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WORKS FOR ME 1

Phage DNA extraction with Monarch kit and digestion to single nucleosides

COMMENTS 0

In 1 collection

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ABSTRACT

This protocol details high-molecular-weight DNA extraction from bacteriophages using the NEB Monarch HMW DNA Extraction Kit for Tissue. Following DNA extraction, DNA is digested down to single nucleosides using the NEB Nucleoside Digestion Mix.

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PROTOCOL CITATION

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COLLECTIONS

Protocol collection: Phage DNA isolation and chemical analysis

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PARENT PROTOCOLS

Part of collection

Protocol collection: Phage DNA isolation and chemical analysis

Degradation of host nucleic acids

1 To degrade any non-encapsulated nucleic acids, treat the concentrated phage lysate with 10 µg/mL RNase A (NEB T3018) and 10 µg/mL DNase I (NEB M0303). Digest for 1 h at room temperature or 4 °C overnight. Store phage at 4 °C.

Phage DNA extraction (using NEB Monarch HMW DNA Extraction Kit f

2 Aliquot 300 µL of concentrated phage into a 1.5 mL Eppendorf tube. This will be used for DNA harvest with the NEB Monarch HMW DNA Extraction Kit for Tissue (NEB T3060L), using the modifications for bacteriophages suggested by NEB in their **extremely** helpful technical note.

TechNote_Fast_efficient_isolation_phage_gDNA_Monarch_HMW_DNA_Extraction_Kits.pdf

3 Add 300 µL HMW gDNA tissue lysis buffer and 20 µL proteinase K to the aliquoted phage. Incubate at 56 °C for 45 minutes on a Thermomixer at 250 rpm. If you only have a static heat block, vortex every 10 min.

4 Add 300 µL of protein separation solution with gentle mixing. Spin down at 16 × g for 10 min.

5 Move aqueous layer (around 900 µL, organic is around ~50 µL at bottom) to a fresh 1.5 mL Eppendorf tube. Add two DNA capture beads to aqueous layer. Then add 550 µL of isopropanol to the tube to begin precipitation.

6 Put the Eppendorf tube onto a rotator set at the slowest speed, and rotate for 5 min to bind DNA to beads. If

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you do not have a rotator, do 25 slow and gentle manual inversions.

7 After DNA binding, pipette off the liquid from the beads and wash the beads twice with 500 µL of DNA wash buffer.

8 Move the beads to the bead retainer, and pulse very quickly in a bench-top centrifuge to remove any residual liquid from the beads.

9 Quickly move the beads to a new clean tube, and add 100 µL of Elution buffer. Elute at 56 °C for 5 min. After elution, transfer the eluate to a fresh tube and Nanodrop.

Nucleoside digestion

10 Use a Nucleoside Digestion Mix (NEB - M0649S) to digest 1 µg of DNA. Each reaction should contain 1 µL of Nucleoside Digestion Mix and 2 µL of 10× Nucleoside Digestion Mix buffer.

11 Perform reactions in 20 µL total volume and incubate at 37 °C for 1 h, followed by enzyme inactivation at 80 °C for 15 min. Keep digested samples at 4 °C until ready for downstream use or analysis.

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