Freezing *Dictyostelium* strains

In order to repeat and extend work done in the lab, it is important to have clones that are reliably pure and stable. The stores should be of single pure clones, and should be kept out as little as possible. This minimizes the amount of unintentional evolution happening in the lab.

Our *Dictyostelium* strains are stored at -80°C as spores suspended in 60% glycerol (v/v) in glass or plastic freezer vials. The glycerol makes it easier to remove materials from the vials without letting them thaw.

Preparing sterile freezer vials

Make a solution of 60% glycerol (v/v) in RO water. Put 1.0 ml glycerol solution into each freezer vial. Screw the vial caps halfway on. Autoclave the vials on slow exhaust. See the guide on the Dicty lab wall for appropriate autoclave times. After the vials have cooled, screw the tops on completely.

Freezing clones (2 methods)

The plates that you are freezing away should be free of contamination and have fully developed fruiting bodies. There are two methods of freezing away strains. The first method is most commonly used when the fruiting bodies are large enough or close enough to the lid of the petri dish to bang. Bang the plate upside down on the bench until a large amount of the spore mass has dropped on the lid. Wash the spores off the lid with about 4 ml KK2 buffer. Pipette the spore solution several times to ensure adequate spore number in the solution. Place approximately 1.5ml of the spore solution in the freezer vial. Make at least two vials for each strain.

The second method is used with species that do not have tall fruiting bodies, such as *D. rosarium*, so that banging would not work. A plastic scraper is used with the blunt, angled side down to divide the plate into half, making sure that the agar is not disturbed. Scrape up half of the plate and place it into a 1.5ml eppendorf tube already filled with 1.0ml KK2. Vortex the tube and then add another 0.5ml of KK2. Shake the tube and then pipette it into the prepared freezer vial. Repeat with the other half of the plate for a second vial.

When using the 1mL plastic vials, autoclave vials with 500µl glycerol solution. Fill the eppendorf tubes with 500µl KK2, add the spores (from half a plate), vortex, and pipette into the prepared freezer vials. Alternatively, you can scrape an entire plate, add the spores to 1 ml KK2, vortex, and pipette 500µL of the mixture into 2 prepared freezer vials.

Use a paper label to identify the vial. It should have the strain, species, strain location, your name, and date frozen. Wrap scotch tape around the label to keep it from falling off. Label the top of the vial, place it in your freezer box and store in the -80°C freezer.