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2024 SEA Faculty Meeting Abstract

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Double-flooding and frogging: improvements in the delivery of PHAGES and GENES at the University of Ottawa

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The SEA-PHAGES program has been running at the University of Ottawa (in Ottawa, Canada) since 2019 (cohort 12), and expanded to include the GENES program in 2023. While the SEA-PHAGES program was initially run with a smaller group of students, it is now a required laboratory class taken by 80 students each year. The 80 students are divided in five sections that have phage hunted on a variety of different hosts: *Arthrobacter globiformis* 2979 and 2880, *Arthrobacter sulfureus*, *Mycobacterium smegmatis*, *Streptomyces avermitilis* and *Streptomyces coelicolor*.

Over the years, we have developed protocols to supplement the Phage Discovery Guide. Of note are two modifications that we believe have improved the delivery of and preparation for both PHAGES and GENES.

Double-flooding refers to flooding a webbed plate once, collecting the lysate, and then flooding the same webbed plate a second time. This modified flooding procedure typically yields lysate with similar titre from both the first and second flood and has halved the number of plates required when students are amplifying phages. Although we have observed phage-specific variations, we adopted this uniformly in our course in 2023. We are currently testing if webbed plates made with only top agar can yield lysates with similar titres as double agar plates. We have also tested a method in which the top agar is scraped off webbed plates and found it can improve titres by ~10-fold.

In GENES, we use sa multi-pronged tool, referred to as a “frogger”, to rapidly spot (or “frog”) transformed *M. smegmatis* onto aTc plates to investigate gene cytotoxicity or *E. coli* onto selection plates for rapid screening of bacterial two-hybrid assay hits. While slightly more challenging, the frogger can also be used to spot dilutions of several phages onto double agar plates to assay immunity or host range. Although this method is not as precise as individually spotting bacteria or phages, it removes a significant time constraint in screening.