

General Growth and Phage Isolation Considerations for Additional Hosts

As you pilot new Actinobacteria, we recommend that you begin by utilizing protocols and techniques developed for *M. smegmatis* mc²155, which are available to you in the SEA-PHAGES Resource Guide or on phagesDB, with these following *key exceptions*:

- Begin by growing new Actinobacteria *in PYCa media, and at 30°C*. The protocol for preparing PYCa media can be found at phagesDB. Once you are proficient in working with the new host, you may test alternative growth media and temperatures.

Other notes to consider:

- Most of these Actinobacteria are slower growers than *M. smegmatis* mc²155. Incubation time vary by strain.
- With incubation at 30°C, the growth time is longer than *M. smegmatis* mc²155.
- When growing the bacteria, clumping can be an issue, so Tween can be added in the *initial* culture inoculated from a single colony. Tween must be omitted when growing cultures to plate for phage as Tween can interfere with phage infection.
- Consider your source of phage. Bacteria from these genera are readily found in soil as well as other environments such as sewage and in waterways, hence it is likely that phages are also present.
- For enrichment, consider varying the amounts of media, bacteria, phage sample (e.g. soil or sewage) and incubation time. For example, the enrichment sample could be incubated for > 3 days.
- Consider including a spot test, in addition to direct plating, with your enriched phage sample. For this, use a similar dilution set of 10⁰ – 10⁻⁴ of your enriched phage sample as you would for direct plating. The lower dilutions may yield individual plaques in the spot test that will allow you to start the purification process more readily.
- All enriched samples should be autoclaved prior to disposal.
- Many of these Actinobacteria are pigmented, ranging in color from yellow to orange.