

## Slug Racing Using Spores

### Preparation

1. 24 hours before starting the tower, inoculate SM broth with 2 loopfuls of KA in 1L flasks. 600 ml of broth per flasks will ensure that the KA will have oxygen.
2. The morning of the tower experiment, pour the overnight KA into 250ml centrifuge tubes.
3. Centrifuge for 10 minutes at 10,000 rpm, then pour off the supernatant.
4. Scrape the pellet into a sterilized 250ml bottle. Any remaining KA can be removed by adding 1ml of KK2 to the centrifuge tube, vortexing to mix, and then added to the bottle.
5. Add enough KK2 to the bottle so that the KA is still concentrated but is pipettable (usually 8-15ml). Vortex to thoroughly mix the KA and KK2.
6. Place starving plates under the hood with the lids ajar to dry for at least thirty minutes.
7. Once plates are dry, use a sharpie to mark off the area for the concentrated KA on the bottom of the plate.
8. Spread 50 $\mu$ l of the KA in the area, making sure that the liquid does not seep around the edges of the plate or from the designated area.

9. Allow the plates to dry undisturbed for 15-20 minutes.

### Spore Count

1. Plate out spores from frozen stock (refer to “Plating from Frozen Spore Stocks” in the protocol notebook).
2. Collect spores using sterile loop (collect approximately 3 loopfulls where the loop is very full of liquid), and suspend in 300 micro liters of SM broth in a 1.5 ml Eppendorf tube.
3. Make a 100X spore dilution (10ul of spore sol + 990ul of KK2). Count spores using a hemocytometer. Count at least 100 spores. Note the number of spores counted and number of squares counted.
4. Calculate the number of spores/ml using the following formula:  
$$10,000 \times (25 / \# \text{ of squares counted}) \times \# \text{ spores} \times \text{dilution factor}$$

### Tower Set-up

1. Pipette the amount of spores wanted ( $5 \times 10^6$  spores is recommended) in the middle of the bacteria and spread by slowly shifting the plate back and forth until the area is covered.
2. Place an empty Petri plate down and cover with a black circle. Then randomly stack the experimental plates on top of each other with a black circle in between each one. Write down the order of the plates for your records. Make sure that the plates are completely lined up with the bacteria line farthest from you.
3. Wrap the plates with the tower cylinder, making sure that the pin holes are opposite of the bacteria line.

4. Cover the top with aluminum foil and tape to prevent light from entering.
5. Allow to develop for approximately two weeks.

#### Taking down Tower

1. Count fruiting bodies using a grid (see attached)
2. Separate the plate into equal halves by cutting the plate with an exacto knife.
3. Sterilize a modified spatula, and cool in EDTA-KK2. Use the flat end to scrape together fruiting bodies.
4. Collect the fruiting bodies into cap of 1.5 ml eppendorf tubes. Make one tube per side.
5. Wash each side with 750 micro liters of EDTA-KK2 and scrape remaining fruiting bodies into the liquid. Pipette liquid from the plates to the eppendorf tubes.
6. Add another 750ul of EDTA-KK2 and repeat 5.
7. Close tubes and vortex to suspend spores in liquid. Spores are very hardy and should be vortexed very thoroughly to break up clumps and ensure an accurate count.
8. Count spores using hemocytometer.