakage.
4. Invert the plate four times to mix.
5. Leave the plate at room temperature for 15 minutes to precipitate the extension products.
6. Place the plate in a table-top centrifuge with plate adaptor (Centrifuge 5810R, Eppendorf) and spin it at 3000 rpm for 30 minutes.
7. Remove the adhesive tape and discard the supernatant by inverting the plate onto a paper towel.
8. Invert the plate and spin up to 700 rpm, then remove from the centrifuge.
9. Rinse the pellet by adding 60µl of 70 % ethanol to each well.

10. Seal the plate with adhesive tape and invert the plate four times to mix.
11. Place the plate in a table-top centrifuge and spin at 2000 rpm for 14 minutes.
12. Remove the adhesive tape and discard the wash onto a paper towel that is folded to the size of the plate.
13. Place the inverted plate with the towel into the table-top centrifuge and spin up to 700 rpm.
14. Remove the plate and discard the paper towel.

Note: Pellets may not be visible. Make sure the samples are protected from light while they are air drying.

Loading the samples for electrophoresis

1. Re-suspend the pellets in 10µl of Hi-Di Formamide and mix by vortexing.
2. Denature the DNA at 95°C for 2 minutes and immediately place on ice for 10 minutes.
3. Centrifuge the plate to ensure the sample is positioned at the bottom of the wells.
4. Assemble the plate and place the plate assembly on the autosampler.
5. Run the samples using the appropriate Sequencing Module.