Chloroform Phenol Phage genome isolation

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1 Works for me

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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 **0.5 Molarity (M)** EDTA (final concentration **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 Molarity (M)**

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