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WORKS FOR ME 1

Phage DNA extraction with phenol-chloroform 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 phenol-chloroform. 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 phenol-chloroform)

2 Strip away phage capsids with 1 µl of Proteinase K (NEB P8107S) + 0.05% SDS. Incubate at 55 °C for 60 minutes on a Thermomixer at 250 rpm. If you only have a static heat block, vortex every 10 min.

3 In fume hood, add an equal volume of phenol – chloroform – isoamyl alcohol mixture, pH 8 (Sigma Aldrich 77618) to the digested phage. If the volume of phage is low, consider bringing up the volume to 500 µl with 1x TE buffer. Incubate at room temperature for 10 minutes, vortexing regularly to keep phenol mixed in.

4 Spin sample at maximum speed at 4 °C for 10 minutes.

5 In the fume hood, transfer the aqueous layer (top layer) to a new tube. Make sure to avoid the interface, and do not transfer any any debris or organics to the new tube. Add equal volume of chloroform to the sample and invert to mix.

6 Spin sample at maximum speed at 4 °C for 5 minutes.

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7 Repeat steps 5 and 6, extracting the aqueous layer with more chloroform.

8 Transfer aqueous layer to new tube. All subsequent steps can be performed on the benchtop.

Isopropanol precipitation

9 Add a 10% volume of 3 M sodium acetate to the sample, and an equal volume of isopropanol. Invert to mix, and move to 4 °C for 2 hours (can be left overnight).

10 Spin sample at maximum speed at 4 °C for 30 minutes.

11 DNA will be a glassy smear or pellet on the side of the tube. Carefully pipette off the liquid.

12 Wash pellet with 1 mL freshly-made 70% ethanol (room temperature, not cold). DNA will start to turn white. Check to make sure the DNA isn't stuck on the sides of the tube. If it is, wash it down with the 70% EtOH.

13 Spin sample at maximum speed at 4 °C for 5 minutes.

14 Carefully pipette off the liquid, and wash with 500 µl of 70% ethanol. Spin sample at maximum speed at 4 °C for 5 minutes.

15 Carefully pipette off the liquid. Do short spins, and pipette with smaller and smaller pipette volumes until the all the ethanol is removed. Let dry for 5 minutes or less.

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16 Resuspend pellet in 30 µl of nuclease free water. It can help to warm the sample to 50 °C. The volume of water can be adjusted if you anticipate a large DNA yield.

Nucleoside digestion

17 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.

18 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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