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We use this protocol and it's working

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Dye-terminator DNA sequencing V.3

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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

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- 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**

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 in PCR tubes (or 96-well plate) with BigDye Terminator cycling kit and forward or reverse primers.

30m

Component	Volume (μl)
v3.1 Ready reaction mix	1
5X Sequencing buffer	1.5
20 μM F/R Primer	0.5
Template (plasmid or cleaned PCR product)	50-150 ng DNA (plasmid or PCR product)
Nuclease-free water	to 10 μl

BigDye Terminator Cycling reaction

5x reaction buffer: 400 mM TRIS, 10 mM MgCl₂

- 2 Run the following thermal cycling protocol:
1. 1 min at 96 °C
 2. 30-40 cycles: 96 °C for 10 seconds, 50 °C for 5 seconds, and 60 °C for 4 min.
 3. Hold at 4-12 °C.

4h

Purification

1h 30m

- 3 Transfer PCR reaction to eppendorf tube. To the reaction, add 2.5 μL of 125 mM EDTA (make sure it touches bottom of tube).

- 4 Add 30 μ l of 100% ethanol, mix well (inversion).
- 5 Incubate at room temperature for 15 minutes.
- 6 Centrifuge at 4 °C at max speed for 30 minutes.
- 7 Discard supernatant and add 50 μ l of ice-cold 70% ethanol.
- 8 Centrifuge at 4 °C at max speed for 5 minutes.
- 9 Discard supernatant and allow to air-dry in the dark for >15 minutes.

Prepare for sequencing

- 10 Resuspend the pellet (likely transparent) in 7.5 μ L HiDi Formamide (add to any empty wells). Incubate at RT for 5 minutes then transfer to plate. Spin down briefly.
- 11 Incubate plate at 95 °C for 3 minutes (denature) then place immediately on ice. Spin down briefly.

12 Submit for sequencing on 3130X genetic analyser (Applied Biosystems). Keep samples on ice.