Protocol for Phage DNA Extraction with Phenol:Chloroform

In this protocol you extract the genomic DNA from the phages in a lysate. The lysates are "dirty" in that they contain spent media components, cell wall debris, flagella, nucleic acids, bacterial proteins and unassembled phage proteins in addition to the phage themselves. There are many methods for purifying phage DNA from the rest of the lysate, This protocol is recommended specifically for phages that have not resulting in high-yield or high-quality DNA from a kit (like the Promega Wizard DNA Clean-up Kit used in the DNA Extraction Protocol). For best results, adhere to the recommendations for incubation times listed in each step.

Day 1: PEG Precipitation

1. Take 1 volume of phage high titer lysate (see below) and add PEG (10% PEG-8000, 1 M NaCl final concentration) at a ratio of 1:2 precipitant:lysate. Mix gently by inversion.
    a. As a rule of thumb, 1 ml of phage lysate with a titer of 1 x 1010 contains about 0.5 µg of phage DNA, assuming the phage has a 50 kb genome. Typically 10 ml of a high-titer phage lysate is used for DNA extraction. More or less lysate (up to 20 ml) may be used depending on the phage stock titer and the expected genome size of the phage. For low-titer phage stocks, up to 20 ml of lysate may be used. With high titer stocks of large-genome phages, use 10 ml of lysate or less.

2. Incubate at 4°C overnight (this is ideal, can also do 60 minutes on ice if you need to work faster). Most phages are stable like this for several days.

Day 2: DNA Extraction

1. Centrifuge the PEG-precipitated sample at 10,000 *xg* for 30 minutes.
2. Use 5mM $MgSO_4$ to re-suspend the pellet, pipetting gently up and down. AVOID introducing BUBBLES during the resuspension. Be sure to rinse down the sides of the tube to obtain all of the pellet. Transfer to a labeled 2 mL epi tube.
3. Using 500 µl of the concentrated sample: add 1.25 ul of DNaseI and RNase (20 mg/ml) and incubate at 37°C for 1hour.
    a. (You can scale all volumes up if more than 500 uL are needed for resuspension).
4. Add 1.25 µl of Proteinase K 20 mg/ml stock (20µg total) and 25 µl 10% SDS stock (0.5% final concentration) and 20 ul of 0.5 M EDTA pH 8.0 (20 mM final). Mix and incubate 1 hour at 60°C.
5. Allow to cool to room temperature.
6. Add an equal volume of **phenol:chloroform** (1:1) and invert several times.
7. Spin 3000 *xg* (6000 rpm on microfuge), 5 minutes room temperature.
8. Carefully transfer the supernatant with a wide-bore pipette tip to a fresh, labeled 2 mL epi tube.
9. Add an equal volume of **phenol:choroform** (1:1), invert.

10. Centrifuge as above and transfer the supernatant again.
11. Add an equal volume of **chloroform**, invert.
12. Centrifuge as above and transfer the supernatant to a fresh, labeled 2mL epi tube.
    a. Depending on volume here you may need to use a 15mL falcon tube
13. Add 1/10 volume of 3 M NaOAc (pH 7.5), and 2.5 volumes of ice cold ethanol (100%). Mix well and incubate at -20°C overnight.
    a. A fast method allows incubation on ice for 15-30 minutes.

Day: 3 DNA Precipitation

1. Centrifuge in a benchtop microfuge at max speed 20 minutes (10000 rpm for 15ml tubes as tube rating allows, 15000 rpm on the microfuge).
2. Carefully remove supernatant and fill tube halfway with 70% ethanol (made from 100% with *purified nuclease free water*), spin at max speed for 2 minutes.
3. Repeat the above step one time (2nd 70% wash).
4. Remove as much ethanol as possible without disturbing the pellet – good idea to hold onto the supernatant until DNA recovery has been confirmed.
5. Leave tube open on bench ~ 15-30 minutes to let ethanol disperse.
6. Dissolve in TE buffer (~ pH 7.6).