Phage genomic DNA extraction – modified Promega Wizard method

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, and in this protocol we will use a modified method with commercial DNA extraction kit, the Promega Wizard DNA Clean-up kit (Promega catalog #A7280). If you publish using this method, please use the following citation: Summer EJ, 2009. Preparation of a phage DNA fragment library for whole genome shotgun sequencing. Methods Mol Biol 502:27-46; PMID 19082550.

As a rule of thumb, 1 ml of phage lysate with a titer of 1 x 10^{10} 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 or else the column will be overloaded and clog. The columns have a maximum capacity of about 30 µg of DNA.

Reagents required

Promega Wizard DNA cleanup kit (cat# A7280)
Phage lysate (10-20 ml, >10^9 PFU/ml)
Nuclease solution (20 mg/ml DNase I, 20 mg/ml RNase A)
Precipitant solution (30% w/v PEG-8000, 3 M NaCl)

-Preparation (200 ml): in a clean bottle add 110 ml ddH_2O and 35 g NaCl, dissolve. Add 60 g PEG-8000, cap bottle and shake. Incubate bottle in a 50 – 60 ºC water bath for about 2 h, shaking occasionally. Remove and let cool to RT, shaking occasionally. Solution should be clear or slightly turbid. Add ddH_2O to 200 ml, store at RT.

Resuspension buffer (5 mM $MgSO_4$ in water)
80% (v/v) isopropanol
Sterile molecular biology-grade water

Materials required

50 ml centrifuge tubes
Sterile 1.5 and 2 ml microcentrifuge tubes
3 cc syringes
Heat block, 80 ºC

1. Prepare your phage lysate by plate lysate or liquid culture methods. Lysates should be clarified by filter sterilization (0.45 or 0.22 µm). Lysates may be in broth (LB, TSB, etc) or in λ-dil.
2. Place the lysate into a clean centrifuge tube. Add 0.5 µl of nuclease solution per ml of lysate (10 µg/ml DNase & RNase final). Incubate the lysates at 37 ºC for 30 min, or at RT for 2 h.

3. Add precipitant solution to the lysate at a rate of 1:2 precipitant:lysate (10% PEG-8000, 1 M NaCl final). Mix gently by inversion. Incubate on ice for at least 60 min; precipitation works best when incubated at 4 ºC overnight. Most phages are stable in this state for up to several days.
    a. Alternative method: phages may be pelleted from the lysate by centrifugation in a normal high-speed centrifuge (e.g., about 7,000 x g overnight) or in an ultracentrifuge (e.g., about 1 hour at 50,000 x g). dsDNA phages have weights of about 400-1000 S, and pelleting time (in hours) can be calculated from the rotor's k-factor by the formula: **time = k/S.**
4. Centrifuge the precipitated phage lysate at 10,000 x g, 4 ºC, 10 min.
5. Carefully pour off the supernatant and retain the pellet. The pellet may be transparent or opaque, and may be spread up the wall of the tube.
6. Resuspend the pellet in 500 µl of resuspension buffer (5 mM $MgSO_4$) by pipetting gently up and down; be sure to rinse down the sides of the tube to obtain all of the pellet.
7. Transfer the resuspended phage to a labeled 1.5 ml microcentrifuge tube.
8. Centrifuge for 5-10 sec to pellet any insoluble particles. Transfer the supernatant to a new labeled 2 ml microcentrifuge tube.
    a. Optional step: some bacteria, such as *Staphylococcus aureus*, produce heat-stable nucleases that are resistant to denaturation. The nuclease will be present in the phage precipitate and degrade the phage DNA once it is released from the phage capsid. In these cases, the nuclease can be degraded by addition of proteinase K. To each 500 µl aliquot of resuspended phage, add 10 µl of 0.5 M EDTA pH 8 and proteinase K to a final concentration of 100 µg/ml. Incubate at 50 ºC for 30 min. Allow the tube to cool to RT and continue to the next step.
9. Thoroughly resuspend the purification resin contained in the Promega Wizard kit (swirl gently, do not shake) and add 1 ml of resin to the phage suspension. Mix by inverting the tube 5-6 times.
10. Label a 1.5 ml microcentrifuge tube for each DNA prep you are extracting, and place into a tube rack with the lid open. Place a Wizard minicolumn into each tube.
11. Remove the plunger from a 3 ml syringe, attach the syringe barrel to the minicolumns in the tube rack, and leave them standing in the tube rack. Place the plunger on a clean paper towel on the bench.
12. Pipet the resin/lysate mix into the syringe. Holding the syringe over a waste beaker, insert the syringe barrel and push the resin into the minicolumn. Keep pressing until all the liquid has been forced through the resin. A slow flow rate usually means a good DNA yield.
13. Detach the minicolumn from the syringe and place it back into its microcentrifuge tube. Remove the plunger from the syringe, then reattach the syringe barrel to the minicolumn.
14. Wash the column by adding 2 ml of 80% isopropanol to the syringe. Holding the syringe over a waste beaker, insert the syringe barrel and push the isopropanol

through the minicolumn. Keep pressing until all the liquid has been forced through the resin.

15. Remove the syringe from the minicolumn and discard the syringe.
16. Cut the lid off of a new 1.5 ml microcentrifuge tube, label it and place the minicolumn into it. Centrifuge for 2 min at 13,000 x g, RT to dry the resin.
17. Cut the lid off of a new 1.5 ml microcentrifuge tube, label it and place the minicolumn into it. Place the tube + minicolumn into the microcentrifuge. Pipet 100 µl of water, preheated to 80 ºC, into the top of each column and immediately centrifuge at 13,000 x g, RT for 1 min to elute the DNA.
    a. Check the elutate volume after the spin. If it is much less than 100 µl, add another 100 µl of heated water and spin again.
18. Obtain a new 1.5 ml microcentrifuge tube and label it. Transfer the eluted DNA from step 17 into the new tube. Discard the minicolumn. Close the lid and store at -20 ºC.