# The Novel Repression System of Cluster M Mycobacteriophages

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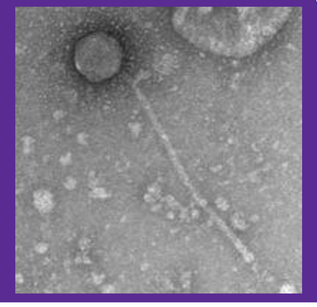

## ABSTRACT

Mycobacteriophages, or phages, are viruses that infect *Mycobacterial* hosts, such as *Mycobacterium abscessus*. They are genetically diverse, categorized into clusters, and have one of two known replication cycles: lytic or lysogenic. A lytic replication cycle is characterized by the phage attaching to the cell wall of its bacterial host and injecting its genetic material (DNA) into the cell. The viral DNA then hijacks the natural transcription mechanism of the host to produce more viral particles. Once particles are packaged into virions, they lyse the cell to escape and go on to repeat this cycle. Temperate phages, such as the cluster M studied here, are capable of lytic and lysogenic replication. During lysogeny, the injected viral DNA will integrate into the bacterial chromosome, activate a repression system, and go dormant, replicating with the bacteria's natural replication process. Known repression systems, such as those of the cluster A phages, often have one gene responsible for binding DNA to stop viral transcription. Thus far, research on cluster M phages has failed to produce a single gene responsible for repression. The research efforts of the Gainey Laboratory focus on investigating a noncanonical system in which repression is caused by the interaction of at least two genes. In this case, IPhane7 (M1) gene products 2 (a protein of unknown function) and 72 (a WhiB-like family transcription factor) are being experimentally tested for repression activity via an efficiency of lysogeny assay. It is believed that gene 2 acts as a noncanonical repressor by binding gene 72, causing a conformational change that prevents normal gene transcription. Therefore, expression of gene 72 alone should increase lytic replication, and in turn *decrease* the efficiency of lysogeny, whereas expression of gene 2 should *increase* the efficiency of lysogeny.

## GOALS / OBJECTIVES

- To clone gp 2 and its intergenic region (an active promoter) and gp 72 in front of the intergenic region of gp 2 each in a pMH94 plasmid with hygromycin and a pMH94 plasmid with Kanamycin resistance.
- To then electroporate the above plasmids into *M. smegmatis* $mc^2155$ cells, along with a control plasmids not containing genes.
- To perform an efficiency of lysogeny (EOL) assay on just IPhane7 virus to determine its baseline.
- To perform an EOL assay to determine how each gene in their respective plasmid affects IPhane7 baseline EOL and assess whether repression is taking place.

## METHODS

### IPhane7 Virus Stock Growth and Titering

- Serial dilution of IPhane7 virus stock in phage buffer $10^0$ to $10^{-4}$
- Each dilution of the virus was then used to infect *M. smegmatis* cells
- Virus and cells were mixed with top agar and plated on 7H10 agar plates and incubated overnight
- Plates with apparent webbing were flooded with ~6mL of phage buffer and filtered (0.22uM) to generate a virus stock.
- Top agar containing *M. smegmatis* added to 7H11 agar plates and virus diluted from $10^0$ to $10^{-8}$ was spotted on to determine the virus titer.
- Process repeated until a concentration of at least $10^{10}$ pfu/mL was achieved
- Pilot EOL assay was then performed

### Plasmid Assembly & Electroporation

- Hifi assembly will be used to build plasmids, one containing gp 2 (Figure 1), one containing gp 72.
- Transformation into *E. coli*
- Plasmid recovery via NEB Monarch Plasmid Miniprep Kit
- Plasmid confirmation via Sanger sequencing

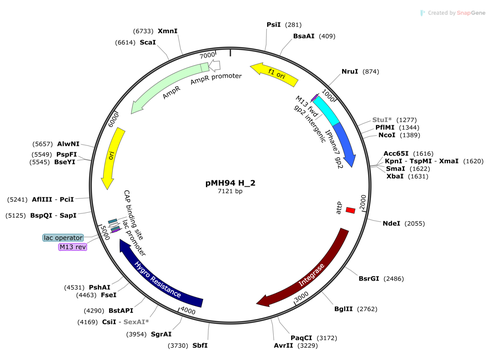


**Figure 1.** Map of the pMH94 plasmid containing hygromycin resistance, IPhane7 gene 2 and its intergenic region which contains an active promoter. *Image created by SnapGene Viewer Version 7.2.1.*

- Electroporation into *M. smegmatis* cells with 4 days of incubation
- Colonies picked and prepared for liquid culture
- Preliminary EOL experiment with plasmid-containing cells

## RESULTS

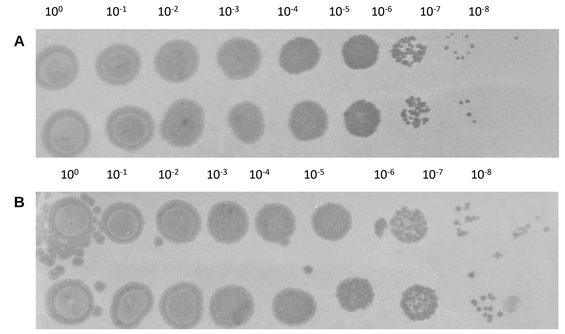


**Figure 2. A.** Viral titer performed on a flooded $10^{-3}$ plate of IPhane7. **B.** Viral titer performed on a flooded $10^{-4}$ plate of IPhane7. Concentration was calculated for each flood by counting the pfu/mL and multiplying by the dilution factor. Floods were then combined and concentrations averaged together to form 4.75 x$10^{10}$ pfu/mL of pure virus.

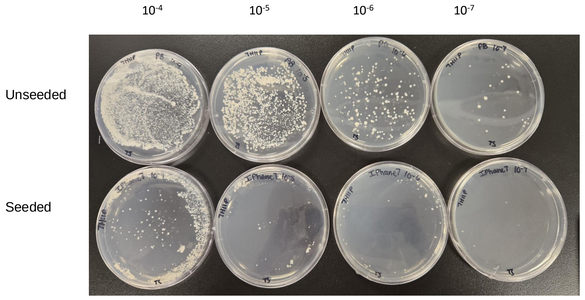


**Figure 3.** Results of the IPhane7 efficiency of lysogeny experiment. Unseeded plates contained 100 µL of phage buffer, seeded plates contained 100 µL of IPhane7 virus, and all plates had 100 µL of *M. smegmatis* cells spread on top of 7H11 agar plates. After 3 days of incubation, EOL was calculated by counting colony-forming units on the $10^{-7}$ plates, dividing the number of colonies on the seeded plates by the number of colonies on the unseeded plates, and multiplying by 100. The baseline EOL for IPhane7 was calculated at 15%.

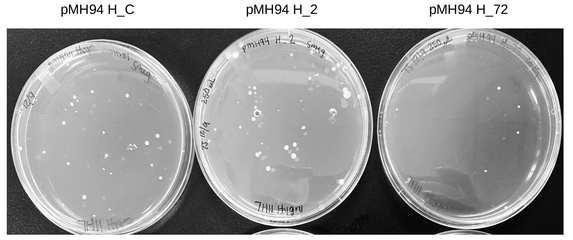


**Figure 4.** The results of electroporation of select plasmids into *M. smegmatis* cells. Growth was observed on all plates indicating a successful electroporation.

## CONCLUSIONS AND RECOMMENDATIONS

- The EOL of IPhane7 is 15% when plates are seeded using a virus concentration of $10^{10}$ pfu/mL.
  - This will need to be repeated in triplicate to confirm this percentage.
- Cloning of gp 72 into pMH94 hygro was unsuccessful as determined by sanger sequencing.
  - Cloning will be repeated with new primers and confirmed with Sanger sequencing before repeating transformation and electroporation.
- Once all plasmids have been confirmed and introduced to *M. smegmatis* cells an EOL experiment will be conducted to determine whether gp 2 increases the EOL, confirming its repression abilities.
  - This assay will be conducted the same as the baseline EOL experiment, but the *M. smegmatis* cells will have our select plasmids.

## References

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