Bacteriophage DNA Organic Extraction

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Method Article

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Abstract

This protocol is designed for the extraction of bacteriophage (phage) DNA used in downstream applications such as whole genome sequencing. For this purpose, the phage lysate is treated with DNase I and RNase A to degrade any DNA or RNA that is not enclosed by the viral capsid (e.g., from bacterial origin), and then EDTA and SDS are added to inactivate the DNase I/RNase A along with the addition of proteinase K to digest the viral capsid. Thereafter, protein debris are removed by sequential extraction with phenol-chloroform and precipitation with ethanol, which increases DNA concentration and removes excess salts. Compared to commercial kits, the price per sample is lower for this protocol, without entailing greater complexity or yielding inferior results.

Introduction

DNA extraction is not only relevant for phage work but is also used in many other areas of microbiology. Genomic analysis of a phage provides relevant information regarding, for example, genomic relatives or lysogeny proteins. In addition to general DNA extraction kits, there are also special phage DNA extraction kits (e.g., Norgen Biotec Corp., Thorold, Canada; cat. no.: 46800). In contrast to these commercial kits, there are also independent extraction protocols. Our non-commercial protocol can be adapted and expanded according to individual needs (see Troubleshooting).

Reagents

1. Chlorophorm (ACROS Organics, Geel, Belgium; cat. no.: 15821001)
2. DNase I (Roche Diagnostics, Mannheim, Germany; cat. no.: 10104159001)
3. Double-distilled water (ddH₂O)
4. Ethanol absolut (VWR International, Pennsylvania, USA; cat. no.: 85651.320)
5. Ethylenediaminetetraacetic acid disodium salt (EDTA-Na₂; Sigma-Aldrich, St. Louis, USA; cat. no.: E5134)
6. Ice
7. Proteinase K (Thermo Fisher Scientific, Waltham, USA; cat. no.: 10504885)
8. RNase A (Roche Diagnostics; cat. no.: 10109142001)
9. Sodium acetate (NaOAc 3 M pH 5.2; Thermo Fisher Scientific; cat. no.: 10190890)
10. Sodium dodecyl sulfate (SDS; Sigma-Aldrich; cat. no.: L3771)
11. Phenol (Carl Roth GmbH, Karlsruhe, Germany; cat. no.: 0038.2)
**Equipment**

1. Centrifuge (e.g., Eppendorf 5430 R; Eppendorf SE, Hamburg, Germany; cat. no.: 5428000205)
2. Centrifuge tube (15 ml; Falcon® Corning Inc., Corning, USA; cat. no.: 352096)
3. Freezer
4. Ice bucket
5. Microcentrifuge (e.g., Eppendorf 5415 R; Eppendorf SE; cat. no.: 22 62 140-8)
6. Microcentrifuge tube (2 ml; Eppendorf SE; cat. no.: 0030120094)
7. Pipette controller and serological pipettes (1 ml)
8. Pipettes and pipette tips (10 µl, 100 µl and 1000 µl)
9. Refrigerator
10. Shaker thermoblock (e.g., Thermomixer Comfort 5355 Block 24 x 2.0 ml; Eppendorf SE; cat. no.: 5355 000.011)

**Procedure**

1. Add 1 ml of phage stock solution ($10^9$-$10^{10}$ PFU/ml) to a 2 ml microcentrifuge tube.
2. Add 10 µl of DNase I (1 mg/ml) and 10 µl RNase A (5 mg/ml) to the tube.
3. Place the tube in a shaker thermoblock and incubate for 60 min at 37 °C.
4. Add 40 µl of 0.5 M EDTA-Na$_2$ to the tube.
5. Add 2.5 µl of Proteinase K (20 mg/ml) to the tube.
6. Add 50 µl of 10 % SDS to the tube.
7. Place the tube in a shaker thermoblock and incubate for 1 h at 56 °C.
8. Let the tube cool down to room temperature (approx. 20 min).
9. Transfer the entire volume of the microcentrifuge tube into a 15 ml centrifuge tube.
10. Add 1.1 ml of phenol to the centrifuge tube, seal it with the lid and invert it 5 times.
11. Centrifuge the tube for 5 min at 22 °C and 4,000 g.
12. Transfer the upper aqueous phase into a new 15 ml centrifuge tube.

13. Add the same volume of phenol/chloroform in a ratio of 50/50 (e.g., for 0.9 ml of extracted volume in step 12, add 450 µl phenol and 450 µl chloroform).

14. Centrifuge the tube for 5 min at 22 °C and 4,000 g.

15. Transfer the upper aqueous phase into a new 15 ml centrifuge tube.

16. Add the same volume of chloroform (e.g., for 700 µl of extracted volume in step 15, add 700 µl chloroform).

17. Centrifuge the tube for 5 min at 22 °C and 4,000 g.

18. Repeat steps 16 and 17.

19. Transfer a maximum of 550 µl of the upper aqueous phase into a 2 ml microcentrifuge tube.

20. Add a maximum of 55 µl (1/10\textsuperscript{th} of volume in step 19) sodium acetate to the tube.

21. Add a maximum of 1375 µl (2.5x volumes after step 20) ethanol (95 %).

22. Incubate the tube for 30 min on ice in a freezer (-20 °C).

23. Centrifuge the tube for 20 min at 4 °C and 14,000 g.

24. Remove the supernatant and add 2 ml of ethanol (70 %) to the tube.

25. Invert the tube 10 times and incubate it for 5 min at room temperature.

26. Centrifuge the tube for 5 min at 4 °C and 14,000 g.

27. Remove the supernatant and let the pellet dry on air (approx. 20 min).

28. Resuspend the pellet in 20 µl ddH\textsubscript{2}O.

29. Store the solution in a refrigerator at 4 °C until further use.

Using a spectrophotometer (e.g., NanoDrop 1000 Spectrophotometer; Thermo Fisher Scientific, Waltham, USA; cat. no.: ND-1000), the concentration of phage DNA and its purity can be evaluated.

**Troubleshooting**

Step: 23 & 26
Problem: No pellet visible
Possible reason: Low phage starting concentration
Solution: Increase the phage concentration at step 1

Step: 23 & 26
Problem: No pellet visible
Possible reason: Pellet may be difficult to visualise (colourless)
Solution: Continue extraction and determine DNA concentration at the end

Problem: No DNA present
Possible reason: RNA virus
Solution: Requires a different protocol

Further modifications of the protocol might include the addition of isoamyl alcohol to the phenol/chloroform (step 13; ratio: 25 ml : 24 ml : 1 ml) and the chloroform (step 16; ratio: 24 ml : 1 ml) steps, as an anti-foaming agent and to support the DNA purification. Additionally, the duration of the ethanol treatment could be increased to 6 h at -20 °C (step 22).

**Time Taken**
Approximately 4:20 h will be required to complete the DNA extraction, including 3:30 h wait time.

**Anticipated Results**
With this protocol, clean phage DNA can be sufficiently extracted and used in downstream analysis. Depending on the phage, the phage production method or the initial phage concentration, extracted DNA concentrations ranging from 50 to 1000 ng/µl (260/280 ratio: ~1.8) can be expected.

**References**
   https://dx.doi.org/10.17504/protocols.io.7kvhw6


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