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Chloroform Phenol Phage genome isolation

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ABSTRACT

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

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

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PROTOCOL integer ID:
28021

- 1 Use cleaned phage stock from the [phage stock](#) protocol. Add 50 μ L DNase I 10x buffer, 1 μ L DNase I (1 U/ μ L), and 1 μ L 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