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HOLTGRIEVE ECOSYSTEM ECOLOGY LAB
PREPARATION OF SAMPLES FOR BULK CARBON AND/OR STABLE ISOTOPE ANALYSIS

INTRODUCTION

This protocol describes the process to prepare samples of muscle tissue, hair or soils for carbon and nitrogen stable isotope analysis. Samples are generally freeze dried, ground into a homogenous powder, and packed into isotope tins. The desired sample masses are set according to UW IsoLab machine limitations; these may need adjustment if samples will be run in another lab (i.e. OCN 446).

Note: if running on NACHO (OCN 446) standards also need to be packed. General guidelines on packing standards are also included in this document.

SAFETY

Chloroform, methanol, acetone, and ethanol are considered acutely toxic chemicals. Chloroform can affect the respiratory and central nervous systems with repeated and/or prolonged exposure. Methanol, acetone, and ethanol can induce dizziness and shortness of breath. All of the above chemicals are flammable. Always wear the proper personal protective equipment, including gloves and eye protection. Familiarize yourself with the MSDS and SOPs for all chemicals prior to starting this protocol.

MATERIALS

- Tins (tin or silver depending on desired analysis)
- Microspatula
- Microbalance (FSH 333) (key obtained from HEEL drawer by the computer)
- Forceps
- Ethanol or acetone in squeeze bottle
- Kimwipes
- Vials
- 96-well plates
- Samples
- Document for recording mass and sample location
- Gloves

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PREPARING REAGENTS

Be sure there is sufficient ethanol or acetone.

PREPPING SAMPLES FOR STABLE ISOTOPE ANALYSIS

1. Clean samples by removing all debris, shells, exoskeletons, bones, etc.
2. Freeze dry or oven dry (FSH 333)
3. Grind to fine powder. There are 3 methods one may use to grind:
    a. mortar and pestle
    b. Wig-L Bug (protocol to come)
    c. ball and mill grinder (FSH 333) *preferred
    d. scissors

PACKING SAMPLES INTO TINS

1. Check out microbalance in stable isotope prep room (FSH 333) in blue notebook on table by the balance.
2. Turn on microbalance, if other machines in the room (freeze dryer or oven) are running, you may want to allow the balance about 1 hour to warm up before using.
3. Make sure station and tools are clean before starting, you can do so by wiping down with kim wipes and acetone/ethanol
4. With clean forceps place tin on microbalance (for carbon/nitrogen bulk isotope analysis be sure to use tin tins, for hydrogen bulk isotope analysis use silver tins)
5. Tare microbalance with doors closed
6. Remove tin from balance and place desired mass of sample in tin (masses differ according to minimum nitrogen content of sample). Be careful not to spill any sample on to the microbalance. If sample spills use mini-vacuum (SUCTION ONLY) to clean.
7. Carefully place the tin back onto the microbalance to ceck the mass.
8. Record mass
9. Close tin by pinching top and folding top corners down to push sample to bottom of the tin with the forceps. Fold tin with forceps to small ball or cube with no sharp edges and place in labeled 96- well tray. Be careful not to puncture tin to prevent sample spillage. If you see or fear a crack in the tin **and no material has fallen out**, it is OK to put the entire tin in a new second tin and re-pack fold the new tin with the previous tin inside.
10. Record sample location and mass placement in tray.

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11. Clean tools with acetone/ethanol
12. Repeat steps 4-12 for subsequent samples.
13. Cover microbalance with plastic slip when done.
14. [If running EA interfaced with NACHO] Be sure to also pack standards (Glutamic Acid 1, Glutamic Acid 2, and salmon)

PREPPING STANDARDS FOR OCN 446 – CE Instruments 2500 NAElemental Analyser interfaced with Thermo Scientific Delta V Plus IRMS (NACHO) (assuming you are expecting somewhat normal d13C (-40 to -10) and d15N (-3 to 20))

Terms
Sequence: A discrete number that represents to total number of times the EA fires and moves material to the mass spectrometer.
Requisition: A single time the EA fires and moves material though the system and to the mass spectrometer.
Blank: Empty crumpled/folded tin (no material inside)
Blankblank: A requisition is completed but there is literally nothing in the specified well. Meant to give the sequence one extra run in the rare occasion that the autosampler does not advance.
Dummy: a tin with some mass (just to ensure peaks on machine)
GA1: Glutamic acid 1
GA2: glutamic acid 2
SAL: salmon

1. A full sequence of the carousal (50 drops) will consist of 24 samples/unknowns, and a series of 25 standards.
2. A sequence should start with a dummy, blank, GA1 QTY set, three standards, eight samples, three standards, eight samples with a blank in between(i.e. four samples -> blank -> 4 samples), three standards, eight samples, three standards, QTY set, blank, and a blankblank (nothing)
3. GA1 QTY set includes three GA1 tins with differing masses (0.14mg, 0.56mg, 0.70mg)
4. GA2 QTY set includes three GA2 tins with differing masses (0.14mg, 0.56mg, 0.70mg)
5. Three standards (STDs) include some combination of GA1, GA2, and SAL in a randomized order within the set.
6. Number of QTY sets will depend on number of sequences you are planning to run
7. Number of STD sets will depend on number of samples you are planning to run

SUBMISSION TO ISOLAB (JOHNSON 302-303)

1. Label trays
2. Submit request online

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3. Email list of sample ID, mass, tray position
4. Bring hard copy of sample mass and tray position to IsoLab when submitting samples

RUNNING ON NACHO (OSB 446)

1. Pack samples
2. Pack standards
3. Contact lab manager (Terry Rolfe on NACHO availability)
4. Consult Running NACHO for EA protocol

GUIDELINE TO SAMPLE MASSES

Target 40 μg of nitrogen and 200μg of carbon. When running both C and N, set the target for N as it is much easier to deal with too much C versus too little N.

Target masses for common materials:

- Animal tissue = 0.450 mg
- Collagen = 0.300 mg
- Soil = 2.000 mg
- Hair = 0.400 mg
- Plant = 1.500 mg
- Insect = 0.700 mg

To find the target mass of a different material/tissue, you need to know the % Carbon or Nitrogen (by mass) of your material and the instrument-specific target N mass (e.g., 40 μg).

- knowing the material is 8% N by mass (from previous literature): 0.04 mg = 8%N x sample.dry.weight
- 0.04/0.08 = 0.5 mg of sample (dry weight)
- Target mass of material = 0.340 mg

TISSUE TYPE SPECIFICS TIPS

Muscle tissue

1. Preparing
    a. Rinse with DI
    b. Oven or freeze dry
    c. Grind with mortar and pestle or ball and mill grind
2. Packing- 333 FSH

Soil

1. Remove rocks, twigs, debris as best as possible with sieves/forceps
2. Ball and mill grind each sample
3. Freeze dry samples to ensure full dryness

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4. Packing- 333 FSH

Hair

For cleaning hair samples:

2:1 chloroform:MeOH — Using a clean, ashed 500 mL graduated cylinder and working in the fume hood, first add 500 mL of chloroform to the designated 1 L glass bottle. Rinse cylinder with a few mL of methanol (MeOH) and discard into waste. Next add 250 mL of MeOH to the glass bottle.

1. Cleaning of hairs- FSH 232
    a. Place desired amount of hairs in tube
    b. Fill with chloroform: methanol in fume hood
    c. Vortex
    d. Place samples in water bath at 50 °C for 12-18 hours
    e. In fume hood pour out and dispose of chloroform: methanol – beware of hairs
    f. Rinse with another round of chloroform: methanol, vortex
    g. Add DI water, vortex, dispose with chloroform: methanol
    h. Rinse with DI water, vortex, pour off
    i. Dry samples in oven (FSH 333) at 60 °C 12-18 hours or whenever fully dry
2. Prepping hairs
    a. Use scissors to cut up hairs into small pieces
    b. Make sure all hairs get homogenized
3. Packing- 333 FSH