eBgDy™ Terminator Cycle Sequencing Mix

-For Sanger Sequencing

eBgDy™ Mix contents and storage:

Contents	Volume	Storage
eBgDy™ Mix	2x 20 ml	Store at -20°C to -80°C

Description:

eBgDy™ Mix is a direct replacement for Applied Biosystems' BigDye™ v3.1. Current BigDye™ v3.1 users can switch to eBgDy™ Mix seamlessly without changing current protocols. No new software or mobility shift file needs to be installed. No new calibration or validation is needed either.

eBgDy™ Mix also brings many advantages over BigDye™ v3.1

- **Faster reaction cycle:**
  3 min extension time, instead of 4 min, is need for EBgDy™ Mix, which can be further reduced to 2 min if needed.
- **Higher dilution factor:**
  As little as 0.15µl of eBgDy™ Mix can be used with success in a 5 µl total volume reaction (Fig. 1)
- **Higher success rate:**
  Especially for difficult templates (Fig. 2)

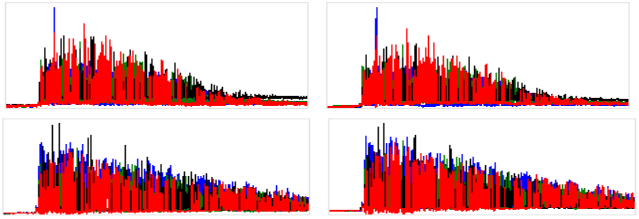

Fig. 1 Top panel: Raw sequencing signal of a normal template using 0.15 µl BigDye™ v3.1 in duplicate. Bottom panel: Raw sequencing signal of the same normal template using 0.15 µl eBgDy™ Mix. Total reaction volume was 5 µl.

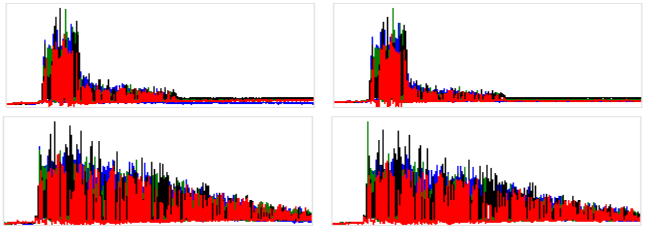

Fig. 2 Top panel: Raw sequencing signal of a GT rich template using 0.15 µl BigDye™ v3.1 in duplicate. Bottom panel: Raw sequencing signal of the same GT rich template using 0.15 µl eBgDy™ Mix. Total reaction volume was 5 µl.

Protocol:

This protocol only covers the reaction set up and the cycle sequencing parameters. For detailed instructions on how to prepare the templates, template concentrations, purity and sequencing reaction purification etc. please keep your current protocol unchanged or refer to Applied Biosystems' manual.

1. Set up reactions in 200 µL single PCR tube or 96 and 384 PCR plates according to the following formula. We only recommend 5 µL and 10 µL reaction volume. Please read the footnotes carefully.

Component	Standard reaction (5 µL)	Standard reaction (10 µL)
Template	2 µL[1],[2]	2 µL[1], [2]
Primer (5 µM)	1 µL	1 µL
5x Sequencing Buffer [3]	0.85 µL	1.7 µL
eBgDy™ Mix [4] [5]	0.15 µL	0.3 µL
Water [5]	1 µL	5 µL
Total volume	5 µL	10 µL

[1] e.g. 150-300ng/µL of dsDNA

[2] Concentration of template may affect volume, if template volume differs, adjust the volume of water in the reaction mix.

[3] The volume of 5x buffer needed per reaction is determined by the following formula:

$$\frac{5x\ (\text{Volume of 5x buffer} + \text{Volume of Terminator mix})}{\text{total reaction volume}} = 1$$

[4] Maximum of 0.5 µl or 1.0 µl eBgDy™ Mix are recommended for 5 µl or 10 µl reactions respectively. **Use more than 0.5 µl or 1.0 µl eBgDy™ Mix gives less than favorable results.**

[5] Alternatively, ready mix cocktail can be made by mixing 100 volumes of water, 85 volumes of 5x Sequencing Buffer and 15 volumes of eBgDy™ Mix. Use 2 µl or 4 µl of the ready mix for each reaction of 5 µl or 10 µl respectively.

2. Perform Cycle Sequencing:

Parameter	Stage/step				
	Incubate	30 cycles[1]			Hold
		Denature	Anneal	Extend	
Temperature	96°C	96°C	50°C	60°C	4°C
Time	01:00	00:10	00:10[2]	03:00[3]	∞

3563 Investment Blvd., Suite 2
Hayward, CA 94545

[1] 30 cycles takes 2 hours on a Thermo Veriti™ 96-Well Thermal Cycler.

[2] Research showed longer anneal time facilitates sequencing GT rich templates.

[3] Extension for 3 minutes allows reading length of 1000bp - 1200bp. Shorter extension times can be used for short templates.

3563 Investment Blvd., Suite 2
Hayward, CA 94545