

## CELL CULTURE PROTOCOLS

### THAWING DICTYOSTELIUM CELLS:

1. In a 10 cm plate, put 10 mL of HL-5 + P/S.
2. Take 500  $\mu$ L of media from dish and put into tube of frozen cells.
3. Pipet cells up and down before transferring cells to dish quickly. Minimize time cells are at room temperature with DMSO by changing media as soon as they attach.
4. Thaw a small volume with the same media and place them at the corners of the plate.
5. Change media to HL-5 +P/S + 10  $\mu$ g/mL G418 (Gentomycin).
6. Add 200  $\mu$ L uracil (SOX) per plate.
7. Incubate at 18°C until confluency (1-4 days).

\*Cells should grow in 2 days-2 weeks.

### FREEZING DICTYOSTELIUM CELLS:

1. Harvest cells at  $2 \times 10^6$ - $4 \times 10^6$  cells/mL in fresh HL-5. (10 mL plate was confluent so take out the media and add 5 mL new media)
2. Resuspend the cells by pipeting back and forth.
3. Aliquot 1 mL of each suspension in 1.5 mL eppendorf tubes on ice.  
Add 110  $\mu$ L DMSO\*. Mix and keep on ice for one hour.
4. Incubate tubes immediately into -20°C freezer for 2 hours.
5. Transfer to -70°C freezer taking care to keep tubes cold.

\*DMSO should be at room temperature, fresh and only stored in glass.

### DEVELOPMENTAL PLATES:

1. Spin down around  $1 \times 10^8$  cells (about a confluent 10 cm plate). (Spins should be done at 1500xg for 10 mins).
2. Wash two times with 5 mLs of starvation buffer.
3. Resuspend in 500  $\mu$ L of starvation buffer.

4. In a 60 mm plate, place 2-3 pieces of Whatman filter paper.
5. Over this lay 1 piece of grid paper.
6. Wet the filter paper and grid paper thoroughly with starvation buffer but so that there is no excess liquid in the plate.
7. Spread cells evenly over grid paper. Place in a dark and humid place.
8. Harvest all material by scraping with a spatula.
9. To harvest just spores, gently brush the top of the fruiting bodies with a pipet tip or spatula.
10. Store spores in 100  $\mu\text{L}$  starvation buffer at  $-80^{\circ}\text{C}$ . One plate should give 2-4 tubes of spores.

#### PICKING DICTYOSTELIUM COLONIES FROM 10 CM PLATE:

1. Aspirate the media from the plate and use a marker to circle the colony.
2. Use 1  $\mu\text{L}$  of media to resuspend the colony.
3. Pass the putative clones into a 24-well plate filled with 500  $\mu\text{L}$  of HL-5 media.