Andersen Lab Nematode Field Sampling Protocol

Barcode Generation

The script below generates labels for use with Avery Durable ID Labels with TrueBlock® Technology, 61533.

Barcodes are generated using a script called generate_labels.py in the Hawaii2017 repo (LINK). The script can be run after the requirements are installed. Be sure you have the latest version of pip prior to installation as well. Navigate to the repo directory and run:

```
pip install --upgrade pip
pip install -r requirements.txt
```

Then you can run the script by running:

```
python scripts/generate_labels.py
```

The number of labels, label prefixes, and type of label can be modified by editing these three lines in the script:

```
# Configuration
LABEL_USE = "a_61533" # Type of label
LABEL_SETS = {'C': 120, 'S': 240} # Label prefixes and counts
```

The script will output a set of labels and an associated count as defined the the python dictionary LABEL_SETS. The script will output two PDF files:

- C-labels.pdf (120 labels)
- S-labels.pdf (240 labels)

The PDF files are saved in the same directory as the script.

Organize materials before the day of collecting:

Fanny pack to organize equipment and sampling bags. We put the external battery and cord, 50-100 labeled ziplock bags (wrapped in groups of 25), temperature and humidity detector (LINK), MoonCity MD912 moisture detector (LINK), Etekcity IR thermometer (LINK), and a snack in the fanny pack. We carried a backpack per group to collect all filled bags with samples. If the weather is hot (>30ºC), you might want to carry a soft-side cooler and ice pack to keep collected samples cool during the hot hike. We made sure each team had at least two extra 9V batteries as well as a first aid kit.

We found that collections went more efficiently with two people per team. One person performed data entry to the Fulcrum app (LINK), carried the ambient humidity/temperature detector, took the picture of the sample, and distributed the labeled empty sampling bag. The second person identified items to

sample, recorded the substrate moisture and temperature, and collected the sample. The first person would scan bags to be used in the next collection before the next collection was identified. This step sped up sampling significantly.

1. Clean the detector prongs of the MD912 moisture detector by rinsing in water and rubbing with a clean paper towel. Repeat this procedure each night as well. Be careful not to get the inner mechanism wet. Throughout the day, make sure the device is set to setting 4. The “m” button adjusts this setting. Also, check each recording to make sure the device is not set to “Hold”. It should change when you touch it to your skin.

2. Label ziplock bags with collection (C) labels. Wrap in groups of 25 with a rubber band.

3. Plan hikes, parking sites, time, weather, etc. Bring water and food for the day.

4. Download google maps for hike or better yet for the entire island (once per trip).

5. Set phone to airplane mode. The Fulcrum app GPS positions are pretty off compared to the GPS position extracted from pictures. This setting ensures that you use less power and get more precise GPS measurements.

Sampling collection:

Equipment and team jobs should be recorded for each day. Because equipment can be subtly different or researchers might use them differently, the team record should have who was on which team, who entered data and carried which pieces of equipment (item number), and who collected the samples and carried which pieces of equipment (item number).

1. Identify a substrate to collect. We looked for clear evidence of rot (dark and slightly damp leaves, collapsed fruits, etc.).

2. Open the Nematode Field Sampling app.

3. Add a collection by pressing + (Shown in figure). This step and step 4 are most efficiently done before identifying a substrate to collect.

4. Scan the collection bag barcode.

5. Take a picture of the sample using the app. Consider taking an additional picture of the tree/plant for future identification.

6. Enter the sample substrate description from the drop down. If the substrate is new, enter it into the description section after selecting “Other”. Add any notes about the substrate (type of plant, etc.).

7. Enter landscape and skyview from the drop down menus. If performing a gridsect, go to the section below and record the remaining information.

8. Make sure the ambient temperature and humidity detector is not on hold. Record ambient temperature and humidity. Enter into the app.

NB: Make sure that probe tip does not get wet with rain or dew. To toggle between ºC and ºF, turn off and then on and hold the max button for five seconds. It will change when the button is released. Try to keep the ambient tool outside of a bag and away from your body, as this changes the reading.

9. Point the IR thermometer at the substrate and press the trigger. Record the substrate temperature in the app.

NB: Make sure you are no more than 14 inches from the substrate while recording the temperature.

10. Make sure the moisture detector is recording and not on hold by pressing (gently) onto your skin. The reading should go to 20-30%. Check that the detector is set to 4. Record substrate moisture by sticking the two prongs into the substrate. If the substrate is too small, enter -1 into that field in the app.

NB: The angle of recording matters. Just do your best to average a measurement for the substrate.

11. Pick up the substrate by inverting the ziplock bag and using it as a “glove”. Seal the bag.

NB: Try to only collect the substrate of interest to make plating out more precise (*e.g.* if you are collecting an isopod, try to avoid collecting soil). Also, a tablespoon of material is sufficient to plate out.

12. Place the bag in a cooled container that stays around 15-20ºC. We used soft-side coolers with ice packs both in our backpacks and in the vehicles.

13. Proceed to the next sample.

Gridsect collections:

1. Identify an area to perform a gridsect collection.

2. Sample the center of the area with the “Gridsect” option set to “Yes”.

3. For the center point, set the “Grid sect direction” to A and the “Gridsect Radius” to 0.

4. Put the gridsect sampling tool into the ground at the point where A-0 was collected. Point the “A” direction north.

5. Extend the string along the A direction and sample every meter up to three meters (marked by red straws on the gridsect tool). Make sure to modify the gridsect direction and radius fields as you sample.

6. Continue sampling through the F direction.

After collecting nematodes:

1. Clean all equipment (especially the moisture detector), replace batteries, empty bags to make sure that all items have been removed, re-freeze cooler packs.

2. Note which C numbers were used for the days collections. These data can be used later to double-check GPS positions and isolation locations.

Plating out nematode samples:

1. Label spotted 10 cm. plates with the C labels collected that day. Place the collection bag with substrate on top of that labeled plate.

2. Using a spoon or gloved hands, spread the substrate around the bacterial spot. Try not to cover the bacterial lawn with substrate because then it is hard to pick worms off of it later.

3. Put the plate in a rack or table space that is consistently 15-25ºC to incubate for 24 hours.

Picking nematodes from samples:

1. Under dissecting microscope, pick up to six hermaphrodite/female nematodes per sample to labeled S plates (one animal per S plate).

2. Note the number and stage of animals on the sample plate. Also, look for males.

3. Record the data about each strain in the Fulcrum Nematode selection app.

4. Wrap S plates in parafilm. Keep plates in a place where the temperature is 15-25ºC until shipping.