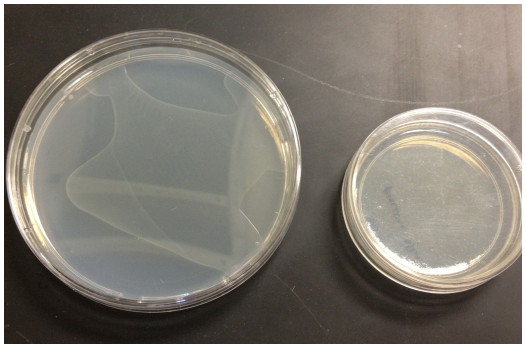
Andersen Lab Basic Worm Maintenance Protocols

by Robyn Tanny October 2013

Chunking

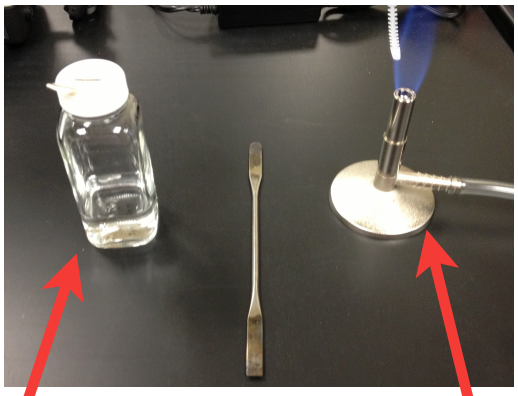
1. Make sure the bottom of the plate to which you are moving the worms is labeled with the strain name and date.

2. Have both your old plate (starved worms, no bacteria) and new plate (no worms, bacteria) ready:



New Plate Old Plate

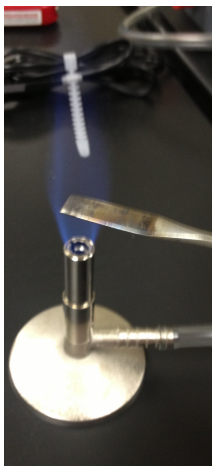
3. Have the necessary tools ready:

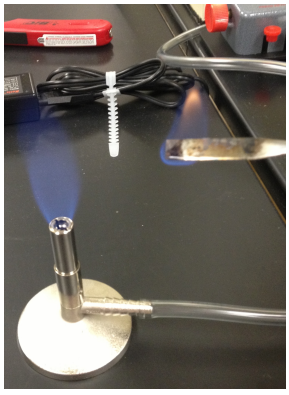


Jar of 95% Ethanol Lit Bunsen Burner

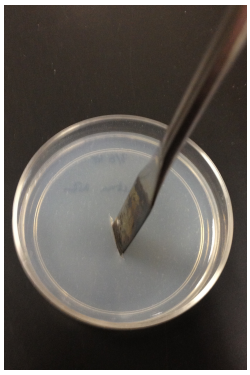
Stainless Steel Spatula

4. Place the spatula in the jar of 95% ethanol and then pass the spatula through the flame to sterilize it.





5. After the flame has extinguished from the spatula, use the sterilized spatula to cut through the agar in the center of the old plate and cut out a ~1 cm^2 area:

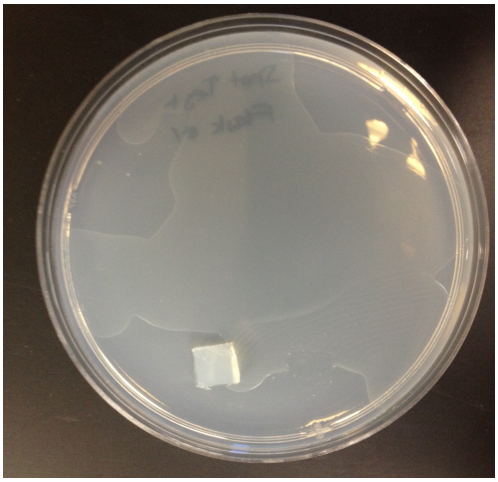


Making the first cut

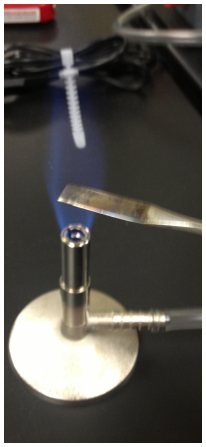
A ~1 cm^2 area

6. Remove the cut-out area using the spatula:

7. Place the agar chunk from the old plate **worm-side down** at the edge of the bacterial lawn on the new plate:



8. After you place the agar chunk down, hold the spatula in the flame of the Bunsen burner for 5-10 seconds to melt/burn any remaining agar and worms:



9. Place the spatula back in the ethanol to sterilize again before preparing the next sample.

Cleaning by bleaching - use this technique to remove **bacterial contamination**

1. Chunk a 1 cm^2 area from the contaminated plate to a labeled 6 cm plate.

2. After **1-2 days** when there are gravid adults, place 15 µl of bleach solution (recipe below) at the edge of a new, labeled 6 cm plate (as far from bacteria spot as possible).

3. Place a minimum of ten gravid animals into the bleach (up to 20 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.
   a. You can add a little more bleach (~10 µl) after you the place the adults if some of the initial bleach absorbed while you were picking the gravid adults.

4. After ~24 hrs, move L1s from the bleach plate to a clean, labeled 6 cm plate. You want between 10-20 L1s per plate - the more, the better.
   a. If you think you will need this strain a lot, pick 10-20 L1s to multiple plates.

5. Parafilm the plates with the L1s to keep them clean.

Cleaning by chunking - use this technique to remove **mold contamination**

1. Chunk a 1 cm^2 area from the contaminated plate to a labeled 6 cm plate.

2. After 10-30 min, pick 20-30 animals to a new, labeled 6 cm plate.
   a. If the original contaminated plate is more than a month old, chunk a larger chunk (5 cm^2) to the new plate to increase the likelihood of transferring live worms. It might take longer (up to 24 hours) for the worms to crawl from the chunk to the new plate.

3. If there was a lot of contamination, you might want to repeat steps 1 and 2 to allow the worms a second chance to crawl away from the mold.

4. Wrap your final clean plate with parafilm.

Notes about long-term storage of strains:
1. Strains should be kept at 15°C for long-term storage.
2. There should be at least four “clean”, parafilmed plates for each strain, especially RIAILs and wild isolate strains used for assays.
3. After a strain has been at 15°C for three months, one of the plates can be chunked to four new 6 cm plates.
4. After the strain has been at 15°C for one year, it should be re-thawed if needed.
5. Avoid repeated passages of strains as much as possible. Advantageous alleles will be selected quickly in most lab procedures.

Bleach Solution

Reagent	Amount Needed
NaOCl (from Fisher, cat #SS290-1)	2 ml
10 M NaOH	0.5 ml
dH_2O	up to 10 ml

• store at 4°C