

Identifying New Wild Isolates

- 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.

- If the mites are slow-moving, proceed to step 2 below (but pick quickly!).
- 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

- If there are no mites on the plate, pick up to 30 animals onto a new 6 cm 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.
- After 4-5 days, check if the plate has a selfing, hermaphroditic population and if the animals have a two-bulbed pharynx. If so, genotype the animals via Sanger sequencing.
- Lyse and PCR the worms for genotyping. (see below for lysis and PCR thermocycler conditions):
 - Pick five animals from each plate for lysis.
 - Use primers oECA202 and oECA305 for the amplification.
 oECA202: 5'-GCGGTATTTGCTACTACCAYYAMGATCTGC
 oECA305: 5' -GCTGCGTTATTTACCACGAATTGCARAC
 - Perform the reaction in 40 μ L.
 - 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	-

- After the PCR is finished, electrophorese 5 μ L of the amplification products on a 1.5% agarose gel.
 - A rhabditid species should generate a band that is \sim 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;
- oECA305: 5' -GCTGCGTTATTTACCACGAATTGCARAC
 - 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. If the species is *Caenorhabditis elegans*, *Caenorhabditis tropicalis*, or *Caenorhabditis briggsae*, clean the original strain by bleaching 8-12 gravid animals. 24 hours later, pick three L1s each to a single 6 cm plate. This will generate three independent isofemale lines.
- If your original plate is starved, you may need to chunk to a new plate to generate gravid animals for bleaching.
10. If the nematode strain is one of the above *Caenorhabditis* species, move forward with **one** of the three isofemale lines. The others can be discarded.
- At this point, we will name the strain, freeze it, 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	1 min	72°C
5	Go to step 2, repeat 34 times	
6	5 min	72°C
7	hold	15°C