BigDye Terminator v3.1 Cycle Sequencing Reaction

Purpose: To convert template DNA to a format that is compatible with the Applied Biosystems 3500 Genetic Analyzer.

Materials and Reagents:

- BigDye™ Terminator v3.1 Cycle Sequencing Kit, Thermo Fisher Cat# 4337455
- Template DNA
- Primer (3.2 µM)
- Molecular Grade water (DNase, RNase Free)
- Performa DTR Gel Filtration Cartridges, Edge Bio Cat# 98780

Before Starting

- Ensure your DNA has been purified after isolation
- Quantify your DNA (if applicable)
- Determine the quantity of DNA you should input into the reaction using this chart:

DNA 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
Single-stranded DNA	25–50 ng
Double-stranded DNA	150–300 ng
Cosmid, BAC	0.5–1.0 µg
Bacterial genomic DNA	2–3 µg

*Note: We typically use 150 ng for plasmid DNA

- Protect the BigDye Sequencing mix from light as they can autofluoresce.

Protocol:

1. Completely thaw the BigDye Terminator v3.1 Cycle Sequencing tube and your primers and store on ice.
2. Vortex each tube for 3 seconds then briefly centrifuge to bring down any liquid in the cap.
3. Combine all components as indicated:

Reagent	Quantity
BigDye Terminator 3.1 Reaction Mix	8 µL
Primer (3.2 µM)	2 µL
Template DNA	Determined in the chart above
Molecular Grade Water	
	Total Volume: 20 µL

*Note: This reaction can be cut in half for a 10 µL total volume reaction

4. Seal tubes and vortex for 3 seconds then briefly centrifuge to bring down any liquid in the cap.
5. Set the thermocycler to run the following program:

Temperature	Time	
96°C	1:00	
96°C	0:10	x25 cycles
50°C	0:05	
60°C	4:00	
4°C	∞	

After completion of the reaction, clean samples up with the Performa DTR Gel Filtration Cartridges using the following steps:

1. Centrifuge the Performa Gel Filtration Cartridge for 3 minutes at 850 x g.
2. Discard the original 1.5 mL tube and transfer the cartridge to the provided 1.5 mL microcentrifuge tube.
3. Add the sample directly to the top of to the column. Be sure to avoid touching the gel with the pipette tip.
4. Close the cap and centrifuge for 3 minutes at 850 x g.
5. Remove the cartridge and discard it. Retain the sample in the 1.5 mL tube and label it.

Your sample is now ready to be submitted for sequencing!