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Freezing a strain of C. elegans for long-term storage V.1

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We use this protocol and it's working

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Abstract

Freezing down a strain of C. elegans for long-term storage. The larval stage is the best at surviving freezing. It does not make sense to freeze down post-reproductive stages as they will not give rise to new worms after defrosting.

Materials

MATERIALS

- M9 solution for nematode culture
- 2X freezing buffer for nematodes
- NGM plate seeded with bacteria
- Cryovial 2.0ml round base internal **Catalog #**121277

Cryovials come in different colours with different catalog numbers: 121277, 121278, 121279, 121280

Before start

Have a plate with many larval stage worms or an overnight Falcon tube with many arrested L1s.

1 If starting with a Falcon tube of arrested L1 worms, add an equal amount of 2X freezing buffer (ideally adding 3 ml to 3 ml) and distribute 1 ml to each of five cryovials. Work in sterile conditions.

If starting with a plate full of early stage worms, collect worms, wash three times in M9, resuspend in 3 ml and add 3 ml freezing buffer 2X and distribute 1 ml to each of five cryovials. Work in sterile conditions.

The tubes should be labelled with the strain name and the date, at least.

2 Put the cryovials in a polystyrene box. This allows the temperature to decrease by one degree per minute. Put the polystyrene box in the -80 C freezer.

3 The following day, put a cryovial in a foam floater, put the foam floater in a glass with warm water to thaw the tube. Once thawed, pour the content of the tube onto an NGM plate seeded with bacterial food.

4 The following day, check that the NGM plate contains many living worms, at least 60. The more, the better. Once this has been checked, three tubes can be stored in the appropriate place in the -80 C freezer and one tube can be stored in liquid nitrogen as the master stock. When the last of the tubes in the -80 C freezer has been used up, it is time to freeze down the strain again.

5 The protocol is worded differently below:

6 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 15ºC, they will be ready to freeze in 1week. b. If you are growing the worms at 20ºC, they will be ready in about 4 days.

7 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 phenotypee. No contamination (if contaminated, the worms will continue to eat andgrow so there will be no stalling at the L1 stage)

8 Add about 5 mL of M9 to each plate.

9 Give each plate a swirl to loosen worms still stuck to the agar and then tilt plateson their lids so the liquid drains to one side of the plate.

10 Using a glass pipette, collect the liquid (worms and M9) in a 15 mL conical tube(about 10 mL total).

11 Pellet the worms for about 1 minute at full speed in a clinical centrifuge.

12 Aspirate as much of the supernatant as possible without disturbing the pellet.

13 Add about 15mL of M9 and resuspend the pellet.

14 Again, spin for about 1 minute at full speed. Repeat steps 7 and 8 if desired.

15 Aspirate all but about 3 mL (or 4.5 mL when using three plates) of M9.

16 Add an equal amount of freezing solution.

17 Briefly agitate the vial to suspend the worms and aliquot the worm suspensioninto 6 cryovials (9 cryovials with three plates) clearly labeled with the strainname, your initials and the date.

18 The styrofoam container that 15 mL conical tubes comes in is useful for freezingworms. The insulation provides the slow decrease in temperature required forsurvival. Place the cryovials into the styrofoam, and cover with another. Securewith tape or rubber bands and label the outside for future reference.

19 Store in your –70°C freezer space.

20 Test thaw the worms about 1 month later to ensure a successful freeze. A goodfreeze is when at least more than 10 worms survive. Pick several survivors on toa fresh plate to make sure they can produce progeny of the correct phenotype. Besure to maintain the line while waiting for the results of the test thaw.

21 • 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.