

Protocol: Phage enrichment

The goal of this method is to enrich your sample for phages capable of infecting your desired host. This is done by removing the endogenous bacteria from your sample and adding it to bacterial culture media and a growing culture of your host, and then incubating it. If even a single phage capable of infecting the inoculated bacterial strain is present in the sample, it will replicate to levels which should be detectable by normal plating techniques.

The volumes used for enrichment can vary, from as little as 1 ml of sample to many liters. Increasing the enrichment volume increases the sensitivity of the procedure, so if you expect your phages to be rare in the sample, a larger enrichment volume is needed. The method below describes enrichments in a convenient volume for many labs: 50 ml in a 250 ml culture flask. This volume can be scaled up or down depending on your individual needs and capacity.

I. Fluid samples (water, sewage influent, etc.)

1. Centrifuge your sample at 8000 x g, 10 min.
2. Filter the clarified supernatant through a 0.45 or 0.22 μm filter to remove any endogenous bacteria. For small-scale samples this may be done with a syringe filter or Millipore "Steriflip" unit; larger samples can be filtered with vacuum-driven filter flasks or tangential flow units. Store at 4 °C.
 - a. Your sample is now sterile and must be handled aseptically from now on.
 - b. Note that filtered sewage may still contain human pathogenic viruses such as Hepatitis A or Norwalk virus; handle these samples accordingly.
3. Add 10 ml of filtered sample to 40 ml of sterile broth medium in a 250 ml culture flask, inoculate with 100 μl of a fresh overnight host culture. Incubate this enrichment culture under whatever conditions the host favors, usually overnight is sufficient for most bacteria; longer incubations may be required for slow-growing bacteria.
 - a. In order to enrich larger sample volumes in smaller culture volumes, the sample may be added to concentrated broth medium. For example, use 25 ml sample + 25 ml 2X broth, or 40 ml sample + 10 ml 5X broth.
 - b. The amount of host culture to add is not critical, but it should be enough that would normally produce a saturated culture over the period of the enrichment if it were inoculated into the same volume of unamended broth. Typically inoculations of 1:1,000 to 1:100 are used.

II. Solid samples (soil, sewage sludge, etc.)

1. Add your solid sample at a 1:4 (w/v) ratio to sterile broth medium (e.g., 50 g soil + 200 ml broth) in a beaker or centrifuge bottle. Shake or stir the resulting slurry for 1-2 hrs at RT.
2. Centrifuge the slurry at 8000 x g, 10 min. Filter-sterilize the supernatant as described in step I-2. Store at 4 °C.
 - a. The phages from the solid sample should have eluted into the broth medium.

- b. These supernatants are often difficult to filter-sterilize; samples may be spun harder (e.g., 12,000 x g, 10 min) or pre-filtered using a sterile paper filter (e.g., Whatman 597 ½ pre-folded filters).
3. Because the sample was processed using culture medium, the sample can be aliquoted into a culture flask and inoculated with host culture directly. Add 50 ml of filter-sterilized sample to a 250 ml culture flask, inoculate with host culture and incubate as described in step I-3.

III. Processing enrichment cultures

1. Remove 10 ml of the enrichment culture and place into a sterile 15 ml Falcon tube, centrifuge at 8,000 x g, 10 min. Filter sterilize the resulting supernatant through a 0.22 µm syringe filter, store at 4 °C.
 - a. Even if an enrichment culture appears turbid, it may still contain phage. Process all enrichment culture regardless of appearance following enrichment.
2. If you have several enrichments, it is usually a good idea to screen them for the presence of phage by simply spotting 10 – 20 µl of each undiluted enrichment to a lawn composed of the same host used for enrichment (see the *Plating out Phage* protocol). Those cultures that yield a clear zone under the spot probably contain phage and should be plated out and subcultured (see the *Subculturing Phage* protocol).