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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  0.5 Mass Percent 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  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