Soft Agar Freezing of *C. elegans*

Robyn Tanny, June 2020

Freezing:

1. Microwave soft agar until fully melted (no chunks!). Confirm that agar is not contaminated —sometimes fuzz balls of mold are not visible until agar has melted.
    - Make sure the cap is loosened
    - Be careful when microwaving: the agar at the bottom can melt and boil before the rest of the agar. This can lead to explosions in the microwave! I heat for 10-15 seconds, swirl the bottle, heat again, etc.

2. Place agar in 50˚C water bath for 1 hour or more. Be sure agar has equilibrated to 50˚C before proceeding.

3. Prepare your tubes. For each strain, label four cryogenic tubes. In the fourth tube, aliquot 0.75 of "normal" freezing solution (see recipe below). Place tubes on ice for a few minutes before starting the freezing process.

4. Using ~3.75 mL of M9, wash animals off 10 cm recently starved plate (lots of L1s, no dauers).
    - If you are using multiple plates for one strain, you will need to start with more M9 and serial transfer the M9 between plates. The goal is to end up with ~3 of worm mixture.
5. Add 0.75 mL of worm/M9 mixture to each of four tubes.
    - Three of the tubes will be stored at -80C as a working stock. The fourth tube, the one with "normal" freezing solution will be stored in the liquid nitrogen tank.

6. Chill tubes on ice 5 min.

7. Add 0.75 mL soft agar (50˚C) to each of the three tubes that do not contain "normal" freezing solution. Invert to mix. Chill on ice 5 min.

8. Transfer tubes to styrofoam holders, then place at -80˚C. After ≥ 24 hours, transfer tubes to permanent storage location (-80˚C or liquid nitrogen).

Thawing:

Maintain a metal tube-holder block at -80˚C (same ones used in heat blocks; must fit cryotubes).

Transfer cryotubes from their storage location to the metal block. Bring block to your bench. Working one strain at a time, remove a cryotube and warm it briefly in your hands. Flame a metal spatula as you would for chunking. Scoop out a plug of frozen agar and transfer it to a 6 cm plate.

Viability declines with each thaw. On the third or fourth thaw, thaw the entire tube and distribute across two or three 6 cm plates.

M9 (1L)

3 g KH_2PO_4
6 g Na_2HPO_4 anhydrous (or 11.3 g $Na_2HPO_4 \cdot 7H_20$)
5 g NaCl
1 1 M $MgSO_4$
H_2O to 1 L

Autoclave. $MgSO_4$ sometimes precipitates out of solution. After solution cools, swirl to dissolve.

Soft Agar Freezing Buffer

9. Place a 1 L **glass beaker** on a scale and add:
    - 300 g glycerol
    - 4 g Difco Agar
    - You must use a glass beaker to heat the solution enough to dissolve the agar.

10. Bring up ~0.95 L with M9. Add stir bar and heat/stir to dissolve agar.
    - This may take a long time; need to heat up to 200°C-215°C.

11. Allow solution to cool (still warm, but cool enough to handle).

12. Transfer to graduated cylinder and bring up 1 L.

13. Return solution to glass beaker and stir to mix.

14. Transfer back to graduated cylinder and pour into to glass bottles, 100 ml per bottle. Autoclave 20 min.

15. Store at room temperature.