Mix Experiment Protocol

Spore Count

1. Plate out spores from frozen stock (refer to "Plating from Frozen Spore Stocks").
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 x (25/ # of squares) x # spores x dilution factor
5. For each clone you wish to obtain cells from, you will now prepare multiple growing plates that have different numbers of spores added. The logic is that these will develop at different times so that you can always catch one set of plates at the correct stage. Therefore, plate out spores with 300 micro liters of Ka in different dilutions. For example, you can use: 3.0 x 10^6 spores/plate, 1.0 x 10^6 spores/plate, and 3.0 x 10^5 spores/plate.
6. Allow plates to develop for approximately 48 hours. The exact number will vary depending on the clones you are using.

Cell Count

7. When the plate with the most spores starts to aggregate (10^6), you should harvest cells from the plate that is one dilution down (3x10^5). *Dictyostelium* cells are brownish, not white like bacteria. If plates are still white, not many cells are present.

8. Time is very important once the cells are removed from the plate. The cells will get sick if left in liquid for too long, which may affect results. SO BEFORE YOU HARVEST THE CELLS:
    a) Prepare starving plates: Place 1.5% agarose in KK2. Microwave in 20 second intervals swirling flask between microwaving. **Exercise caution as solution may boil over when disturbed.** Plates are 10 ml each. LABEL PLATES
    b) Obtain and label tubes you will use. Add 990ul of KK2 to eppendorf tubes for cell counting dilution.

9. Scrape plates using slanted end of white scraper. Make sure scraper is sterilized by rinsing with ethanol and allowing to air-dry. Scrape into 50 ml Falcon centrifuge tubes.
10. Add 10 ml of KK2 to the tube and shake/vortex as little as possible to break up the cells (vortexing kills cells).
11. Top off tubes to 45 ml KK2 and centrifuge at 1000 RPM's for 3 mins.
12. Pour of supernatant and shake upside down gently to remove excess liquid. Be careful not to lose pellet.

13. Repeat steps 11 and 12.
14. Resuspend pellet in 600 micro liters of KK2.
15. Count cells using a 100-fold dilution. Do at least two hemocyctometers per clone. While you are doing this, swirl the centrifuge tubes with your cells occasionally to prevent cells from becoming anaerobic.
16. Calculate the number of cells/ml using the following formula:

    10,000 x (25/ # of squares) x # cells x dilution factor

17. Dilute cell solutions to 1.0 x 10^8 cells/ml or to desired concentration.
18. Combine equal volumes of pure clone cell solutions to create the 50:50 mix solution. Be sure to briefly vortex the cell solutions before pipetting to ensure accurate sampling of the solutions.
19. Plate out 200 micro liters of the pure clone solutions/pure clone plate, and 200 micro liters of the mix solution/mix plates onto the starving plates. Make multiple plates for both the pure and mix solutions.
20. Clones should fruit in approximately 24 hours.

Spore Count

21. Sterilize a modified spatula, and cool in EDTA-KK2. Use the flat end to scrape together fruiting bodies.
22. Collect the fruiting bodies into cap of 1.5 ml eppendorf tubes. Make one tube per starving plate.
23. Wash each starving plate with 750 micro liters of EDTA-KK2 and scrape remaining fruiting bodies into the liquid. Pipette liquid from the plates to the eppendorf tubes.
24. Add another 750ul of EDTA-KK2 and repeat 23.
25. 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.
26. Count spores using hemocytometer.