**dsDNA Phage Genome Extraction Protocol**

* **Required Reagents**
  + 100 mg/mL Lysozyme
  + 10 mg/mL Rnase A
  + DNase I (NEB, M0303S)
  + 10x DNase I Buffer
    - 100 mM Tris, pH 7.6
    - 25 mM MgCl2
    - 5 mM CaCl2
  + 10% SDS
  + 10 mg/mL Proteinase K
  + Phenol:Chloroform:Isoamyl Alcohol (25:24:1)
  + Chloroform
  + 100% EtOH
  + 3 M Sodium Acetate
  + 70% EtOH
* **Required Equipment**
  + Water bath set at 37 °C
  + Water bath set at 75 °C
  + Water bath set at 55 °C
  + Microcentrifuge

1. In a 2 mL microcentrifuge tube, add the following:
   * 783 μL of high-titer phage lysate (~1011 -- not purified by CsCl gradient with dialysis)
   * 9 μL of 100 mg/mL Lysozyme (1 mg/mL final).
   * 9 μL 10 mg/mL RNase A without EDTA (0.1 mg/mL final concentration).
   * 9 μL of NEB DNase I (2 U/μL per 100 μL reaction)
   * 90 μL of 10x DNase I Buffer.

**Note:** The lysozyme is added to lyse any host cells still present. The RNase and DNase are added to remove any remaining host-cell nucleic acids. EDTA is left out of the reaction to enable DNase to function properly. Steps 1-6 can be omitted for CsCl purified phage lysate. Rescale the volumes as necessary.

1. Mix by inverting the tubes several times or by pulse vortexing, and Incubate at 37 °C for 90 minutes.
2. Inactivate DNase I by incubating at 75 °C for 10 minutes.
3. Let the sample cool for 5 minutes.
4. Add 10 μL of 10 mg/mL Proteinase K (0.1 mg/mL final concentration) and 50 μL of 10% SDS (0.5% final), and mix by inversion.
5. Incubate at 55 °C for 1 hour.
6. Let the sample cool to room temperature.
7. Add 960 μL (an equal volume) of Phenol:Chloroform:Isoamyl Alcohol [25:24:1] , and mix by inverting the tube several times until the sample is cloudy.

**Note:** The phenol:chloroform:isoamyl alcohol will be in two phases in the bottle. You want to draw from the bottom layer as the top is for buffering purposes.

1. Centrifuge at 12,000 RPM for 10 minutes at room temperature using a microcentrifuge.
2. Transfer the aqueous layer to a new 2 mL tube (~750 μL is a good starting point). Be careful not to aspirate any of the organic layer.
3. Add an equal volume of chloroform and mix by inverting the tube several times. Using chloroform rather than phenol:chloroform:isoamyl alcohol a second time helps to remove phenol, which can cause a peak shift as seen in a spectrophotometer.

**Note:** In certain circumstances, you may find you need to do a second phenol:chloroform:isoamyl alcohol and/or chloroform step, but for this protocol, you should only need a single treatment of each.

1. Centrifuge at 12,000 RPM for 10 minutes at room temperature using a microcentrifuge.
2. Transfer the aqueous layer to a new 2.0 mL tube (~550 μL depending on how much was transferred from the step above).
3. Add two volumes of 100% Ethanol and then 1/10 volume of 3 M Sodium Acetate. Mix by inversion.
4. Incubate at -20 °C for a minimum of 1 hour.
5. Centrifuge at 14,000 RPM (or max speed) for 10 minutes at room temperature (4 °C is more ideal, but room temperature is sufficient) in a microcentrifuge.
6. Carefully decant the supernatant into a waste container.
7. Add 500 μL of 70% Ethanol, being careful not to disturb the pellet. Carefully invert the tube once or twice to wash the pellet.
8. Centrifuge at 14,000 RPM (or max speed) for 3 minutes at room temperature in a microcentrifuge.
9. Carefully decant the supernatant into a waste container. Pipette off any significant volume of ethanol that remains.
10. Air-dry the pellet for ~15 minutes or until no droplets of ethanol are visible. Do not over-dry the pellet, as it will make it difficult to dissolve it later.
11. Resuspend the pellet in 50-100 μL of DI-H2O (or a tris buffer) or a sufficient volume to ensure the DNA goes into solution.
12. [Optional] Incubate the tube at room temperature for 30 minutes (this incubation sometimes help the DNA pellet to go into solution if the pellet was over-dryed).
13. Check the DNA concentration on a spectrophotometer.
14. Store the DNA at -20 °C.