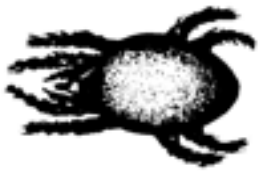
November 2013

Identifying New Wild Isolates

1. After plates arrive in the lab, look through them *before* removing parafilm. First, determine if there are worms on the plate. It is easiest to observe worm tracks in the bacteria. If there are **no** worms on the plate, throw the plate in the biohazard *with* the parafilm on. If there are worms, check for mites. If you see mites, determine if they are slow-moving or fast moving.
   a. If the mites are slow-moving, proceed to step 2 below (but pick quickly!).
   b. If the mites are fast-moving, set the plate in a separate container. When you have gone through all the plates and determined those that should not be opened in the lab, take those plates to the BSL3 room (Pancoe 3353) to pick off the worms. Perform step 2 below in the BSL3 lab room.



An example of a mite

2. If there are no mites on the plate, pick off as many worms as possible from each plate onto a 6 cm plate with a maximum of 30 worms on the new plate. Make sure to wrap the new 6 cm plate in parafilm before storing. Wrap the 10 cm in parafilm and dispose in biohazard. Pay attention to nematodes of different shapes, colors, movement characteristics. Pick all similar worms to one plate. We often find multiple species on the same isolation plate.

3. Regularly check the plates for gravid females. Prepare three isofemale lines by picking one gravid female to one 6 cm plate three separate times. Wrap plates in parafilm.

4. Allow the plates to starve.

5. Chunk the starved isofemale plates to new 6 cm plates.

6. One day after chunking:
   a. Pick 20 animals from each plate to a new, appropriately labeled plate.
      - The purpose of this is to help remove mold contaminants.

   b. Lyse and PCR the worms for genotyping. Follow the procedure for Genotyping by Single Worm Lysis, with the following specifics:
      i. Pick five animals from each plate into a single cap.
      ii. Use primers oECA202 and oECA305 for the amplification.
          oECA202: 5'-GCGGTATTTGCTACTACCAYYAMGATCTGC
          oECA305: 5' -GCTGCGTTATTTACCACGAATTGCARAC
      iii. Use **ExTaq** polymerase from Takara (cat# RR001) as the enzyme and the corresponding 10X Buffer (make sure to note whether or not you need to add $MgCl_2$).
          - We are using ExTaq at this step to ensure high polymerase fidelity for sequencing.
      iv. Perform the reaction in 30 µl. Appropriately scale up all of your reagents for a final volume of 30 µl but use only 2 μL of the lysis reaction.
      v. Make sure to include N2 as a positive control! Do several N2 lyses so that you have one N2 reaction to load for each row on your agarose gel.

7. After the PCR is finished, electrophorese the amplification products on a 1.5% agarose gel.
   a. A rhabditid species should generate a band that is ~1.6 kb.

8. For *only* the animals that had an appropriately sized PCR product (i.e. - the same size as the N2 positive control PCR product), excise any PCR products (but not the N2 products!) of the appropriate size using a razor blade.
    - Make sure to wipe the razor blade with 95% ethanol between each use. You do not want to contamination between samples.

9. Extract the DNA using the Qiagen Gel Extraction kit (cat# 28706). Elute each of the samples in 30 µl of sterile water.

10. Prepare gel-extracted samples for sequencing with the following specifics:
    a. You can use either one of the following primers:
        oECA305: 5' -GCTGCGTTATTTACCACGAATTGCARAC
        oECA306: 5' - CACTTTCAAGCAACCCGAC
    b. Prepare the DNA template (i.e. - the gel-extracted DNA) according to the directions provided by your sequencing company of choice. Note that your PCR product is approximately 1.6 kb.

11. Once you have the sequencing results, use the NCBI BLAST web interface to identify the closest species match.

12. If the sequence is good such that sequences from both primers overlap each other, proceed with freezing (below). If the sequence is not good (the sequences from both primers do not overlap, there are a lot of unknown nucleotides, etc), repeat the lysis and PCR using the ExTaq polymerase to confirm the species identification.

13. If the nematode strain is *Caenorhabditis*, then we want to name the strain, freeze it, and collect a high-quality DNA sample. You only need to keep one isofemale line from each isolated species. If you want to keep the others, that is fine too. Redundancy is always preferred for precious reagents. When naming the strain, please record all information about isolation date, time, GPS location, elevation, temperature, name of both person who isolated from nature and processed in the lab, etc. Please be as detailed (even with pictures) as you can. To freeze and collect DNA, chunk the strain to one 10 cm plate for freezing and then five HGMA plates for DNA isolation. Follow lab protocols both of the next steps (see FreezingThawingWormsForLongTermStorage and DNAEasyGenomicDNAPrep).