



PREPARATION



ISOLATION



PURIFICATION



AMPLIFICATION



EXTRACTION



CHARACTERIZATION



SEQUENCING



ANNOTATION



PHAMERATION



FURTHER DISCOVERY

Growing *Mycobacterium smegmatis* mc² 155

OBJECTIVE

To grow and maintain a plate stock of *M. smegmatis* mc²155, obtain a smooth starter culture for amplification using Tween®80, and grow bacteria for experiments.

BACKGROUND

Mycobacterium smegmatis, "*smeg*," is used as the host bacterium. It is a non-pathogenic, common soil organism that forms colonies on an agar plate in around 4 days. Because it is resistant to the antibiotic carbenicillin (CB), CB is added to the growing medium to kill other bacteria. To inhibit molds and yeasts growth, cycloheximide (CHX) is also added. In liquid culture, Tween®80 is added to the initial cultures to minimize clumps. It is excluded from subsequent subcultures, however, because it may inhibit phage infection.

smeg has a wide temperature range, but is typically grown in baffled flasks at 37°C on a shaker, which allows aeration of the culture. The bacteria can also be grown at room temperature using different methods, such as with a stir bar and stir plate. While these methods may take longer, they still give the same end results. A saturated *smeg* culture is tan-white, and smells like dirt.

APPROXIMATE TIME NEEDED

37°C Incubation

Plate Culture: ~3 days
Tween®80 Culture: ~24hr
Liquid Culture: ~3 days*

Room Temperature Incubation

Plate Culture: ~1 week
Tween®80 Culture: ~3 days
Liquid Culture: ~3 days

MATERIALS NEEDED

Equipment

- 37°C incubator
- 37°C shaker OR room temperature stir plate
- Pipettors, micro- and serological
- Sterile, capped test tubes (>10mL)
- Sterile, capped Erlenmeyer flasks (>25mL with or without stir bar – read below)

Consumables/Reagents (See online media preparation guides)

- Agar Plates
- 7H9 (90 mL/100 mL culture)
- ADC (10 mL/100 mL culture)
- 0.1M CaCl₂ solution (1 mL/100 mL culture)
- CB solution (100 µL/100 mL culture)
- CHX solution (100 µL/100 mL culture)
- Tween®80 solution (250 µL/100 mL culture)
- Appropriate micropipette tips
- Inoculating loop or sterile equivalent
- Serological pipettes

HELPFUL TIPS

- *If culture agitation ceases before it has reached stationary phase, biofilms may form. Biofilm formation will lead to clumping *smeg* in bacterial lawns.
- Mycobacteria are named for their mycolic acids—long chain fatty acids branching from their cell walls that give them a waxy coating. If given the chance, the cells will stick together and form small clumps that appear as pellicles. Overtime, large clumps form and the culture will not be usable for plating. Tween®80 (a detergent and nutrient source) is used to minimize the clumping.
- Streak for isolation from the first culture received. This is done to maintain a stable, retrievable culture in the lab. Use single isolated colonies for amplifying *smeg*.
- Starter liquid cultures propagate the dry, wrinkled colonies of *smeg* into a smooth, milky, non-clumping liquid culture. Addition of Tween®80 is the most successful way to do this.
- *smeg* liquid cultures are the working cultures used for isolation, purification, and amplification of mycobacteriophage.
- Excellent aseptic technique is necessary for continued success.
- Learning the smell, color, and surface appearance of *smeg* is essential for success in growing *smeg*. Cultures of *smeg* are very stable and can be maintained for an extended period of time.

PROCEDURES

1. In order to grow *M. smegmatis* mc²155 on a plate, streak for isolation using a small inoculum from the liquid culture received. You will always want to start your culture from a single clone. Use best practices of aseptic technique.
 - a. Streak for isolation using standard protocol, one of which is the [T-streak](#) method.
 - b. Incubate for at least three days at 37°C until several isolated colonies have grown before picking. They should be light tan colored with a waxy consistency. Alternatively, this step can be performed at room temperature, though it will take more time for colonies to grow. You may need to parafilm the plates as you incubate to deter loss of moisture.

- c. Parafilm plates and store at 4°C for continued use.
2. Prepare initial liquid cultures from plate stocks (7H9 WITH Tween®80).
 - a. As mentioned above, *smeg* tends to clump when grown in liquid culture. To combat this, initial stocks grown from plates should be grown in growth media plus Tween®80. This stock is considered to be the “First Generation” of growth, and should be used to make working stocks of *smeg*.

- b. The recipe is as follows:

To 1 bottle 7H9 (90 mL), add:

10 mL ADC

1 mL 0.1M CaCl₂

100 µL CB

100 µL CHX

250 µL Tween®80

This mixture should be stored at 4°C.

- c. If access to a shaking incubator is available, aliquot 2 – 5 mL of the above medium into a sterile test tube and inoculate with a touch of a colony. Incubate this shaking at 37°C overnight.
 - d. If access to a shaking incubator is not available, this step can also be performed by aliquotting 5 mL of the media into a 25 mL non-baffled sterile Erlenmeyer flask with a stir bar, then inoculating with a touch of a colony, and stirring at room temperature over a longer period of time (>2 days). This culture can be saved at 4°C for use in multiple subcultures.
3. Prepare *smeg* culture for phage infections (follow above recipe without Tween®80).
 - a. When growing *smeg* for general plating, omit Tween® to optimize phage infection. To prepare this growth media, simply follow the above recipe, but do not add Tween®80. Any amount between 5 – 500 mL can be grown at a time.
 - b. Obtain a baffled Erlenmeyer flask that is **5 times the volume** of the culture to be prepared. For example, use a 25 mL flask for a 5 mL culture.
 - c. This culture is inoculated with 10 – 100 µL of culture from the original Tween®80 stock. The culture can be incubated shaking at 37°C overnight, or using a stir bar and stir plate, as described in step #2c and #2d.