PHAGEHUNTING PROGRAM

Making Serial Dilutions

OBJECTIVE
Create standard dilutions of a phage sample in order to perform plaque assays or titers.

BACKGROUND
Serial dilution is an important technique in many areas of experimental biology, and working with phages is no exception. Because phage stocks are often quite concentrated, it is necessary to accurately and precisely dilute them in order to titer phage or perform other experiments, such as lysogeny efficiency assays or host range tests.

APPROXIMATE TIME NEEDED
~ 15 – 20 minutes

MATERIALS NEEDED
A liquid sample of phage, such as a plaque pick or lysate
Microcentrifuge tubes, sterile
Phage Buffer w/ 1mM CaCl₂
Vortex
Appropriate pipettors with sterile tips

HELPFUL TIPS
• It is important to vortex well after each dilution to ensure that the subsequent dilutions will be accurate.
• Make sure to pipette accurately; air bubbles or sample left in the tip will throw off the series.
• This procedure outlines how to do 10-fold serial dilutions, however the volumes of buffer and phage may be modified in order to do 2-, 5-, 100-, or 1000-fold dilutions.
• Dilutions should keep at 4°C for a while, but experience has shown that it’s better to make them shortly before using them.

PROCEDURES

1. Aseptically aliquot 90 µL Phage Buffer w/ 1mM CaCl₂ into one microcentrifuge tube for each dilution that will be made. (For example, if making dilutions for a titer, prepare 10 tubes with 90 µL Phage Buffer w/ 1mM CaCl₂.)

2. Label the tubes with the dilution number.

3. Remove 10 µL of the “neat” (undiluted) phage sample and add it to the first dilution tube (labeled 10-1). Vortex for five seconds to mix.
4. Remove 10 µL with a new pipette tip from the now-mixed $10^{-1}$ tube and add to the $10^{-2}$ tube. Vortex for five seconds.

5. Repeat this process until the required number of dilutions has been made.