

Sequencing with BigDye Terminator v3.1 Cycle Sequencing Kit

The BigDye Terminator v3.1 Cycle Sequencing Kit provides the required reagent components for the sequencing reaction in a ready reaction, pre-mixed format. You need only provide your template and the template-specific primer. These reagents are suitable for performing fluorescence-based cycle sequencing reactions on single-stranded or double-stranded DNA templates, on polymerase chain reaction (PCR) fragments, and on large templates (for example, BAC clones).

1. The BigDye terminators are very bright, so the premix can be diluted. A full reaction is 8 μL of premix in a 20 μL reaction. You can save reagent by doing "mini reactions" - i.e. 4 μL in a 10 μL reaction. However, I would suggest that you consider 4 μL in a 20 μL reaction (or 2 μL in a 10 μL reaction) to be your "full" reaction. If you have success with this formulation, then try 2 μL in a 20 μL reaction, or 1 μL in a 10 μL reaction. At MBSU we use the 2 μL in a 20 μL reaction as our standard reaction.

2. When you dilute your premix, use buffer, do not use water. Applied Biosystems sells a 5X dilution Buffer:

PN 4336697 - 1 mL
PN 4336699 - 28 mL
PN 4336701 -233 mL

OR, you can use this buffer which gives good results:

200 mM Tris, pH 9.0
5 mM MgCl_2

This buffer is at 2.5X, which is the same as the premix, so just use it in place of the premix. For example, if AB says a full reaction is 8 μL of premix in a final volume of 20 μL , and you want to use only 2 μL of premix, then add 6 μL of buffer to your reaction.
($8/20 \times 2.5 = 1$)

3. Since you are using less premix, it is easy to saturate the amount of dNTP's and ddNTP's in your reaction, resulting in a top-heavy, short run. You should aim for 100 - 400 ng of plasmid template, and 50 - 200 ng of PCR product template in a 20 μL reaction. Use less if you are doing "mini" reactions. Use 5 pmol of primer (1 μL of a 5 μM solution.)

premix:	2 μL
buffer:	6 μL
template:	50 to 400 ng
primer:	3 to 5 pMol
H ₂ O:	to a final volume of 20 μL

4. Our standard cycling protocols are:

Regular protocol:

96°C	30 sec
50°C	15 sec
60°C	2 min (1 min works OK too)
25 cycles	

High GC protocol:

96°C	30 sec
60°C	2 min
30 cycles	

5. Proper clean-up of your samples is very important. When you bring us the pellets for loading, there must only be DNA in the tube or else the samples will not run well due to salts or other interfering reagents. Moreover, there could be large dye blobs of unincorporated Dye Terminators which obscure the base calls. You can purify your samples with a commercial spin-column, or use ethanol precipitation, which is what we use. Here is our protocol:

Add 2 μ L 1.5M NaOAc/250 mM EDTA to a 1.5 mL microfuge tube.

Add the 20 μ L sequencing reaction. Mix by pipetting up and down.

Add 80 μ L 95% EtOH. Vortex.

Leave on ice (or at 4°C, or at room temperature) for 15 min.

Centrifuge at 4°C for 15 min. Aspirate the supernatant.

Add 500 μ L 70% EtOH to the tube. Vortex thoroughly.

Centrifuge for 5 min. Aspirate the supernatant.

Dry the pellets in the speed-vac for 5 min, or air dry.