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Dye-terminator DNA sequencing

Diep R. Ganguly[1]

[1]The Australian National University

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Diep R. Ganguly
The Australian National University

ABSTRACT

This protocol (based on the BigDye® Terminator v3.1 Cycle Sequencing Kit) is for performing terminator cycling sequencing reactions for Sanger sequencing of amplified PCR products or plasmid DNA on the 3130X genetic analyser (Applied Biosystems).

BigDye Terminator v3.1.pdf

MATERIALS

NAME	CATALOG #	VENDOR
Antarctic Phosphatase - 1,000 units	M0289S	New England Biolabs
96 well PCR Plate Non-skirted	MPS-499	Phenix Research
Nuclease-free water (e.g. MilliQ or HPLC grade water)		
primers		
EDTA		
10 mM dNTPs	10297-018	Life Technologies
Ethanol	100983	Merck Millipore
BigDye™ Terminator v3.1 Cycle Sequencing Kit	4337454	Thermo Fisher
Exonuclease I (E. coli)	M0293S	NEB
Hi-Di™ Formamide	4311320	Thermo Fisher Scientific

BEFORE STARTING

Optimize PCR cycling (if sequencing amplified PCR products) to ensure your reaction produces a single product. Perform gel excision or PCR clean-up with the potential inclusion of incubating with Antarctic phosphatase and Exonuclease 1 to dephosporylate and degrade unincorporated dNTPs in PCR reactions to prepare templates for DNA sequencing.

Terminator cycling reaction

1 Perform sequencing reaction with BigDye Terminator cycling kit and either forward or reverse primers, or both.

Component	Volume (µl)
2.5X Reaction Ready Mix	4
5X BigDye Sequencing buffer	2
20 µM F/R Primer	1
Template (plasmid or cleaned PCR product)	150-300 ng dsDNA or approx 10ng PCR product (see BigDye manual)
Nuclease-free water	to 20 µl

BigDye Terminator Cycling reaction

2 Set the following protocol and allow thermal cycler to reach 96 °C:
1. 1 min at 96 °C
2. 30 cycles consisting of: 96 °C for 10 seconds, 50 °C for 5 seconds, and 60 °C for 4 min.
3. Hold at 10 °C.

Purify products

3 To the PCR reaction, add 60 µL 100% ethanol and 5 µl 125 mM EDTA.

4 Incubate at room temperature for 15 minutes.

5 Centrifuge at 4 °C at max speed for 10 minutes.

6 Remove as much supernatant as possible, then allow to air-dry in the dark for 10-15 minutes.

Resuspend products and submit for sequencing

7 Resuspend the pellet (likely transparent) in 20 µL HiDi Formamide (add 20 µL to any empty wells). Spin down briefly.

8 Submit for sequencing on 3130X genetic analyser (Applied Biosystems).