STABLE ISOTOPE PREPARATION PROCEDURE

SAFETY: See associated COSSH form. Always wear PPE (lab coat, safety glasses and nitrile gloves). Inform safety officer of any spills.

7 % HCl – mild burns, irritant
Acetone – highly flammable, inhalation can cause dizziness, severe eye damage
First aid – run with water for ~20 minutes, seek medical help for eye incidents.

CHEMICALS REQUIRED:

- MilliQ water
- Acetone (Analar)
- 7 % hydrochloric acid (Analar)

EQUIPMENT REQUIRED:

- Pestle and mortar
- Foil
- Al caps
- Microscope
- Eppendorf tubes
- Drying cabinet/oven
- Crucibles or plastic pots
- Fine forceps
- Balance / scales

NOTES:

In general make sure lab area is clean! Wear latex gloves. Do not lean over samples/cores or breath on them (can wear face mask). Make sure all implements are clean and DO NOT use paper towels! but put everything on tin foil.

Use milliQ water

Use analytical grade chemicals only (not GPR)

To clean equipment:
Do not touch any tools by hand, always wear gloves and touch only handles etc. Scrub under running water, then acid wash (HCl, prepped for isotopes), then rinse 5x with milliQ. Either air dry or use analytical grade acetone.

Separate protocols exist for N and C as the acid treatment in C affects nitrate concentration, but is needed to remove carbonates which would otherwise affect C total. You can use a trial run to see if acid treatment affects your N readings, and if not use the C prep method.

PROCEDURE:

Subsample 0.5-1.0 cm^2 of sediment (amount will vary depending on how organic rich the material is, this volume is for gyttja).

Fill ultrasonic bath with milliQ water and put to heat.

For Nitrogen:

1. Transfer half of the sub-sample to a clean, labelled crucible or plastic pot and dry at <40°C. Alternatively, samples can be freeze-dried overnight if facilities are available.

2. Break up the sediment pellet and discard any rootlets or quartzy particles, using metal forceps (do not directly touch the sample). You may need to do this under a microscope.

3. Grind the dry sample to a fine powder, using a glass or agate pestle and mortar. Clean the pestle and mortar between samples by washing in distilled water with a brush, rinsing with acetone, sonicating in ultrapure water at about 45° C and allowing to dry with the residual heat from the sonicator water.

For Carbon:

1. You can put the samples in the ultrasonic bath to help separate the sediments for 10 minutes before sieving (make sure the heater is not on, or at least below 40°C).

2. Sieve the remaining half of the sub-sample at 500 µm using MilliQ water into a clean, 50cm3 centrifuge tube. Clean sieves in the ultrasonic bath in between uses (5-10min), do not touch the sieves – use the tongs to put in/out of bath then rinse 5x with MilliQ. Do not burn the sieves to clean them. Sieve back and forth between two or more tubes until sample finished. [Can probably skip this step!]

3. Discard rootlets, including any that have floated to the surface of the tubes.

4. Centrifuge, discard supernatant and if more than one tube has been used per sample reunite the sediments.

5. Make up a batch of 7% HCl using MilliQ water and analytical grade HCl. Add a small amount of 1M HCl, whirlimix, then top up with approximately 30cm3 of HCl.

6. Leave cold over-night (or warm for 30 min), discard any floating rootlets, then centrifuge, decant.

7. Wash with MilliQ water, whirlimix, centrifuge and decant. Repeat 5 washes (some say only wash 2-3 times). At each stage, discard any floating rootlets.

8. Whirlimix and leave to dry overnight at <40°C. Can whirlymix to spread up sides to aid drying – occasionally hard to then get sediments off side and scratch tubes.

9. Grind the dry sample to a fine powder, using agate or glass pestle and mortar.

It is REALLY important to grind samples to very fine powder and to make sure that the sample is homogenised – if it is not heavier bits will separate out in storage and this will affect the reproducibility of results.

Store all excess isotope samples in a dehumidifier.

Weigh out samples into tin/Al capsules using a fine balance. You will need a scoop, 2x tweezers, a clamper, a metal plate to hold the caps and air dusters with refill cans. Mass the samples to 5-20mg from test run. Place weighed capsule into labeled eppendorf tube.