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HOLTGRIEVE ECOSYSTEM ECOLOGY LAB
PREPARATION OF BULK ISOTOPE SAMPLES

INTRODUCTION

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

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)
- Forceps
- Ethanol or acetone in squeeze bottle
- Kimwipes
- Vials
- 96-well plates

PREPARING REAGENTS

Be sure there is sufficient ethanol or acetone.

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PREPPING SAMPLES FOR BULK ISOTOPES

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 ISOTOPES

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 (carbon/nitrogen bulk isotopes be sure to use tin tins, for hydrogen bulk isotopes use silver tins)
5. Tare microbalance with doors closed
6. Remove tin from balance and place desired weight of sample in tin (weights differ according to minimum nitrogen content of sample). Be careful not to spill any sample on to the microbalance. If sample spills use vacuum (SUCTION ONLY) to clean.
7. Record weight
8. 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.
9. Record sample placement in tray.
10. Clean tools with acetone/ethanol
11. Repeat steps 4-12 for subsequent samples.
12. Cover microbalance with plastic slip when done.
13. [If running EA on NACHO] Be sure to also pack standards (Glutamic Acid 1, Glutamic Acid 2, and salmon)

SUBMISSION TO ISOLAB (JOHNSON 302-303)

1. Label trays
2. Submit request online
3. Email list of sample ID, weight, tray position

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4. Bring hard copy of sample weights and tray position to IsoLab when submitting samples

RUNNING ON NACHO (OSB 425)
Protocol to come...

GUIDELINE TO SAMPLE WEIGHTS

In accordance to UW IsoLab – target for 40μg of nitrogen and 200μg of carbon (can find in literature)

- 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

In accordance to EA on NACHO (OSB 425) – target for 0.04 mg nitrogen and .20-.20 mg for carbon

- Example of how to calculate target mass: 0.04 mg = 8%N x sample.dry.weight OR 0.04 mg = 12% x sample.dry.weight
- 0.04/0.08 = 0.5 mg of sample (dry weight)
- OR
- 0.04/0.12 = 0.33 mg of sample (dry weight)
- Animal tissue = 0.340 mg
- Huckleberry leaves = 1.000 mg
- Conifer needle = 2.000 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
4. Packing- 333 FSH

Hair

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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