CHISHOLM LAB, MIT (with modifications to this protocol by Lisa Crummett)

**Preparation of Cyanophage DNA from a Cleared Lysate\***

**Protocol Developers**

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**Citing Protocol**

Henn MR, Sullivan MB, Stange-Thomann N, Osburne MS, Berlin AM, Kelly L, Yandava C, Kodira C, Zeng Q, Weiand M, Sparrow T, Saif S, Giannoukos G, Young SK, Nusbaum C, Birren BW, Chisholm SW. (2010). Analysis of high-throughput sequencing and annotation strategies for phage genomes. PLoS One. Feb 5;5(2):e9083.

Yield : 100 ng – 1 μg DNA (\*protocol omits nuclease and proteinase K to minimize phage DNA degradation)

1. Spin 100 ml of phage lysate for 10 minutes at ~16,500xg to pellet any remaining cells and cell debris. Save supernatant (lysate) and discard the pellet.
2. Put 100 ml phage lysate into centrifuge tube or bottle.
3. Add 4 ml *Phage Precipitant.*
4. Incubate on ice for 1 hour.
5. Centrifuge at 10,000xg for 10 min, then decant and discard supernatant.
6. Resuspend pellet in 500 μl *Phage Buffer.*
7. Transfer supernatant to 2 ml Eppendorf tube.
8. Add 1 ml *Purification Resin* and mix by inverting tube.
9. Attach minicolumn to bottom of syringe and add contents of Eppendorf tube.
10. Use a plunger from a 3-ml syringe to push the slurry through the syringe (what comes through the syringe at this point is waste – DNA remains on the column).
11. Remove minicolumn from syringe and pull out plunger.
12. Reattach minicolumn and add 2 ml 80% isopropanol to syringe.
13. Use the plunger to wash the minicolumn, pushing through the isopropanol.
14. Remove minicolumn from syringe and place minicolumn back in first Eppendorf tube (2ml).
15. Centrifuge at 10,000xg for 2 min to remove any remaining liquid.
16. Place minicolumn in new (fully-labeled) 1.5 ml Eppendorf tube.
17. Add 100uL 80°C TE Buffer and immediately centrifuge at 10,000xg for 20 sec. to elute phage DNA.
18. Discard minicolumn and store purified DNA at 4oC or -80oC.

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**Materials used:**

**Phage Precipitant**

33% polyethylene glycol (PEG-8000) 3.3M NaCl (molecular weight: 58.44 g/mol)

*For 800 ml:* 267 g PEG-8000 154.3 g NaCl

H2O up to 800 ml

**Phage Buffer**

150mM NaCl 40mM Tris-HCl (pH 7.4) 10 mM MgSO4

*For 100 ml:*

**TE Buffer**

12.5 ml NaCl (1.2 M stock) 3.2 ml Tris-HCl (1.25 M stock) 1 ml MgSO4 (1 M stock) H2O up to 100 ml

10mM Tris-HCl (pH 7.5) 1mM EDTA

*For 20 ml:* 160 μl Tris-HCl (1.25 M stock) 40 μl EDTA (0.5 M stock)

**DNA Purification Resin (store in box, away from light)**

Promega product A7181 – 250 ml

**Minicolumns**

Promega product A7211 – 250 columns

**Modifications to Chisholm protocol (by Lisa Crummett):**

Note: For both centrifugation steps, I use a high speed Beckman centrifuge that has a swing bucket rotor. I feel that having a swing bucket rotor is especially useful since the host cell pellet is loose and can be fairly messy if it isn't localized at the bottom of the centrifuge tube.

**Step 3**: They add 4ml of phage precipitant per 100ml of lysate and their titers are ~1 x10^8 phage/ml. In the Promega protocol that comes with the reagents, they use 10x that amount (40ml of precipitant per 100ml of lysate but their coliphage titers are ~1 x 10^12 phage/ml. So based on these #s, I use 4ml of precipitant for my isolates that have a titer of ~ 1 x10^8 phage/ml but I use 12 ml of precipitant for my isolates that have a titer of ~ 1x 10^9 phage/ml.

**Step 5**: I do my centrifugation in 50ml tubes so I split up my lysate (minus host cells) into ~30ml volumes between 3 tubes. I don't decant the supernatant after centrifugation because there usually isn't a visible pellet. Rather, I pipet off the supernatant and leave about 1 -1.5 ml of liquid at the bottom of the tube.

**Step 6**: Since their isn't a visible pellet but rather ~ 1- 1.5 ml of liquid remaining in the tube, I add 1ml of phage buffer (not 500ul) and mix this with the remaining liquid by pipetting up and down.

**Step 7**: I transfer this phage liquid + buffer (~2- 2.5 ml) into a 15 ml falcon tube. This is done for each of the 3 tubes and the contents of the 3 tubes are combined into 1 15 ml falcon tube, yielding ~ 7 ml.

**Step 8**: I add 2-3ml of purification resin instead of 1ml because I end up with a much larger volume of phage liquid + buffer than the original protocol would yield.

**Step 10**: Sometimes I have to use two minicolums per isolate instead of one because with *high phage titer isolates*, halfway through filtering your 9ml of phage DNA + phage buffer + purification resin, the minicolumns get clogged up and impossible to push anymore fluid through. So if that is the case, I push the rest of the sample through a second minicolumn and elute from each minicolumn separately. You could pool these products into one tube or keep them separate. I keep them separate because I usually get plenty of DNA from either of the two single minicolumns and the DNA from each tube can always be combined and concentrated later.

I quantified my DNA yield with a **Qubit kit** and plate reader.