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)
- Zymo ZR DNA Sequencing Clean-Up Kit, Cat# D4050 or D4051

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

Last Updated 8/15/23 by Riccardo Racicot

*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 Zymo ZR DNA Sequencing Clean-Up Kit using the following steps:

1. Add 240 µl of Sequencing Binding Buffer to a 5-20 µl sequencing reaction.
    a. Note: Alternatively, 5-20 µl sequencing reaction can be mixed with 240 µl Sequencing Binding Buffer that has already been added to the Zymo-Spin™ IB Column.
2. Transfer mixture to a provided Zymo-Spin™ IB Column in a Collection Tube.
3. Centrifuge at 13,000 rpm (15,000 - 16,000 x g) for 30 seconds.
4. Add 300 µl Sequencing Wash Buffer to the column. Centrifuge at 13,000 rpm (15,000 - 16,000 x g) for 30 seconds.
5. Add 6-20 µl water directly to the column matrix. Transfer the column to a 1.5 ml microcentrifuge tube and centrifuge at 13,000 rpm (15,000 - 16,000 x g) for 15 seconds to elute the DNA.

Your sample is now ready to be submitted for sequencing!