

Identifying New Wild Isolates

1. After plates with substrates 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 a single gravid animal onto a 6 cm plate for a maximum of six plates. This step will generate isofemale lines. Make sure to wrap the new 6 cm plate in parafilm before storing. Wrap the 10 cm plate in parafilm and dispose of it in biohazard.
3. After 4-5 days, check if the plates have a selfing, hermaphroditic population and if the animals have a two-bulbed pharynx. If so, genotype the animals via Sanger sequencing.
4. Lyse and PCR the worms for genotyping. (see below for lysis and PCR thermocycler conditions):
 - i. Pick five animals from each plate for lysis.
 - ii. Use primers oECA202 and oECA1687 for the amplification.
 oECA202: 5'-GCGGTATTTGCTACTACCAYYAMGATCTGC
 oECA1687: 5' -CTGCGTTACTTACCACGAATTGCARAC
 - iii. Perform the reaction in 40 μ l.
 - iv. Make sure to include N2 as a positive control! If you are genotyping many wild isolates, prepare several N2 lysates so that you have one N2 reaction to load for each row on your agarose gel.

	Stock Concentration	Volume to Add	Final Concentration
Lysis Reaction	-	2 μ L	-
Primer 1	4 μ M	4 μ L	0.4 μ M
Primer 2	4 μ M	4 μ L	0.4 μ M
dNTPs	2.5 mM	3.2 μ L	0.2 mM
10X ThermoPol (Taq) Buffer	10X	4 μ L	1X
Taq Polymerase	-	0.2 μ L	-
dH ₂ O	-	22.6 μ L	-

5. After the PCR is finished, electrophorese 5 μ l of the amplification products on a 1.5% agarose gel.
 - A species in the Rhabditidae family should generate a band that is ~1.6 kb.

6. For *only* the animals that had an appropriately sized PCR product (i.e. - the same size as the N2 positive control PCR product), submit the amplified material for sequencing. Submit two reactions for each sample using the following primers;
 - oECA1687: 5' -CTGCGTTACTTACCACGAATTGCARAC
 - oECA306: 5' - CACTTTCAAGCAACCCGAC
7. Once you have the sequencing results, use the NCBI BLAST web interface to identify the closest species match.
8. 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 and use ExTaq polymerase this time to confirm the species identification.
9. Proceed with isofemale lines that are *Caenorhabditis elegans*, *Caenorhabditis tropicalis*, or *Caenorhabditis briggsae*. Choose how many isofemale lines from each substrate that you will save.
10. For each line that you will save, give the strain a unique name.
11. Clean the strains by bleaching 8-12 gravid animals by spot bleaching. 24 hours later, pick 10-20 L1s to a new 6 cm plate.
 - If your original plate is starved, you may need to chunk to a new plate to generate gravid animals for bleaching.
12. At this point, we will freeze the strain and make DNA for whole-genome sequencing.

Thermocycler Conditions for Lysis

Time	Temperature	Number of Cycles
1 hour	60°C	1
15 min	95°C	1
Hold	4°C	1

Thermocycler Conditions for PCR

Step	Time	Temperature
1	3 min	95°C
2	15 sec	95°C
3	15 sec	60°C
4	2 min	72°C
5	Go to step 2, repeat 34 times	
6	5 min	72°C
7	hold	15°C