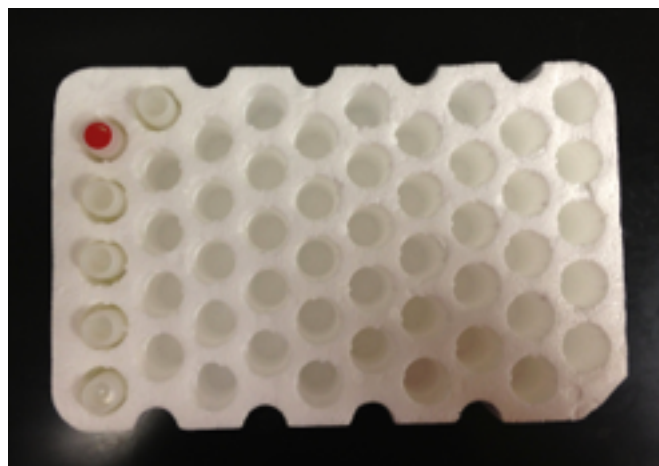


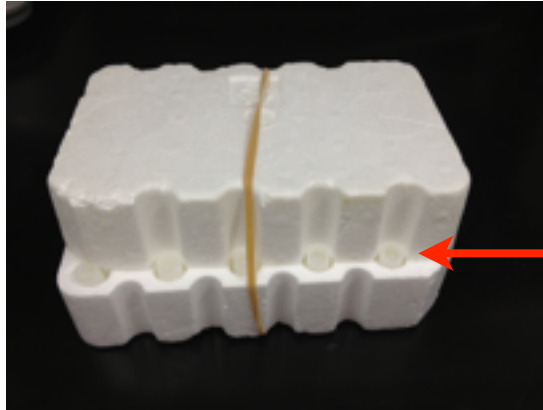
Freezing Worms

by Robyn Tanny (December 2013)

1. Chunk worms to the number of 10 cm plates you wish to freeze for a particular strain.
 - a. For most strains, one plate is enough. For sick strains, more plates will result in a higher timer and therefore a better thaw rate.
 - b. Check the genotype of the strain so that you can follow any phenotypes to make sure the animals are correct.
2. Store the plates at the optimal temperature for your strain (20°C for most strains) until the 10 cm plate is starved.
 - a. The strain is ready to freeze when there is no bacteria on the plate and there are few or no embryos remaining on the plate. Most of the animals should be L1, L2 or dauer.
3. Prepare six cryovial tubes (Fisher 12-565-163N) with cryo-safe labels (Fisher 15930A) with the appropriate information (e.g. - strain name, date frozen, genotype, the initials of the person who froze the strain, etc.)
 - a. To mark the last tube, place a red cap insert (ISC Bioexpress T-2807-R) in one of the six tubes. When this tube is thawed, you must refreeze strain.
4. When the strain is ready to be frozen, use a glass, sterile pipette to aliquot 3 ml of freezing solution (recipe below) into a 15 ml conical vial.
5. Take the lids off all six cryovials.
6. Wash worms off the plate by squirting ~3 ml of M9 (recipe below) onto the 10 cm plate. Either use a Pastuer pipette to transfer the worms or directly pour from the plate into the 15 ml conical. **Make sure not to use more than 3 ml of M9.** If you use less than 3 ml of M9, squirt a little more M9 on the plate and transfer the worms to the 15 ml conical. The final volume in the conical should be 6 ml.
 - a. If you are freezing more than one plate for a single strain, use 3 ml of M9 between all the plates.
7. Use a sterile, glass pipette to pipette the M9/worm/freezing solution mixture up and down once to mix.
8. Use the same sterile glass pipette to aliquot 1 ml of the M9/worm/freezing solution mixture into each of the tubes, replace the caps and place tubes in a 15 ml conical styrofoam container.



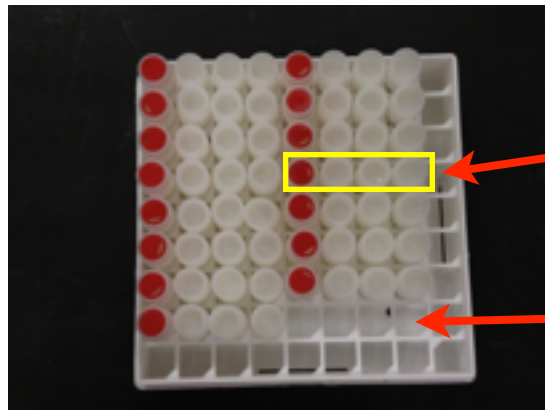
9. Put a second 15 ml conical styrofoam container on top of the first and secure with a rubber band. The styrofoam containers are slightly off-set from each other.



The bottom row of tubes will be exposed.

10. Place the rubber-banded containers in a -80°C freezer.

11. After at least 24 hrs, move four tubes to the a permanent storage box in the -80°C freezer, move one tube to a liquid nitrogen storage box, and thaw one tube as a test thaw.
- a. The red-capped tube is one of the four tubes in the Working Stock Box and is placed as far to the left as possible for that strain.



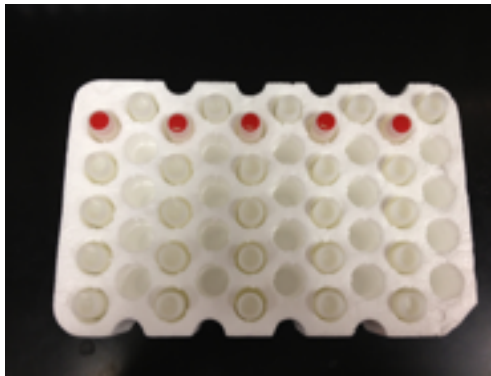
The four tubes for one strain.

The tubes for the next strain go here.

- b. For the test thaw, empty the contents of the tube onto a 10 cm plate just after the contents melt. Check the plate after $\sim 48-72$ hrs. If you see gravid animals and embryos on the plate, the strain survived the thaw. If you do not see gravid animals and embryos, try freezing the strain again, but start with more 10 cm plates (step 1).
- c. As you move the strain into the permanent storage locations in the -80°C freezer and the liquid nitrogen unit, make sure to record the storage position for each strain.

Notes for freezing more than one strain at a time:

1. Aliquot freezing solution into all of the necessary 15 ml conicals at once. Leave the caps on loosely so you can move through the protocol more quickly.
2. Arrange your strain plates and sets of tubes in alpha-numeric order.
3. You can put up to five strains in a single 15 ml conical styrofoam container



4. If you have more than 10 strains to freeze, move the tubes for 10 strains (in their styrofoam containers) to the -80°C freezer before freezing the next set of strains.
 - a. You don't want the animals in freezer solution at room temperature for too long. You want to move them to -80°C as soon as possible.

Thawing and Cleaning Worms

1. Remove the right-most tube of those remaining in the Working Stock Box. If the red-capped tube is the only tube remaining, **you must make sure to freeze new copies of this strain** after the strain is cleaned.
 - a. Follow the freezing protocol as listed above, but you should only need to freeze **five** tubes: four for the Working Stock Boxes and one for a test thaw.
2. Right as the contents of the tube are melting, decant the contents onto a clean 10 cm plate. Incubate the plate at the optimal temperature for the worms.
3. Mark in LabGuru for whom the strain was thawed and the date. Also, delete the appropriate tube from the Working Stock Box database.
4. Once there are gravid animals on the 10 cm plate, bleach the animals to clean any bacterial contaminants:
 - a. Place 15 μ l of bleach solution (recipe below) on the edge of a labeled 6 cm plate.
 - b. Place a minimum of 6 gravid animals into the bleach (10-15 animals is good). The bleach should dissolve the cuticle of the adult worms, releasing the embryos. Leave the plate lid-side up until all the bleach has soaked into the plate. After the bleach has soaked in, move the plate, lid-side down, to the optimal temperature for the worms.
 - c. After ~24 hrs, move L1s from the bleach plate to 4 separate clean, labeled 6 cm plates. You want between 10-20 L1s per plate - the more, the better.
5. Parafilm the clean plates to keep at 15°C.

M9

1. Mix the following:

Reagent	Amount Needed
KH ₂ PO ₄	6 g
Na ₂ HPO ₄	12 g
NaCl	10 g
dH ₂ O	up to 2 L

2. Autoclave on liquid cycle, 30 minutes

- Make sure the volume is maintained during autoclaving by autoclaving in the large autoclave (the autoclave on the left).

3. When the M9 is cool, add:

4. Store in 2 L aliquots

Freezing Solution

1. Mix the following:

Reagent	Amount Needed
Potassium phosphate buffer, 1 M pH 6	100 ml
NaCl, 5 M	40 ml
Glycerol, 100%	600 ml
dH ₂ O	up to 2 L

2. Divide into 250 ml aliquots.

3. Autoclave on liquid cycle for 30 minutes.

4. Add MgSO₄ to a final concentration of 0.3 mM (e.g. for 200 ml of freezing solution, add 60 µl of 1 M MgSO₄).

Bleach Solution

Reagent	Amount Needed
NaOCl (from Fisher, cat #SS290-1)	2 ml
10 M NaOH	0.5 ml
dH ₂ O	up to 10 ml

- store at 4°C