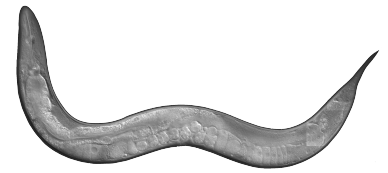




The Andersen Laboratory
Northwestern University
Department of Molecular Biosciences



Standard Operating Procedures

Bacteria-eating nematode isolation procedures

Foreign soil and plant debris includes everything collected outside of the continental United States of America, including but not limited to Hawaii, Puerto Rico, Guam, Mexico, Belize, Costa Rica, Guatemala, Nicaragua, Panama, Venezuela, Brazil, Chile, Argentina, Australia, New Zealand, Indonesia, Philippines, and Singapore. The details of this SOP are based on the conditions of the USDA soil permit and compliance agreement XXX, both issued to Erik Andersen, and must be closely followed. Any deviations from this SOP might lead to the removal of the foreign soil import permit.

This standard operating procedure (SOP) provides a detailed description of the protocols used by anyone collecting, handling, or analyzing foreign soil or plant debris in the isolation of *Caenorhabditis* nematodes in the Andersen Laboratory at Northwestern University.

The isolation of nematodes of the family Rhabditidae and the genera *Caenorhabditis*, *Oscheius*, and *Pristionchus* is quite easy because most species eat bacteria. Many morphologies, including body and pharynx shapes are morphologically distinct from plant parasitic nematodes. When presented with a ready source of bacterial food on an agar plate, Rhabditidae nematodes will leave soil or plant substrates to consume the bacteria (Figure 1). After a few hours on isolation plates, the plant and/or soil material can be removed leaving the nematodes behind.



This SOP can be divided into two parts: (1) isolation of nematodes in the field, or (2) isolation of foreign soil and rotting plant debris in the field then shipment to Northwestern University. Once the material is at Northwestern University, it will be processed in a biological safety level 3 (BSL3) facility, Pancoe building room 3353, to isolate the nematodes. Please see the final page for a description of the BSL3 facility.

Figure 1: Rotting plant debris is placed on agar plates with a lawn of *Escherichia coli* bacteria. The nematodes evacuate the rotting debris to eat the ready source of bacterial food. Plant debris is removed 24 hours later and disposed in the field or in the BSL3 facility's autoclave.

(1) Isolation of nematodes in the field

Permits must be obtained to collect materials from most foreign countries. Plan at least six months in advance to obtain these permits.

Soil and plant debris collection:

1. Identify rotting plant debris, take a digital photograph, and note GPS position before collection.
2. Don disposable nitrile gloves.
3. Label a plastic collection bag with the following information: picture number, date, time, and name of person collecting the sample.
4. Turn the bag inside out.
5. Place your hand inside the bag and pick up the rotting plant debris.
6. Carefully, seal the bag with extra air.

Isolation of bacteria-eating nematodes:

1. Label a fresh agar plate seeded with a bacterial lawn with the picture number, date, time, and name of person collecting the sample.
2. Remove the lid from the plate.
3. Place several pieces of rotting material on the surface of the plate around and touching the edge of the bacterial lawn.
4. Cover the plate with the lid. Oftentimes, the plate will not close all the way.
5. Keep the plate out of direct sunlight and away from scavenging animals for roughly 24 hours.
6. After 24 hours, remove the rotting material and dispose of it.
7. Seal the plate by wrapping parafilm around the outside of the plate.
8. Place the plate in a Ziploc bag and seal for shipment back to the Andersen Laboratory isolation facility.

(2) Isolation of foreign soil and rotting plant debris

Soil and plant debris collection:

1. Identify rotting plant debris, take a digital photograph, and note GPS position before collection.
2. Don disposable nitrile gloves.
3. Label a plastic collection bag with the following information: picture number, date, time, person collecting, and **Contents - Foreign soil and plant debris sample**.
4. Turn the bag inside out.
5. Place your hand inside the bag and pick up the rotting plant debris.
6. Carefully, seal the bag with extra air.
7. Package samples with extra packing materials to ensure safe passage. Double wrap samples in a Ziploc freezer bag for shipment.
8. Seal the box and all cracks with shipping tape.

Transport of foreign samples to Northwestern University:

Following field collection, all samples must be transported only by an individual noted on the USDA permit, Erik C. Andersen. All other individuals must ship their samples to the laboratory.

1. Samples must be carried in sturdy, leak-proof containers, sealed, and marked with a USDA soil and plant debris import sticker.
2. Include copies of the soil and plant debris import permit and the compliance agreement. These documents should be on the outside in the transparent shipping envelope.
3. Foreign samples will be released at the port of entry without treatment. ***However, foreign samples must be used only for nematode isolation performed in the Andersen laboratory in Pancoe 3353 Biological Safety Level 3 (BSL3) room.***
4. Under the conditions of this permit, soil may NOT be used for growing plants. Nematodes must be isolated, cultured, and identified before release from the BSL3 room.

Isolation of bacteria-eating nematodes from soil and plant debris:

This protocol should be followed in the BSL3 room (Pancoe 3353).

1. Label a fresh agar plate seeded with a bacterial lawn with the picture number, date, time, and name of person collecting the sample.
2. Remove the lid from the plate.
3. Place several pieces of rotting material on the surface of the plate around and touching the edge of the bacterial lawn.
4. Cover the plate with the lid. Oftentimes, the plate will not close all the way.
5. Keep the plate out of direct sunlight and away from scavenging animals for roughly 24 hours.
6. After 24 hours, remove the rotting material for disposal.
7. Using a platinum wire pick, transfer the nematodes off of the bacterial lawn to a fresh agar plate seeded with a bacterial lawn.
8. Seal the plate by wrapping parafilm around the outside of the plate.
9. After you observe offspring on the plate, lyse the nematode to prepare DNA.
10. Identify species using rhabditid PCR primers and Sanger sequencing.
11. Freeze the nematode strain using standard techniques and do a test thaw for viability.

Disposal of soil and rotting plant debris:

1. Place samples in 1 L Pyrex beakers.
2. Place beakers in autoclave.
3. Close autoclave door securely.
4. Turn on the autoclave.
5. Initiate a 30-minute cycle to sterilize the samples.
6. Wait until the pressure and temperature have dropped and the door can be opened.
7. Check to make sure that the autoclave tape has black lines indicating that the temperature and pressure have been sufficiently high as to ensure sterilization.

8. Once the beaker has cooled, pour the contents into a Ziploc bag.
9. Seal the bag and place bag in trash.

Biological safety level 3 facility (Pancoe 3353):

To gain access to this room, please contact Dr. Robert Lamb to get permission. The keys must be logged out and back in from above Olga Rozhok's desk in Cook Hall room XXX.

1. Make sure the green safety light is illuminated before entry.
2. Use the Marlok key to unlock the outer door (Figure 2).
3. Once inside, close the outer door before trying to open the inner door (Figure 3). You can not have both doors open at once.
4. Inside the room you will find a sterile biosafety hood (Figure 4), a closed circuit air system, no windows, a closed system water system (Figure 5), and an isolated autoclave (Figure 6).
5. Use the dedicated dissecting microscope to transfer nematodes from isolation plates to fresh agar plates.
6. Clean up after yourself!



Figure 2: outer door



Figure 3: inner door



Figure 3: sterile hood



Figure 4: sink



Figure 5: autoclave