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Chloroform Phenol Phage genome isolation

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

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Abstract

Protocol of the isolation of genomic DNA from phage lambda and phage T7.

1 Use cleaned phage stock from the **phage stock** protocol. Add 50 µL DNase I 10x buffer, 1 µL DNase I (1 U/µL), and 1 µL RNase A (10 mg/mL) for 01:30:00 at 37 °C without shaking to remove E. coli DNA and RNA.

2 Add 20 µL of [M] 0.5 Mass Percent EDTA (final concentration [M] 20 Molarity (M)) and incubate for 00:15:00 at 75 °C to deactivate DNase I and RNase A

3 Add 2 µL Proteinase K (20 mg/mL) and 50 µL SDS 10% to digest the phage capsid and incubate overnight at 56 °C without shaking.

4 Add equal volume phenol/chloroform/iosamyl alcohol (25:24:1) and mix well (do not vortex, as genome is easily damaged)

5 Centrifuge at room temperature for 00:10:00 at 10000 x g

6 Carefully take aqueous phase

7 Add equal volume chloroform/isoamyl alcohol (24:1) and mix well (do not vortex, as genome is easily damaged)

8 Centrifuge at room temperature for 00:10:00 at 10000 x g

9 Carefully take aqueous phase

10 Add 1/10 volume of Sodium acetate [M] 3 Mass Percent

11 Add 2,5 volume of 100% Ethanol

12 Incubate at -20 °C for 00:30:00

13 Centrifuge for 00:10:00 at 4668 x G

14 Discard supernatant

15 Rinse pellet with 70 % ethanol

16 Dry pellet. Heating to 50 °C increases drying.

17 Dissolve pellet in MQ water