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Isolating bacteriophage from environmental samples

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Protocol status: Working

We use this protocol and it's working

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Abstract

There are two methods a quick and dirty method, and the technically right method that selects for lytic bacteriophage.



Quick and dirty isolation

2d 0h 5m

1 Collecting environmental samples

Collecting samples does not have to be in a sterile container. Often I will wash old water bottles or use Ziploc bags to collect samples.

The more sample collected, the better. You can shake to homogenate the sample

2 Incubating to grow phage

5mL broth (typically LB or TSA)

1mL bacterial strain (the strain you would like to isolate phage against)

1mL environmental sample

Incubation

overnight room temperature 24hrs

The incubation time and temperature will be dependent on bacterial type

1d



3 Filtration of phage from sample

Syringe filter (5-10mL syringe with luer lock) with 0.22µm filter (25mm diameter is fine)

Remove the plunger from the syringe

Attach 0.22µm filter

Dump or pipette incubated sample into syringe

Carefully push the plunger down. Do not let the plunger go backwards

If resistance is met, it is best to stop and change the filter. The filter can be removed and a new filter can be screwed on.

5m

4 Spot the samples on plates with bacterial strain

I will edit this to have the spot protocol linked in as soon as it's published.

1d



The technically right method for isolating phage

6d 0h 15m

5 *This protocol is usually to isolate predominantly lytic phage. This process usually selects for the predominant lytic phage that infects the host bacteria. This is also very close to the SEA-PHAGE protocol from the manual.*

6 Collecting environmental samples

Collecting samples does not have to be in a sterile container. Often I will wash old water bottles or use Ziploc bags to collect samples.

The more sample collected, the better. You can shake to homogenate the sample



7 Filter out the phage from an environmental sample

10m

Filtration through filter

Push desired amount of environmental sample through a 0.22µm filter

The more sample you use, the more organisms inside, as well as phage! Therefore, you can increase your chances of getting phage.

Filtration through centrifugation

Centrifuge the desired amount of environmental sample 5,000rpm 10 minutes 4°C

Pipette off the supernatant into the container you want to incubate the sample in

You can also do both centrifugation and filtration with a 0.22µm filter

8 Incubate to grow phage

5d

Try to add a significant amount of nutrient broth to the environmental sample.



25mL broth

10mL environmental sample

500-1,000µL bacterial strain (you can also use a bacterial strain cocktail such as various *E. coli* strains).

Incubation

Incubate at the temperature of the bacteria shaking for 3-5 days.

Check each day

Because phage kill bacteria, you won't actually be looking for cloudy growth, but for more transparent growth. You can wait the whole 5 days, or if you see transparent growth, you can pull the sample.

9 Filter the sample

5m

Filter your sample with a 0.22µm filter

You can filter the entire thing with a larger filter, or you can syringe filter a portion (~5-10mL) of the sample.

10 Spot or plaque assay

1d

I will edit this to have the spot or plaque assay protocol linked in as soon as it's published.

