



## Rapid Isolation of Bacteriophages: Steps Towards Phage Therapy

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### Introduction

With the advent of antimicrobial resistance worldwide, there is renewed interest in studying bacteriophages and using them as a possible source of controlling raging bacterial infections. This approach is known as phage therapy and is attracting renewed interest as a topic of study. Bacteriophages are viruses that infect bacteria. Bacteriophages, phages for short, are the most abundant organisms in the biosphere, approaching an estimate of  $\sim 10^{31}$  particles in total. They can be found in soil, wastewater and sewage. In this protocol phages can be isolated from virtually any source by using bacteria to fish them out. Initially waste water is collected from a point source then the phages are enriched using a suitable bacterial host strain such as *Escherichia coli* or indeed any microorganism that is found in sewage especially, such as *Staphylococcus aureus*, *Klebsiella* sp, *Campylobacter* sp or *Pseudomonas* sp. The phage is enriched by inoculating an overnight culture and growing it 37°C overnight with shaking.

The phage is then harvested in 1 ml aliquots by centrifugation at 12K rpm for 5 mins and the supernatant is passed through a 0.22µm filter into a sterile microfuge tube. Three drops of chloroform are then added to lyse any remaining bacteria and also help with long term storage of the phage. At this point the phage is titered by inoculating different amount of the phage with 1 ml of host bacterium and 3 ml of sterile top agarose (0.7 % agarose), mixed thoroughly by vortexing and plating onto fresh LB plates. This is incubated at 37°C overnight. On the following day, one should be able to observe plaques, which are zones of clearing due to lysis of the host bacterium. If the plaques are well separated, then the phages can be picked using a Pasteur pipette fitted with a bulb. The plaque is then deposited in 1 ml of sterile buffer containing 10 mM Tris (pH 8.0) and 1 mM EDTA (pH 8.0) also known as TE buffer (pH 8.0). The plaques are allowed to diffuse out of the agar plug and then stored with a drop of chloroform. This phage

is then used to inoculate another round of infection overnight so that a large quantity of phage can be harvested and purified. This is done by flooding the plate with 2 ml sterile TE (pH 8.0) and gentle rocking of the plate on a shaker. After an hour the solution is then harvested and clarified by centrifugation at 5 K rpm for 2 min. The supernatant is the phage prep that is stored with a drop of chloroform indefinitely.

The genomic DNA is isolated from phage using a lysis buffer (100 mM Tris:HC pH 8.0, 150 mM Sodium chloride, 60 mM EDTA and 1% SDS). To a 2 ml microfuge tube is added 800 µl of phage prep and 800 µl of Lysis buffer and mix by inversion, then add 20 µl of Proteinase K (20 mg/ml) is added incubated at 55°C for 30 min. The lysate is centrifuged at 10 K rpm for 10 min at room temperature. Transfer 800 µl of supernatant to two fresh 1.5 ml tubes and 80 µl of 3M Sodium acetate pH (5.2) is added to each tube and another equal volume of isopropanol (800 µl) is also added and mixed by inverting the tube 3 - 4 times. The mixture is centrifuge at 10 K for 10 min at RT and the supernatant is decanted and the tubes are well drained. The DNA pellet is washed with addition of 1 ml of ice-cold 70% ethanol and centrifuged at 10 K for 3 min. The supernatant is discarded and the pellet is dried in a Speedvac for 10 minute. The pellet is resuspended in 50 µl of TE buffer and use accordingly either for gel electrophoresis or down-stream processing.

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