VERSION 1

DEC 06, 2019

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.97jh9kn

Protocol Citation: Diep R Ganguly 2019. Dye-terminator DNA sequencing. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.97jh9kn>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: Dec 06, 2019

Last Modified: Dec 06, 2019

PROTOCOL integer ID:
30667

Dye-terminator DNA sequencing V.1

Diep R Ganguly¹

¹University of Pennsylvania

Diep R Ganguly

University of Pennsylvania, The Australian National Universi...

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).

ATTACHMENTS

[BigDye Terminator v3.1.pdf](#)

MATERIALS

MATERIALS

Antarctic Phosphatase - 1,000 units New England Biolabs Catalog #M0289S

96 well PCR Plate Non-skirted Phenix Research Catalog #MPS-499

Nuclease-free water (e.g. MilliQ or HPLC grade water) Contributed by users

primers Contributed by users

EDTA Contributed by users

10 mM dNTPs Life Technologies Catalog #10297-018

Ethanol Merck Millipore Catalog #100983

BigDye™ Terminator v3.1 Cycle Sequencing Kit Thermo Fisher Catalog #4337454

Exonuclease I (E. coli) NEB Catalog #M0293S

Hi-Di™ Formamide Thermo Fisher Scientific Catalog #4311320

Keywords: Sanger sequencing, Dye-terminator sequencing

BEFORE START INSTRUCTIONS

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 dephosphorylate 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.

Comp onent	Volu me (µl)
2.5X Reacti on Ready Mix	4
5X BigDy e Seque ncing buffer	2
20 µM F/R Prime r	1
Templ ate (plas mid or clean ed PCR produ ct)	150- 300 ng dsDN A or appro x 10ng PCR produ ct (see BigDy e manu al)
Nucle ase- 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).