SOP#105: Preparation for DNA Extraction of Soil Samples (>10 mL)

Purpose: To liberate DNA and tissue from soil samples greater than 10 mL in volume for subsequent filtration (SOP#102), extraction (SOP#100) and eDNA analysis (SOP#101).

Materials and Reagents Required:

Nitrile or Latex Gloves	50% Bleach solution in 400 mL beaker
Forceps, measuring scoopula or small spoon	dH_2O in 400 mL beaker for rinsing equipment
Kimwipes/ Clean Paper Towels	Autoclaved ddH_2O (450 mL per sample)
500mL Nalgene Bottles (one per 100 mL of soil)	50 mL Pipettes (one per soil sample)
Black Permanent Marker	Clean cheese cloth
Stainless steel scissors	

Estimated time: 2 to 24 hours for sample suspension and settling, 20 minutes per sample for filtering

Important Notes or Considerations:

1. Nitrile or latex gloves should be worn to avoid sample contamination. Between handling separate soil samples, gloves should be thoroughly wiped with 70% ethanol or changed for a fresh pair.
2. Benchtop should be wiped with a 10% bleach solution, followed by a 70%ethanol solution.
3. All implements should be rinsed well with a 50% bleach solution, then rinsed with copious amounts of distilled water and wiped with a paper towel in between handling each soil sample.
4. Nalgene bottles should be pre-labelled with a black permanent marker.

Methods:

1. Using a clean scoop, add up to 200 mL of soil sample into a Nalgene bottle pre-labelled with the sample name and DNA Processing Number (DPN).
2. Fill Nalgene bottle containing sample to 450 mL with autoclaved double distilled H_2O, secure cap and shake vigorously for 30 seconds.
3. Let sample settle for 2 to 24 hours at 4°C. Repeat steps 2-4 for the remaining samples.
4. Using sterilized scissors, cut clean cheese cloth into pieces at least twice the diameter of the Nalgene bottles. Stack cheesecloth pieces away from sample processing area to avoid cross contamination.
5. Set up filtration unit according to Helbing lab SOP#102. Sample water will be pipetted directly from the Nalgene bottles to filter funnels.

6. Once the suspended sample has settled, there may be organic matter floating at the top as well as settled at the bottom of the bottle. Insert a double layer of cheesecloth into the first sample bottle so that the cloth covers the surface of the water.
7. Use a 25 mL pipette to push the cheesecloth down, and **slowly** draw water through the cheesecloth. The cloth layers should prevent suspended particulate matter from clogging the pipette. If the pipette tip gets clogged, gently expel the water to flush the tip and resume drawing water. It is okay to transfer some solid material to the filter funnel.
8. Once filtration of a sample is complete, set bottle aside to dispose of solid sample matter later into yellow biohazard waste pails.
9. Process sample filters according to SOP#102. It should be noted that the filter resulting from this protocol will be exceedingly dirty and, after eDNA extraction (SOP#100), the DNA extract will often need cleaning with a OneStep PCR Inhibitor Removal Kit (Cat#D6030, Zymo Research, 17062 Murphy Ave, Irvine, CA 92614, USA). Use discolouration of DNA isolates to indicate whether the sample should be cleaned prior to IntegritE-DNA qPCR analysis.

Last Updated: November 2020 **Update Author:** Michael Allison