 Protocol for the purification of viral particles from bacterial liquid culture

**MATERIALS**

- 50 ml Falcon tube
- 0.45 μm syringe filter & syringe
- SM buffer
- DNAse I, 10x DNAse buffer
- heatblock
- 20 % SDS
- Proteinase K
- phenol:chloroform:isoamyl
- phase lock gel light tubes
- TE buffer

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**Protocol status:** Working

We use this protocol and it's working

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1. Centrifuge 40 ml of bacterial culture infected with phage at 6000 g for 30 min, remove the supernatant and filter it through a 0.45 μm syringe filter.

2. Centrifuge the filtrate at 35 000 g for 4 h, remove the supernatant and re-suspend the pellet in 600 μl SM buffer.

3. Add 2 μl of DNase I and 20 μl of 10x DNase buffer and incubate at 37°C for 1.5 h.

4. Incubate sample at 65°C for 30 min to inactivate DNAse I.

5. Add 10 μl of 20 % SDS and 40 μl of Proteinase K (20 mg/ ml) and incubate at 37°C for 1 h.

6. After the incubation, mix the sample with an equal amount of phenol:chloroform:isoamyl pH 8.0 (25:24:1) alcohol in a phase lock gel light tubes and centrifuge at 12 000 g for 5 min.

7. Add 600 μl more of the phenol:chloroform:isoamyl alcohol to the tube and centrifuge at 12 000 g for 5 min.

8. Transfer the aqueous phase to a new tube and add 1200 μl cold 100% ethanol.
9 Incubate sample overnight at -80°C, and then centrifuge at 16 000 g for at 4°C for 1 h

10 Remove the supernatant and re-suspend the pellet in 100 μl TE buffer

11 Store at 4°C