

## Freezing *C. elegans* Strains

### **Reagents Needed:**

M9 (common stock)  
freezing solution

### **Procedure:**

*Make sure you begin with healthy, non-contaminated, non-starved worms of the correct phenotype.*

1. Pick 20 young adult worms onto each of two (or three) 10 cm plates that have been seeded with 1 mL OP50 (40 or 60 worms total).
  - a. If you are growing the worms at 15°C, they will be ready to freeze in 1 week.
  - b. If you are growing the worms at 20°C, they will be ready in about 4 days.
2. When the plates are ready to freeze, they should have:
  - a. Little or no food (*just* starved)
  - b. Plenty of L1s and L2s (these are what will survive)
  - c. Eggs on the plate (means that the plate has not been without food for long)
  - d. Worms with the correct phenotype
  - e. No contamination (if contaminated, the worms will continue to eat and grow so there will be no stalling at the L1 stage)
3. Add about 5 mL of M9 to each plate.
4. Give each plate a swirl to loosen worms still stuck to the agar and then tilt plates on their lids so the liquid drains to one side of the plate.
5. Using a glass pipette, collect the liquid (worms and M9) in a 15 mL conical tube (about 10 mL total).
6. Pellet the worms for about 1 minute at full speed in a clinical centrifuge.
7. Aspirate as much of the supernatant as possible without disturbing the pellet.
8. Add about 15mL of M9 and resuspend the pellet.
9. Again, spin for about 1 minute at full speed. Repeat steps 7 and 8 if desired.
10. Aspirate all but about 3 mL (or 4.5 mL when using three plates) of M9.
11. Add an equal amount of freezing solution.
12. Briefly agitate the vial to suspend the worms and aliquot the worm suspension into 6 cryovials (9 cryovials with three plates) clearly labeled with the strain name, your initials and the date.
13. The styrofoam container that 15 mL conical tubes comes in is useful for freezing worms. The insulation provides the slow decrease in temperature required for survival. Place the cryovials into the styrofoam, and cover with another. Secure with tape or rubber bands and label the outside for future reference.
14. Store in your -70°C freezer space.
15. Test thaw the worms about 1 month later to ensure a successful freeze. A good freeze is when *at least* more than 10 worms survive. Pick several survivors on to a fresh plate to make sure they can produce progeny of the correct phenotype. Be sure to maintain the line while waiting for the results of the test thaw.

## Recipes:

### M9 (1L)

\* Common lab stock in worm room.

5.8g Na<sub>2</sub>HPO<sub>4</sub>•7H<sub>2</sub>O

3.0g KH<sub>2</sub>PO<sub>4</sub>

5.0g NaCl

0.25g MgSO<sub>4</sub>•7H<sub>2</sub>O

ddH<sub>2</sub>O to 1L

• Filter (0.22μm) and bottle.

### Freezing Solution (1L)

\* Usually need to make your own.

5.8g NaCl

50mL 1M KH<sub>2</sub>PO<sub>4</sub> (pH 6.0)

240mL glycerol

710mL ddH<sub>2</sub>O

• Bottle and autoclave.

• Add 30μL 1M MgSO<sub>4</sub> per 100mL of solution.

## Notes on Thawing

- It is fine to pour all of the freezing liquid from a vial onto a plate when you are checking for survival.
- If you are thawing a strain for actual use, you cannot do this. You must thaw the vial and let the worms settle to the bottom. With a pipette, suck up the worms at the bottom of the vial getting *as little liquid as possible*. Distribute the worms around the OP50 on a 6- or 10cm plate. You CANNOT centrifuge the worms, because the glycerol will not allow a pellet to form.