

Protocol: Freezing C. elegans (and Soft Agar Solution)

Protocol adapted from Wormbook: http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html

Reagents Required:

M9 (room temperature and a small aliquot on ice)
Soft Agar Solution

Making the Soft Agar Solution:

1. To a 500 ml autoclavable bottle, add (in this order):

Reagent	Amount to add
diH ₂ O	76 ml
100% Glycerol	23.8 ml
NaCl	0.58 g
KH ₂ PO ₄ (potassium phosphate monobasic)	0.68 g
Bacto Agar	0.4 g
5M NaOH	112 µl

2. Autoclave the mixture (**You MUST get trained on how to use the autoclave before doing this!**)
 - i. Make sure the screw-caps are **LOOSLEY** screwed on, allowing gas to escape the bottle.
 - ii. Place a piece of autoclave tape across the cap, making sure one end is taped to the glass bottle.
 - iii. Autoclave for 25 minutes (use "SLOW EXHAUST" setting).
3. Soft Agar Solution can be kept at room temperature indefinitely. Microwave to melt before using.

Harvesting Worms for Freezing:

- Chunk worms onto 6-8 10cm dishes with OP50 bacteria. Note that each plate of worms will become a 1ml cryovial of frozen *C. elegans* in soft agar.
- Allow the worms to grow until the bacteria is just depleted and there are lots of freshly starved young larval-stage worms present. This should be about 4 or 5 days at 20°C. Check plates daily to monitor bacteria depletion.
- On the day of freezing:
 1. Microwave the soft agar solution and allow to cool to 50°C in the water bath.
 2. Wash each plate three times with 3 mL of M9 buffer. Collect worms in 15 mL conical centrifuge tubes. Allow the conical tubes to sit on ice for 30 minutes and pre-chill the centrifuge to 4°C.
 3. Spin at full speed (or 3,000 x g) for 1 minute. Using a glass aspirator, remove as much of the M9 buffer as possible, leaving a pellet of worms at the bottom.
 4. Resuspend in 500µl of ice-cold M9 per plate harvested.
 5. Add 500µl of 50°C soft-agar solution to each cryovial. Add 500µl of resuspended worms to each vial, vortex, and freeze in a cryo container with isopropanol.