FEB 27, 2024

DOI:
dx.doi.org/10.17504/protocols.io.14egn318yl5d/v1

Protocol Citation: Casey A Schlenker 2024. SCN Egg Extraction Protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.14egn318yl5d/v1

Protocol status: Working
We use this protocol and it's working

Created: Dec 28, 2023

SCN Egg Extraction Protocol

Casey A Schlenker[1]

[1]The Ohio State University

Casey A Schlenker

ABSTRACT

OSU full SCN egg extraction protocol

Last Modified: Feb 27, 2024

PROTOCOL integer ID: 92799

MATERIALS

Core processing:
Soil cores
Plastic bin
Mesh screen
Scrub brush

Elutriation:
50 1 L plastic beakers
50 conical tubes
Funnel for tubes
20 and 60 mesh sieves
Water bottle

Cyst grinding:
Plastic tub
200 and 500 mesh sieves
60 mesh PVC sieve
Plastic cup
Funnel
Water bottle

Cyst staining:
6 glass beakers
Pipette
Egg stain
Microwave
Funnel
Water bottle

Counting:
400 mL beaker with stir bar
Stir plate
Dissecting microscope
Pipette
Engraved counting dish

Sucrosing:
Centrifuge
45% sucrose solution

Pipette
Stirring spatula
500 mesh sieve

Core Processing

1 Obtain soil cores, a small plastic bin, and anaccompanying mesh screen. Grab one Ziploc bag/container of a sample and pour it out onto the screen, on top of the bin. Carefully break up large pieces with your fingers by rolling cores against the mesh. Do not crush pieces; break up cores gently to avoid squishing nematodes. Discard rocks or any large organic material such as sticks or plant matter.

2 Using a scrub brush, brush the mesh once done to ensure no dirt sticks to the mesh.

3 Ensure the filtered sample is well mixed and pour the sample back into its Ziploc bag/container. Thoroughly brush the bin into a trash can in order to prevent contamination of other samples.

4 Repeat steps 1-3 for the remainder of the samples.

Elutriation

5 Fill up 50 numbered 1 L beakers with 500 mL of water. Add 100 cm3 of soil to each beaker to bring the volume up to 600 mL. Ensure soil is placed into the beakers in order of their numbering. Place soil in beakers from lowest sample number to highest sample number.

6 Label 50 tubes (2 flats) with sample information. Label them in the same order as the beakers.

7 Place a 20 mesh sieve at an angle on top of a 60 mesh sieve on each elutriator collection funnel. Start with the sample beakers labeled 1-4. Have the labeled tubes and a funnel ready for use. There will be one tube for every sample.

8 Set time on the elutriator to 5:00 minutes

9 Press START before dumping samples or samples will be lost

10 Wash samples into elutriator cones using a water bottle

11 Let the cycle run and complete

12 Rinse the 20 sieve above the 60 sieve. Discard anything left over in the 20 sieve. Pick up the 60 sieve, keep at an angle, and rinse the collected sample into the corresponding 50 mL tube.

13 Repeat 7-12 for the remaining samples. Be sure to rinse mesh and funnel between samples. Store in a refrigerator until grinding.

Cyst Grinding

14 Place a 500 sieve at an angle within a tub within the sink. Nest a 200 sieve on top of the 500. Place a 60 PVC grinding sieve on top of the 200.

15 Select one soil sample and pour it out into a cup. Fill the cup with water and pour into the 60 grinding sieve. Sand may be removed by “swirling” the cup before pouring it into the grinding sieve.

16 Use a drill press with a stopper bit assembly to crush cysts within the 60 grinding sieve and release eggs into the nested 200/500 mesh sieves sitting below the drill press within the tub. Rinse repeatedly while gently grinding until only coarse sand particles remain in sieve.

17 Once sure that all the cysts have been ground, thoroughly rinse the 200 sieve material while holding it above the 500 and remove the sieve. Discard what remains in the 200.

18 Carefully rinse the material caught in the 500 sieve (kept bent at an angle) and rinse into the correct tube using a water bottle. Do not fill the tube above 30 mL; fill any empty space in the tube up to the 30 mL mark using a water bottle. Rinse funnel to make sure all eggs have been collected into tube.

19 Rinse the sieves and funnel. Repeat 14-18 for remaining samples. Note, once removed from cysts, the eggs begin hatching. Keep samples stored in refrigerator and stain within the same day.

Egg Stain

20 Stain samples using either a serpentine or left to right front to back pattern. Ensure that the same sample is put back into the correct tube after staining.

Note

Prepare 1 L of egg stain by combining 0.35 g Acid Fusion Powder, 250 mL Lactic Acid, and 750 mL DH2O

21 Pipette 3 mL of egg stain into each tube.

22 Label six 400 mL beakers 1 through 6 (one for each sample). Pour your six egg samples into their corresponding beakers. Use a distilled water bottle to rinse out tubes completely and ensure the volume of liquid in the beakers does not rise above 50 mL.

23 Set three beakers in the microwave for 90 seconds. When finished, switch to the other 3 beakers and microwave for 90 seconds.

23.1 Samples must boil for 30 seconds for the stain to set. If staining 1 or 2 samples, you must observe the samples in the microwave, and time them to boil for 30 seconds. Do not boil 1 or 2 samples for 90 seconds.

24 Pour the stained egg samples back into the correct tubes, rinsing the beaker with a distilled water bottle in order to be sure that the entire sample is poured back into the tube. Fill up any empty space in the tubes with water to the 50 mL.

25 Repeat 21-24 for the rest of the egg samples, being sure to clean the beakers in between sample sets. Eggs will not hatch after being in the microwave.

Counting

26 Select a stained sample and pour it into a 400 mL beaker with a stir bar. Dilute the sample to 100 mL by adding 50 mL of water to the beaker. Place on a stir plate and stir.

27 Pipette 5 mL of stirred sample into counting dish.

28 Count the number of eggs and empty shells within the engraved area using a dissecting microscope. The engraved area in the dish represents ½ of the total dish area; multiply the counted number by 2 to get the number of eggs in the 5 mL sample.

29 Multiply the number of eggs per 5 mL sample by the dilution factor, typically 20, to get total number of eggs per 100cm3 of soil.

30 Record eggs/5mL, dilution factor, and total eggs per 100cm3 of soil. Discard sample. Rinse beaker in between samples.

31 Repeat steps 26-30 for the rest of the samples.

Sucrosing

STEP CASE

Sucrosing sample if sample is too dirty to count 7 steps

If samples are too dirty to count, they will need to be run through a 45% sucrose solution in order to accurately count them. Obtain a 1-L amount of 45% sucrose solution. More can be made if needed. Sucrosing samples can be done in sets of 4.

32 Select 4 egg samples, take off caps and make sure that there are equal amounts of liquid in each tube. Place the 4 samples into centrifuge so that weights are evenly distributed throughout the machine.

33 Set at a speed of 2800 rpm for 5 minutes (9 Acc/7 Decc) (setting 1). While waiting, ready the sucrose solution, a syringe, and a stirring utensil. While waiting, place four 500 sieves in the sink, set at an angle.

34 Carefully remove the samples from the centrifuge. Using a syringe, extract the water from the tubes, being careful not to disturb the sediment at the bottom.

35 Pour 45% sucrose solution into each tube up to 45 mL mark. Use a stirring utensil to suspend all material in the solution. Rinse stirring utensil before stirring next tube sample. Immediately after stirring, place tubes into the centrifuge so the weight is evenly distributed. Spin tubes for 1 minute at 1500 rpm (9 Acc/7 Decc) (setting 2).

36 As soon as the centrifuge stops spinning, remove samples and place into tube rack, careful not to disturb the samples. Pour liquid from the tubes and into a corresponding 500 sieve, ensuring not to disturb the plug at the base of the tubes. Thoroughly wash each sample in the sieves.

37 Rinse out each tube to remove all silt and sugar, and place back on tube rack. Pick up each sieve, rinse with distilled water bottle, and rinse into correct tube.

38 Store tubes in the refrigerator until counted. Repeat 32-37 for all samples.