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Protocol status: In development

We are still developing and optimizing this protocol

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Soil eDNA Extraction - Omega Mag-Bind / Opentrons OT-2

Stephen Douglas Russell[1]

[1]The Hoosier Mushroom Society

Stephen Douglas Russell

ABSTRACT

This protocol uses Opentrons OT2 automation and the Omega Mag-Bind Environmental DNA 96 kit. It assumes you are starting with a dried soil sample in a 50mL tube that contains beads, per this protocol.

MATERIALS

Equipment:

Opentrons OT-2 Robot - Opentrons - $9,000
Temperature Module (GEN2) - Opentrons - $3,750
Magnetic Module (GEN2) - Opentrons - $3,750
Opentrons 96-well Aluminum Block - Opentrons - $550
P300 8-Channel Electronic Pipette (GEN2) - Opentrons - $2,000

Homogenizer/Vortexer for 50 mL Tubes
Bead Genie - USA Scientific - $2,500
Omni Bead Ruptor w/ 50 mL Carriage - USA Scientific - $7,019 - Ebay - $2,500 - $5,000

Plate Centrifuge (96 well) - Ebay - $100 - $400
Eppendorf MixMate - New ~$2,400 - Ebay - $700 - $1,000

Consumables:

NEST 2 mL 96-Well Deep Well Plate - Opentrons - $220 + $50 shipping per 50 plates - $5.40 per plate
NEST 96 Well Plate 0.2 µL PCR Full Skirt - Opentrons - $247.50 + $55 shipping/tax - $3.03 per plate
NEST 12-Well Reservoirs, 15 mL - Opentrons - $220 + $50 shipping per 50 plates - $5.40 per plate
Opentrons 300uL Tips - Opentrons - $550 + $110 shipping/tax per 100 racks - $6.60 per rack
Opentrons 200uL Filter Tips - Opentrons - $687.50 + $133 shipping/tax per 100 racks - $8.21 per rack
Mag-Bind Environmental DNA 96 Kit - Omega Bio-Tek - $1,179.40 per 4 x 96 - $294.85 per 96 plate - $3.07 per sample

Initial Preparation

1 This protocol makes use of the Opentrons Omega Mag-Bind Environmental 96 protocol found here.

This protocol also assumes you have a dried soil sample in a 50 mL tube with beads already included per the sister sample collection protocol.

2

Sample Preparation - Omega Protocol

3m

3 Place the 50 mL tube with beads onto the bead beating/vortexing unit. Homogenize the sample the 50 mL tube for 00:05:00 . 5m

4 Before starting on the extraction process:
• Set an incubator to 70°C.
• Heat Elution Buffer to 70°C.
• Prepare an ice bucket and prechill P2 Buffer on ice.
• Dilute each bottle of VHB Buffer with 56 mL of 100% ethanol. (This only has to be done one time for the kit).

5 The next 13 steps are derived from the Mag-Bind Environmental Kit protocol.
QMF27.0248.M5645 v9.0 (1).pdf

Briefly centrifuge the E-Z 96 Disruptor Plate C Plus to remove any ceramic beads from the walls of the wells. Uncap the E-Z 96 Disruptor Plate C Plus and save the caps for use in Step 6 below.

6 Add 250 mg soil sample. Need way more here. XXXXXXXXX

7 Add 525 µL SLX-Mlus Buffer and 2 µL RNase A. Seal the plate with the caps removed in Step 4.

Note: SLX-Mlus Buffer and RNase A can be made as a mastermix.

8 Vortex at maximum speed for 00:05:00 to lyse and homogenize samples. For best results, a Mixer Mill, such as Spex Certiprep GenoGrinder 2010 (very, very expensive) or Eppendorf Mixmate (moderately expensive), should be used. 5m

Note: Complete homogenization is critical for best yields

9 Centrifuge at 500g for 10 seconds.

10 Remove and discard caps. Add 53 µL DS Buffer. Seal the plate with new Caps for Racked Microtubes.

11 Vortex to mix thoroughly.

12 Incubate at 70°C for 10 minutes. Briefly vortex the plate once during incubation.

13 Centrifuge at ≥ 2,000g for 10 minutes at room temperature.

14 Transfer 200 µL supernatant to a new set of 96-well Racked Microtubes.

15 Add 67 µL prechilled P2 Buffer and 100 µL cHTR Reagent. Seal the plate with new Caps for Racked Microtubes. Vortex to mix thoroughly.

Note: Prechill P2 Buffer on ice before use. Completely resuspend cHTR Reagent by shaking the bottle before use. If necessary, cut the pipet tip to transfer cHTR Reagent.

16 Centrifuge at ≥ 2,000g for 5 minutes.

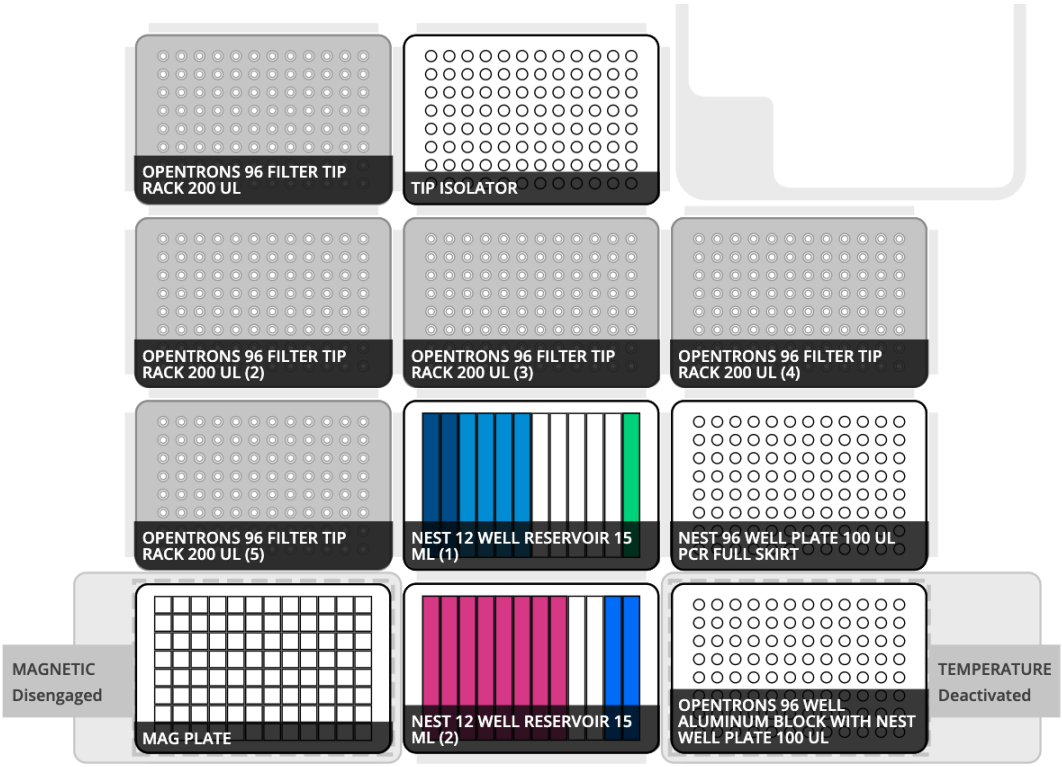
17 Transfer supernatant to a NEST 2 mL 96-Well Deep Well Plate.

Final Extraction via Opentrons

18 The remainder of this section of the protocol will be done with the Opentrons Omega Environmental Extraction protocol on an Opentrons device. The script for the protocol can be found here: 2c62b7.py based on the parameters below. You can alter these parameters and download a new version on the Opentrons protocol page.

The script has the following parameters that you may need to change depending on your use case.
Debug Mode: Enable
Sample Number: 96
P300-multichannel GEN2 mount: left
P20-multichannel GEN2 mount: right
P300 Tip Type: standard
Master Mix 2 Volume: 300
VHB Buffer Volume: 300
Elution Buffer Volume: 100
Magnetic Module Bead Settling Time: 5 minutes
Trash Tip Threshold (number of tips before emptying): 288

Process:
1. Input your protocol parameters above.
2. Download your protocol and unzip if needed.
3. If you have custom labware, upload your custom labware to the OT App by navigating to More > Custom Labware > Add Labware, and selecting your labware files (.json extensions).
4. Upload your protocol file (.py extension) to the OT App in the Protocol tab.
5. Set up your deck according to the deck map. (below)
6. Calibrate your labware, tiprack and pipette using the OT App. For calibration tips, check out our support articles.
7. Hit 'Run'.

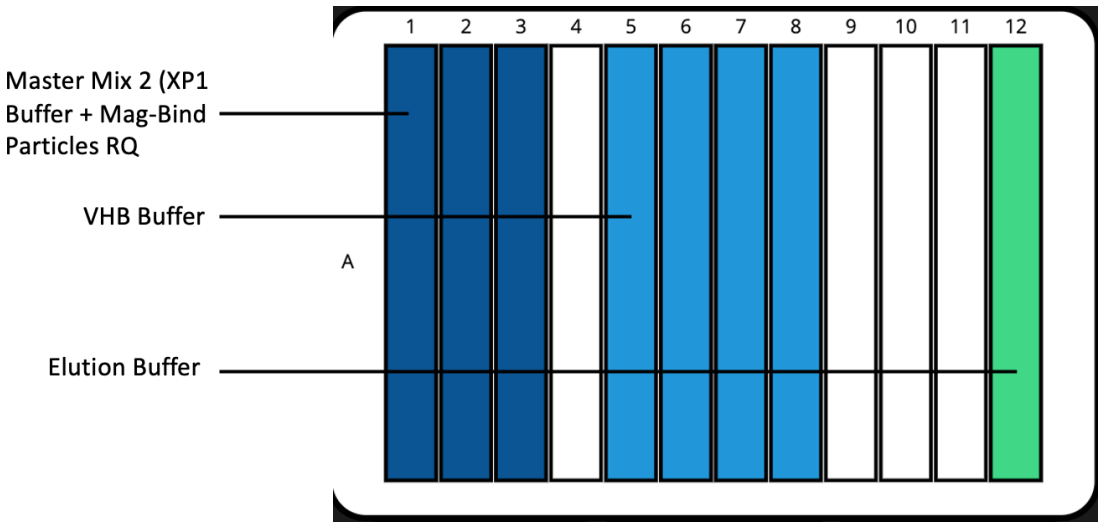


Note: The **Tip Isolator** is simply an empty Opentrons tip rack that is filled with water. The water should be filled up to the level that is enough for the tips to be slightly touching the surface of the water. The top of a standard Opentrons ti rack is detachable from the base (looks like a

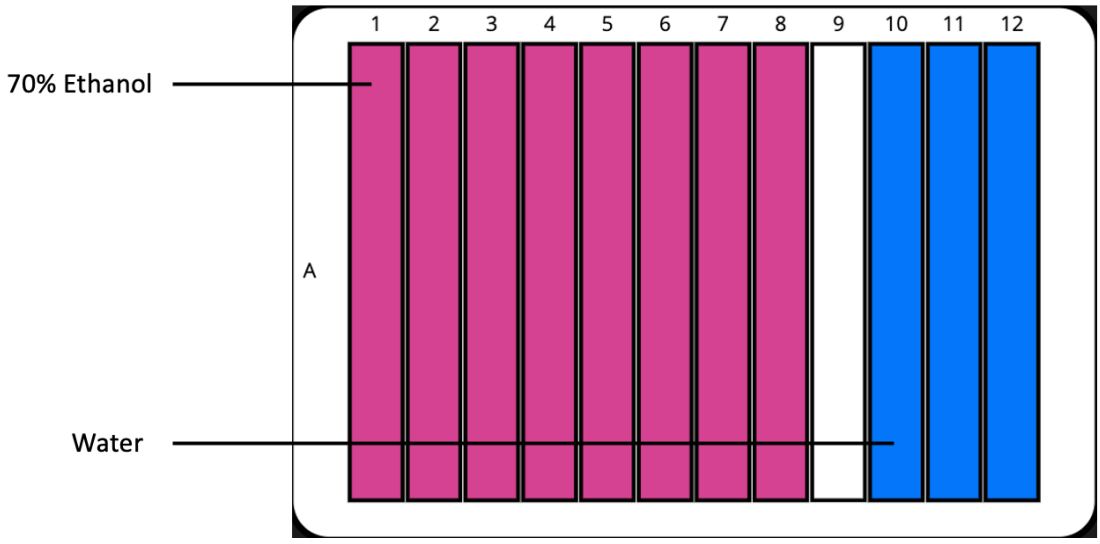
deep well plate). The tip isolator is used to conserve tips at specific steps.

Reagent Setup

- This section can contain finer detail and images describing reagent volumes and positioning in their respective labware. Examples:



Reservoir 1: Slot 5



Reservoir 2: Slot 2

19 The remainder of the protocol is done via automation. It is listed step-by-step so you can follow along with what the machine is doing.

Add 1 volume XP1 Buffer and 20 µL Mag-Bind Particles RQ (Prepared as Master Mix 2). Mixing is performed 10 times thoroughly, referred to as tip mixing (start mixing from the middle, then the bottom, then back to the top)

Note: The master mix is thoroughly mixed and then transferred to 3 columns at a time. It is then mixed again to prevent the beads from settling in the reservoir (repeats every 3 columns of samples)

20 Incubate at Room Temp for 10 minutes while mixing (increase yields). Each column is mixed 2 times via tip mixing. Park tips in the Tip Isolator.

21 Engage Magnetic Module and Delay for 5 minutes (settling time parameter) to allow mag beads to settle on the walls.

22 Aspirate supernatant and discard without touching the magnetic beads. Reuse tips from step 15 and discard tips.

23 Disengage Magnetic Module.

24 Add max volume (300uL on P300 Pipette) of VHB Buffer to NEST 2 mL 96-Well Deep Well Plate, V Bottom (Dispensed at a fast rate to agitate the beads).

25 Tip Mixing is performed and tips are conserved in the tip isolator. (Vortexing steps are substituted with Tip Mixing)

26 Engage Magnetic Module and Delay for 5 minutes.

27 Aspirate supernatant and discard without touching the magnetic beads.

28 Disengage Magnetic Module

29 Add 500 uL of 70% Ethanol to NEST 2 mL 96-Well Deep Well Plate, V Bottom

30 Tip Mixing is performed (reuse tips from step 20).

31 Engage Magnetic Module and Delay for 5 minutes.

32 Aspirate supernatant and discard without touching the magnetic beads.

33 Repeat 27-31 for a second ethanol wash

34 While the plate is on top of the Magnetic Module and is engaged, delay 1 minute and then remove any liquid.

35 While Magnetic Module is engaged, delay for 10 minutes to allow mag beads to dry.

36 Transfer Elution buffer from A12 of Reservoir 1 to plate on temperature module. Heat elution

buffer to 70C on the Temperature Module, then transfer 100 uL of elution buffer to samples.

37 Tip Mixing is performed (Tips are discarded).

38 Engage Magnetic Module and Delay for 2 minutes to allow mag beads to settle on the walls.

39 Transfer 100 uL of clear supernatant containing purified DNA to NEST 0.1 mL 96 Well PCR Plate, Full Skirt.

Purified DNA and Next Steps

40 You now have a plate of purified DNA that is ready to move to PCR amplification. Remove your plate from the Opentrons and seal it manually or with an automated sealer.

41 Store your DNA at -20C until it is ready for amplification.