



PREPARATION



ISOLATION



PURIFICATION



EXTRACTION



CHARACTERIZATION



SEQUENCING



ANNOTATION



PHAMERATION



FURTHER DISCOVERY

Harvesting, Concentration, and Dialysis of Phage

OBJECTIVE

Harvest and concentrate the amplified phage sample, and perform the necessary treatments, including ultracentrifugation, extraction of CsCl band, and desalting through dialysis, to prepare for DNA extraction.

APPROXIMATE TIME NEEDED

Harvesting & Treatment: **~30 minutes**
Precipitation: **Overnight**
Re-suspension: **~30 minutes**
Preparation for Ultracentrifugation: **~15 minutes**
Ultracentrifugation: **16 hours**
Phage Extraction: **~20 minutes**
Preparation for Dialysis: **~20 minutes**
Dialysis: **Overnight**

MATERIALS NEEDED

Equipment

- Microcentrifuge
- Ultracentrifuge
- Refractometer
- Pipettors, serological and micro-
- Camera for documentation
- Ring Stand and clamps

Consumables/Reagents (See online media preparation guides)

- 250 mL conical centrifuge tubes
- 250 mL flat bottom plastic bottles
- Flip-cap ("Eppendorf") and screw capped (O-Ring) microcentrifuge tubes
- 10 mL ultracentrifuge tubes with caps
- Polyethylene glycol (PEG)
- Sodium Chloride, solid (NaCl)
- Phage Buffer – 4 big bottles w/o CaCl_2 (2000 mL) + small bottle w/ CaCl_2 (100 mL)
- Cesium Chloride, solid (CsCl)
- Needles – ½ inch and low gauge
- Syringe
- Dialysis tubing (MWCO 15000), ~4"
- dH_2O
- Serological pipettes
- Appropriate micropipette tips

HELPFUL TIPS

- PEG (polyethylene glycol) may be harmful by inhalation, ingestion, or skin absorption. Avoid inhalation of powder. Wear appropriate gloves and safety glasses.

PROCEDURES

Harvest and Precipitation

1. After the buffer has been siphoned off into two 250 mL conical centrifuge tubes, centrifugation is required to remove agar and cell debris.
2. Centrifuge at 5500 x G for 10 minutes at 4°C to pellet the debris. Be sure to balance tubes and set the rotor code correctly. **Ask for help!** If the speed exceeds 5500 Gs, a different centrifuge tube is required.
3. Decant the supernatant into a 250 mL flat bottom plastic bottle (orange cap). Try to do this in one fluid motion, with the pellet up,

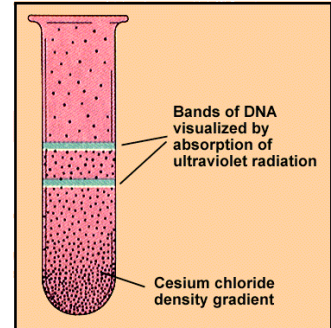


Figure 1: Illustration of Cesium Banding.

so to pour away from the pellet. Remove 10 mL and filter sterilize for a lysate* stock. Titer and keep this lysate at 4°C. Check the remaining volume and add NaCl to make 1M solution. Then add 10% of polyethylene glycol (PEG 8000©). Be sure to **add NaCl first!**

e.g. If there is 265 mL (0.265 L), then

add $0.265 \text{ L} \times 58 \text{ g/mole} \times 1 \text{ mole/L NaCl} = 15.4 \text{ g NaCl}$

add $265 \times 0.1 = 26.5 \text{ g PEG 8000}$.

4. Stir this at 4°C overnight or longer until the PEG and NaCl are in solution.
5. Titer the phage lysate. This is one of the archive stocks that must be submitted with the finished phage. Record the titer directly on the tube.

Phage Re-Suspension

6. The PEG has solvated the solution and pushed the phage out. The phage is now in the pellet.
7. In 2 balanced (by weight) conical centrifuge tubes (blue cap), centrifuge the phage suspension for 10 minutes 5500 x G at 4°C. Be sure to balance tubes and set the rotor code correctly. **Ask for help if needed!**
8. Decant the supernatant back into the orange-capped bottle for now; discard when this procedure is complete. Remove as much PEG from the pellets as possible before adding buffer. Decant quickly. Turn the tubes upside down to drain on a paper towel. Remove any residual PEG with a transfer (dropper) pipette.
9. Re-suspend the pellets in buffer and transfer them to a single 50 mL conical tube. To accomplish this, re-suspend each of the phage pellets in ~4 -6 mL phage buffer/CaCl₂. The pellet should be extricated as quickly as possible. Avoid any PEG on the side of the tube and remove the pellet before it is broken apart. This will minimize the amount of pellet fragments left stuck to the side of the tube. The total volume of both re-suspended pellets should be approximately 10 – 15 mL.

10. Homogenize (break up) the pellet in the buffer **gently**. The pellet should be broken apart but the phage particles should be left in tact. Place the sample on the rocker in the cold room and allow the sample to rock at 4°C for several hours (or overnight). This time can be extended if necessary. At this step, the phage diffuses back into the buffer/CaCl₂ solution and will stay in the supernatant after centrifugation. **Now the phage is in the supernatant!**
11. Centrifuge at 5500 G for 10 minutes at 4° C, save the clear supernatant into a 15 mL conical tube. Remember the phage is in the supernatant at this step.
12. As a second step, add 10 mL of buffer back on this pellet. Homogenize and rock for another overnight period in the cold room. The phage will yield another band, almost the size of the first band it produces. Label this tube as re-buffered and go back to the supernatant in step 10.

Phage Preparation for Ultracentrifugation

13. Back to the supernatant, add adequate amount of cesium chloride (CsCl) to the supernatant following the ratio of 8.5 g CsCl/10 mL supernatant. Mix gently by inverting the tube. As the sample warms to room temperature, the CsCl will solvate much more readily. The CsCl must be totally dissolved in order to do the next step.
14. Using refractometer adjust the density to 1.5 g/mL. Measure, adjust, and repeat until this benchmark is reached (see guidelines to use refractometer below). The desired density or refractive index, n_D , is between 1.3807 and 1.382. **Note:** if this step is skipped, a band will still be obtained, but it's location will not be predictable. If the density is adjusted to 1.5 g/mL, the band will appear in the same place each time. **This step can only be skipped if** the UC tube weighs between 20 and 21 grams. If it does not, proceeding will damage the ultracentrifuge. Do not proceed further.
15. Load and seal the sample into a 10 mL ultracentrifuge tube. Be sure to not put any air bubbles in the tube. In addition, heat seal the tube and make sure it does not leak.
16. Ultracentrifuge at 38,000 RPM for 16 hours. Calculate the speed X G depending on the centrifuge and rotor used.

Phage Extraction

17. Turn off the ultracentrifuge. Carefully remove tubes from the rotor and place them in a secure place. Take out the ultracentrifuge rotor and clean it with water and let it dry on the designated place. Use a wet paper and clean the ultracentrifuge as well. When dry, close the lid. When unsure **exactly** how to do so, **ask somebody who does**.
18. Put the tube in a ring stand firmly.
19. Photograph the band. Use a black background to better visualize the band; binders generally work well. Save the file and tape a copy of the photo in the notebook.



Figure 2: Example of a photograph documenting a CsCl band.

20. First, vent the tube so that as the extraction begins a vacuum is not generated. This is accomplished simply by inserting a short needle into the top of the tube. (The needle acts the vent. Bore size of the needle is not critical, but a length of ½ inches is recommended, since this way the extraction is not interfered by the vent.
21. Remove phage band with a syringe and needle. Insert the needle below the band (about 2 – 4 mm below), beveled edge facing up, close to the band, and slowly pull back the plunger. The needle may have to be moved around to vacuum up the band into the syringe. Use a low gauge needle for this extraction, the finer (higher number) the gauge, the smaller the bore size.
22. Expel the syringe contents (concentrated CsCl phage sample) into a screw capped microcentrifuge tube and label. Use the colored lidded tubes with an O-ring to seal samples.
23. A 100 µL sample of undialyzed phage is to be submitted to the Genome Center for the Archives. Each sample must be a screw capped (O-ring) microcentrifuge tube labeled with the researcher's name, phage name, CsCl, date (including year), and titer. Be sure to seal well. The sample will store at 4°C for a while. The remainder of the sample can be used freely for any experiment. While the phage sample must be dialyzed for any further experiments to proceed, phage stored in CsCl is most stable. Dialyzing 0.5 – 1.0 mL of the band sample is recommended, if such volume is available. This can vary depending on whether the phage concentration is high or low.

Dialyzing Phage Sample

24. Dialyze the 500 µL phage sample at 4°C with stirring overnight.
 - a. Cut a ~4 inch piece of dialysis tubing and put it into dH₂O for few minutes.
 - b. Fill a 1L beaker with phage buffer (2 big bottles) **without CaCl₂**. Insert a magnetic stir bar. Chill the beaker with buffer in the cold room for ~60 minutes.
 - c. Clip one end of dialysis tubing and fill tubing with phage sample (0.5 – 1.0 mL). Clip the other end of the tubing, leaving about ¼ inch room to spare. Place the sample in the beaker.
 - d. Slowly turn up the dial of the stir plate, allow the tube to spin slowly (minimal movement is just fine). Vigorously stirring may cause the tube to be trapped by the stir bar.
 - e. Allow the sample to be slowly spinning and continuously floating. Leave at 4°C overnight.
25. Remove the phage buffer and fill the beaker with fresh phage buffer, dialyze the sample for an additional 2 hours.
26. Transfer sample to a labeled screw-capped microcentrifuge tube.
27. Titer the phage sample (See **TOOLBOX: Titering**). The titer must be at least 10¹³ phages/mL. This sample can be used for DNA extraction (See **EXTRACTION: PCI DNA Extraction**).
28. An additional 100 µL sample of dialyzed phage is to be submitted to the Genome Center for the Archives. Each sample must be in a screw capped (O-ring) microcentrifuge tube labeled with the researcher's name, phage name, date (including year), and titer. Be sure to seal well.