

Freezing worms (Bhalla Lab)

- 1) Make sure worms are clean: no contaminating bacteria or mold! Chunk clean plate of relevant genotype to six NGM plates with spread or spotted OP50. If you are freezing a balanced strain, pick 20-25 of the balanced genotype to each plate to make sure that your balanced genotype is well represented in the frozen population.
- 2) Worms should be harvested off plates one day after the plates have starved so that the population of worms are mostly L1s and L2s with no food in their gut (this reduces the viability of frozen worm stocks). Add ~2mls of S basal to each plate and swirl briefly to dislodge worms. Remove S basal to 15ml conical tube.
- 3) Spin down worms 15-30 sec at 2000RPM in clinical centrifuge. Remove all but 1.5mls S basal and add 1.5mls worm freezing solution. Mix and aliquot 600ul into 5 sterile Nunc vials. Insert colored caps into two of the vials (write strain number on top), and label all the tubes with the strain number AND genotype.
- 4) Freeze all five tubes at -80° overnight. Pull out one of the nalgene tubes and let thaw. Pour out onto a spread NGM plate and let dry. This is your test thaw. **If enough of the worms do not survive the freezing you will need to refreeze the strain.** If the test thaw is successful, enter the strain in the worm lab collection; it will be assigned the BHL strain designation (i.e. BHL1). Three of the tubes will go in to strain collection at -80° . One of these will be the Nunc tube with a colored cap insert on which the BHL strain designation will be written. The other Nunc tube will be placed in the liquid nitrogen container in the basket, cane and position indicated in the strain collection.
- 5) If you need a strain from the collection, check with other members of the lab to see if it is already out. If you pull a strain out of the -80° collection, you are responsible for maintaining it for a year so that if anyone else needs it, they can go to you and not have to pull it out of the strain collection. If you pull out the last strain from the -80° collection, you are responsible for freezing down another three copies to replace it. Strains are to be pulled out of the liquid nitrogen collection only if all strains from the -80° collection are gone or otherwise compromised.