# iGEM - Lysogen Efficiency Testing

## Introduction

This protocol is used to determine the degree of lysogen of a temperate phage.  It was adapted from the SEA-PHAGES discovery program from the uOttawa TMM teaching laboratories.

## Materials

- Phage lysate
- *Staph epi* 71 Culture
- TGY plates
- Phage buffer

## Procedure

### Day 1

1. Prepare bench for aseptic work and assemble supplies.

2. Dilute the phage lysate to 1 x $10^9$ pfu/mL, in a final volume of 500 µL of phage buffer.

3. Seed plates with phage:

    a. Obtain 5 agar plates and label them with the name of the phage, the name of the host bacterium (*S. epi* 71), and one of the following dilutions $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, and $10^{-6}$.
    b. To each of these 5 plates, add 100 µL of your phage sample that has been diluted to 1 x $10^9$ pfu/mL.
    c. Spread the lysate evenly across the plate using sterile glass beads.

4. Prepare a dilution series of the host bacteria:

    a. Arrange 5 microcentrifuge tubes in a rack and label them $10^{-1}$, $10^{-2}$,..., $10^{-6}$.
    b. Fill each microcentrifuge tube with 450 µL of phage buffer.
    c. Add 50 µL of your undiluted host bacteria culture to the tube labelled "$10^{-1}$" and mix by pipetting up and down, and vortexing.
    d. Using a new clean pipette tip, transfer 50 µL of the "$10^{-1}$" sample to the "$10^{-2}$" tube and mix well.
    e. Continue each successive dilution until you get to your last tube.

5. Spread host bacteria on control plates:

    a. Obtain 5 new agar plates and label them with the name of the host bacterium (*S. epi* 71), and one of the following dilutions $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, and $10^{-6}$. Note: these are your control plates.
    b. To each of the 5 new agar plates, add 100 µL of host bacteria at corresponding dilution. Ex; add 100 µL of host bacteria at the $10^{-2}$ dilution onto the plate labelled with that dilution.

c. Spread the culture evenly across the plate, using sterile beads.

6. Spread host bacteria on phage-seeded plates:

   a. Obtain the 5 agar plates that have been already seeded with pahge.
   b. To each of these 5 plates, add 100 µL of host bacteria at corresponding dilution. Ex; add 100 µL of host bacteria at the $10^{-2}$ dilution onto the plate labelled with that dilution.
   c. Spread the culture evenly across the plate, using sterile beads.

7. Incubate the plates at 34$^{o}$C until colonies form. For *S. epi*, should take 1-2 nights.

## Day 2-3

8. Check plates each day for colony formation.

   Colonies that form on the phage-seeded plates are mostly lysogens, though a small fraction of host bacteria may contain a mutation that renders them insensitive to infection by your phage and can also form colonies on the phage-seeded plates.

9. Once colonies are clearly visible, photograph all plates.

10. Calculate the rate of lysogeny, also known as the Efficiency of Lysogeny (EOL).

    EOL = [(CFU on a phage-seeded plate) / (CFU on the corresponding control plate)] x 100
    Note: CFU = colony forming units. The best plates to use for counting colonies contain 20-500 colonies. The efficiency of lysogeny for a temperate phage can range from 0.1% to 100%.