**Phage Genome Extraction Protocol**

* **Required Reagents**
	+ 10 mg/mL Rnase A
	+ 10 mg/mL DNase I
	+ 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)
	+ 100% EtOH
	+ 3 M Sodium Acetate
	+ 70% EtOH
* **Recommended 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 10 μL 10 mg/mL RNase A, 10 μL of 10 mg/mL DNase I, and 90 μL of 10x DNase I Buffer to 790 μL of high-titer phage lysate made from a 30 mL phage prep.
2. Incubate at 37 °C for 1 hour.
3. Inactivate DNase I by incubating at 75 °C for 10 minutes.
4. Let the sample cool for 5 minutes.
5. Add 10 μL of 10 mg/mL Proteinase K and 50 μL of 10% SDS.
6. Incubate at 55 °C for 1 hour.
7. Let the sample cool to room temperature.
8. Add an equal volume of Phenol:Chloroform:Isoamyl Alcohol [25:24:1] , and mix by inverting the tube several times until the sample is cloudy.
9. Centrifuge for 10 minutes at 12,000 RPM in a microcentrifuge.
10. Transfer the aqueous layer to a new 1.5 mL tube. Be careful not to aspirate any of the organic layer.
11. And an equal volume of chloroform, and mix by inverting the tube several times.
12. Centrifuge for 10 minutes at 12,000 RPM in a microcentrifuge.
13. Transfer the aqueous layer to a new 1.5 mL tube.
14. Repeat steps 11-13.
15. Add an equal volume of 100% Ethanol and then 1/10 volume of 3 M Sodium Acetate. Mix by inversion.
16. Incubate at -20 °C for 1 hour.
17. Centrifuge for 10 minutes at 15,000 RPM (or max speed) in a microcentrifuge.
18. Carefully decant the supernatant into a waste container.
19. Add 500 μL of 70% Ethanol, being careful not to disturb the pellet. Carefully invert the tube once or twice to wash the pellet.
20. Centrifuge for 5 minutes at 15,000 RPM (or max speed) in a microcentrifuge.
21. Carefully decant the supernatant into a waste container. Pipette off any significant volume of ethanol that remains.
22. 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.
23. Resuspend the pellet in 50 μL of DI-H2O (or a tris buffer).
24. Incubate at room temperature for ~30 minutes to ensure that the pellet is dissolved. Briefly vortex to mix.
25. Check the DNA concentration on a spectrophotometer.
26. Store the DNA at -20 °C.