Single Nematode DNA Extraction with Worm Lysis Buffer (WLB)

Overview

After collecting the samples of interesting or desired genus/species, nematodes need to be extracted from the soil using the appropriate method to maximize the number of nematodes extracted as well as the cleanliness of the sample/specimens. Once nematodes are extracted, then we should proceed with the sorting and picking of nematodes under a dissecting microscope. This protocol also has specific steps for image capturing of nematode under the compound microscope if morphological vouchers of the specimens are required prior to DNA extraction (see steps 5-9). Tubes should be always kept on ice during the entire procedure.

Materials

200 µl PCR tubes
70% Ethanol
Coverslips (rounds are better)
Glass slides
Hot Plate
Ice
Nematode pick (eyelash, mounted hooked insect pin, etc)
Small plastic petri dish
Small syringe

Procedure

1. Place extracted nematodes (i.e. bulk sample) in a labeled petri dish or watch glass. Under a dissecting microscope determine which individuals to capture.
2. Pick out nematodes of interest and place them in a new/clean and labeled embryo dish with PCR grade water (*salt water for marine samples*).
3. Place a small drop of water in the center of one glass slide
4. Using a nematode pick of your choice (eyelash, mounted hooked insect pin, etc) tease the nematode to the surface of the water and fish it out from the embryo dish.
5. Place nematode in the drop of water trying to sink it to the bottom of the water drop.
6. Place a coverslip at a 45° degree angle with the slide and slowly lower the cover slip over the drop of water containing the nematode (*this is to prevent the nematode from being smashed and from having bubbles under the cover slip*).
7. Move to the compound microscope for image capturing and take the syringe of water to the microscope and add extra water if the slide starts to dry out. If the nematode is very active and does not want to pose for its picture place the slide on the hot plate (55-60 °C) for 10 seconds to immobilize the nematode. Be careful not to leave the slide on the slide warmer too long.

8. Image capturing might be done using different lenses (e.g. 10x, 20x, 100x) depending on the size of the nematode as well as the purpose of the study (i.e. images can be later used for measurement of morphological features). In addition, image capturing might include full body length of specimens and key diagnostic features from the head and tail.
9. Once image capturing is finished, take the slide containing the nematode specimen back to the dissecting microscope and remove the coverslip by adding more water between the cover and the slide. Make sure to observe how the nematode moves when water is added so that the specimen does not get lost.
10. Using your nematode pick, fish the specimen from this slide and place in a new glass slide containing 5 µl of WLB. Depending on the nematode size, you might decide to cut the nematode into 2-3 pieces using a disposable scalpel or blade.
11. With an automatic pipette and a new 20 µl pipette tip transfer the nematode (or pieces) to a PCR tube containing additional 20 µl of WLB. If the nematode is not cut, then it can be also transferred to the PCR tube using the nematode pick.
12. Check the tube under the dissecting microscope and assure the specimen or pieces of it are in the tube. You might have to adjust up and down the focus of the microscope. Place your tubes on ice until the incubation step.
13. Once the desired number of nematodes has been acquired, you might proceed with the incubation step in the ThermoMixer (it can be also done using Thermo Cycler machine). It is important to warm up the equipment lid to 100-105 °C to avoid any sort of evaporation. Incubation will be carried out at 65 °C and 750 rpm for 2:00 hrs followed by 5 min at 100 °C. After that proceed, you might proceed with PCR, otherwise place the samples in - 20 °C for later use.

NOTE: Make sure to use new materials (e.g. scalpel, coverslip, glass slides) for each nematode specimen and clean the nematode pick with ethanol 70% before processing a new specimen.