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Cryopreservation of Nematodes and Revival

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We use this protocol and it's working

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Abstract

This protocols describes how to cryopreserve and revive nematodes. We used this protocols for numerous species. We see best survival rates using this protocol originally developed for *Pristionchus pacificus* nematodes compared to other protocols we tried.

Guidelines

If you see low survival rates for your nematode using this protocol, it can help to optimise (most likely you need to prolong) the time of incubation in the freezing buffer before freezing.
If no worms survived the freezing, you can try to freeze more worms and prepare more plates for freezing for the next time.
Depending on the species of your nematodes, other life cycle stages might survive better the freezing than the usual L2 stage. I have seen adults being the predominant stage of survival for some species. In such cases you want to start with plates containing mostly the surviving life cycle stage of the nematodes.

Materials

- **Freezing solution** (40 ml): 4 ml DMSO, 4 g Dextran add autoclaved water to 40 ml total. Store in 4C degrees; Dextran: D9260 Sigma Aldrich, Dextran from *Leuconostoc mesenteroides,* average mol wt 9,000-11,000 / DMSO: D8418 Sigma Aldrich, Dimethyl sulfoxide, Molecular Biology.
- 1x M9 buffer
- **Thawing solution**: 75 mg L-Glutamine → fill up to 250ml with 1x M9 buffer (store in fridge). L-Glutamine: Thermo Scientific Chemicals, L(+)-Glutamine, 99%, Catalog number 119951000.
- 1x M9 buffer with 1 mM CaCl2
- Centrifuge for 15 ml tubes e.g. Eppendorf Centrifuge 5910R
- Cryo-tubes 1.8 ml e.g. Thermo Scientific™ Nunc™ Biobanking and Cell Culture Cryogenic Tubes; Cat.No. 377267
- 2 styrofoam racks for 15 ml falcon tube
- 15 ml falcon tubes e.g. CELLSTAR®, BLUE SCREW CAP, NATURAL, GRADUATED, WRITING AREA, STERILE, 50 PCS./BAG; Item No.: 188271-N
- transfer pipets e.g. VWR®, Transfer Pipettes, Graduated; VWR Catalog Number: 612-6803
- Rubber bands or tape
- NGM plates seeded with feeding bacteria e.g. OP50

Before start

Assuming that L1/L2 larvae are most likely to survive freezing; take 5 to 10 small plates (60 mm diameter) or 3 big plates (90 mm diameter) of fully grown plates (non starved). Most of the eggs should be hatched and the plates should contain lots of L1/L2 larvae.

Freezing nematodes

3d 0h 17m

1 Wash the worms off in a 15 ml falcon tube with M9 buffer.

2 Spin down for 2 min 450 g at room temperature.

2m

3 Take off M9 and add Freezing solution to a total volume of 7 to 8 ml.

4 Label 5 to 6 1.8 ml cryotubes with the strain number and the month/year

5 Re-suspend worms and add to the cryotubes

6 Take 2 styrofoam 15 ml falcon tube racks for freezing

7 Place the cryotubes in and close with another rack on top.
I use thick rubber bands. You can also use tape.

7.1 Add 3 rubber bands for better hold and add label with the strain number and day of freezing

8 Let the "package" with the worms stand at room temperature for about 15 min.
Do not let it stand longer than 1 hour at room temperature.
This step might need optimisation depending on the species. Some species need a longer incubation time to survive the freezing.

15m

9 Put the package in the ultra low temperature (ULT) freezer

10 Leave it there for at least 3 days before doing a thawing test for survival. Then move the rest of the tubes to the final worm box in the ULT freezer.

3d

10.1 After 3 days, you can perform a thawing test (reviving nematodes) with one of the tubes.

Move the other tubes to their permanent position in a storage box “worm box”. Keep record of box number and position in the freezer.

Revive nematodes

30m

11 Let the cryo-vial stand at room temperature for thawing (takes about 20 min).

12 Shortly vortex the tube after thawing and put the worms which are in the freezing solution, in a 15 ml falcon tube. Then add 10x the volume of **thawing solution.** I usually have about 1 ml in a cryotube, so I add 10 ml of thawing solution.

13 Mix by inverting several times.

14 Spin carefully for 2 min 150 g.

15 Take off supernatant with transfer pipet and fill the falcon tube with M9 containing 1 mM $CaCl_2$.

16 Invert the tube and spin 2 min 150 g @ room temperature.

17 Take off the supernatant with transfer pipet and leave about 0.5 ml plus worm pellet.

18 Add the worms onto new NGM plates, that have been seeded with OP50 or other feeding bacteria.

19 Put the plate in growing temperature and wait. Check the next day for moving worms. Then put them back in the incubator.

20 Check again after 5 to 7 days if the next generation of nematodes is growing. Add the result of this thawing test to your records.
If no worms survived the process, cryopreservation needs to be repeated and the already frozen cryotubes in the ULT freezer thrown away. You can prepare more plates for the next try and try different incubation times for the worms in freezing buffer before freezing.

Protocol references

http://www.wormbook.org/chapters/www_ppageneticprotocols.2/ppageneticprotocols.pdf