## I. Freezing worm stocks

- A. Hypochlorite stock to obtain starved L1s
  - 1. See protocol for synchronization
  - 2. Hatchout time should be 18-36 hours shaking at 20 C
  - 3. Keep a backup stock in case hypochlorite or freezing procedures are lethal
- B. Aliquot 0.5 mL of starved animals in the S-basal solution (containing a minimum of 100 animals) into 5, 2mL cryo tubes labeled with the strain name and date.
- C. Add 0.5 mL of S-basal in 30% glycerol to each tube, cap the tube, and mix 5-10 times by inversion.
- D. Place in Styrofoam container at -80 C and <u>record the location in both your</u> notebook and the reagents database
  - 1. Container should be insulated to cause rate of cooling to be approximately 1°/min
- E. Test thaw a tube of animals
  - 1. Repeat freezing procedure if viability is below 50% for wild-type or non-sickly mutants
  - 2. If successful, update the database "strain frozen?" field