DNA extraction of phages

Introduction

Phage is produced in large quantities (high titer stock, see protocol) by infection of its bacterial host, generally resulting in a lysate containing 10^8 CFU/mL to 10^{11} CFU/mL in marine broth. The phage is then precipitated with Polyethylene Glycol (PEG), so that it can be further concentrated if required, and the phage can be reabsorbed with a nuclease-compatible buffer. Indeed, before extracting the viral DNA (protected by the capsid), the DNA and RNA of the bacterial host must be degraded, particularly if sequencing libraries are to be produced. These nucleases are then inhibited by addition of the chelating agent EDTA, and the proteins can be digested with proteinase K. Phage DNA is then purified by extraction with phenol chloroform and precipitation with ethanol. Commercial kits may be an alternative, but they don't work with all our isolates, so it's best to use the (old) tried-and-tested methods.

Materials

- 5× PEG solution (500 mL)
  - 5 M NaCl stock solution – 250 mL
  - PEG8000 – 100 g
  - $H_2$0d qsp 500 mL
  - Stir until dissolved.
- SM buffer
  - NaCl 100 mM
  - $MgSO_4.7H_2O$ 8 mM
  - Tris-Cl 50 mM pH7.5
- DNAse RQ1 (Promega) and its buffer
- RNAse
- EDTA 0.5N pH 8
- Proteinase K at 20 mg/ml
- SDS 20%
- Mix 1VPhénol 1V (chloroform, isoamyl alcohol 24.1)=> buy this mix
- Chloroform/isoamyl alcohol 24.1 : this can be bought or made in the lab
- Sodium acetate 3N pH 5.4
- Pure ethanol, 70%
- TE: Tris 10mM pH7.5 et EDTA 1mM pH8

Method

Concentration PEG

1. To 40 mL phage (high titer, schizo 10^8 PFU/mL) add 10 mL PEG 5X, mix by inverting tube and incubate overnight at 4°C (conical tube).
2. Centrifuge at 19,000 × g for 60 min at 4°C.
3. Resuspend pellet with 500 µL SM buffer at 4°C, can be stored at 4°C.
4. Compare titer of concentrated and non-concentrated phages: final phage titer?

Digestion nuclease

1. Add DNAse quantity necessary for 1X final buffer (50 µl 10X buffer + 450 µl phage concentrate)
2. Mix by pipetting gently
3. Add 1µl DNAse RQ1 (Promega) at 1 Unit/µl
4. Add 2.5 µl RNAse at 3.5 mg/ml
5. Mix by gentle pipetting
6. incubate at 37°C for 30 minutes
7. Block the reaction by adding 20 µl EDTA 0.5M pH8 to the 500 µl preparation (20 mM final)=> proceed immediately to extraction.

Extraction

1. To 500 µl of nuclease-treated phage add 12.5 µl of a 20 mg/ml proteinase K solution and 12.5 µl of 20% SDS.
2. Mix by gently inverting the tube two or three times.
3. Incubate 30 min at 55°C
4. Add 2V of Phenol/Chloroform/Isoamyl Alcohol (Take an aliquot from the bottle to a falcon and PIPET UNDER THE AQUEOUS LAYER).
5. Mix by gently inverting the tube two or three times.
6. Centrifuge 5 min at 14,000 rpm
7. Recover the upper phase in a 1.5-ml Eppendorf tube, avoiding the whitish cake at the interface with the phenolic phase.
8. Add 500 µl chloroform/Isoamyl alcohol (24 :1).
9. Mix by gently inverting the tube two or three times.
10. Centrifuge for 5 min at 14,000 rpm.
11. Collect upper phase in 1.5 ml Eppendorf tube, avoiding interface
12. Add 1/10 volume of Sodium acetate 3N pH5.4, mix by inverting the tube.
13. Add 2.5 volumes of 100% ethanol, mix by inverting the tube: can you see a jelly blob? if yes, proceed directly to the centrifugation step. If not, incubate at -20°C for at least one hour (you can stop there).
14. Centrifuge for 10 minutes at 14,000 rpm, RT
15. Wash pellet with 500 µl 70% ethanol at room temperature
16. Centrifuge for 5 minutes at 14,000 rpm, RT
17. Re-suspend with 50 µl TE buffer (increase volume if viscous) Incubate 1H à 65°C or ON at 4°C
18. Quantify with nanodrop (pay attention to the 260/280 ratio, which must be between 1.7 and 2).
19. Check DNA quality on a 0.7% migration 50V ON gel at 4°C

Important tips !

Commercial kits can be an alternative, but they don't work with all our isolates, so it's best to use the (old) tried-and-tested methods.

References

1- Cahier K, Piel D, Barcia-Cruz R, Goudenège D, Wegner KM, Monot M, Romalde JL and Le Roux F*. Environmental vibrio phage-bacteria interaction networks reflect the genetic structure of host populations. **Environmental microbiology** 2023 Mar 6. doi: 10.1111/1462-2920.16366.

2- Piel D, Bruto M, Labreuche Y, Blanquart F, Chenivesse S, Lepanse S, James A, Dubert J, Petton B, Lieberman E, Wegner KM, Hussain FA, Kauffman KM, Polz MF, Bikard D, Gandon S, Rocha EPC and Le Roux F*. Phage-host coevolution in natural populations. **Nature Microbiology.** 2022 Jul;7(7):1075-1086.