Collagen Extraction for Stable Isotope Analysis  
(from Mark Clementz, UCSC)

Equipment/Chemicals

*Equipment* – scalpel, balance, 10 ml glass vials with Al foil lined caps, Al foil, glass beakers, 1-5 ml pipette with glass pipette tips, tweezers, tin capsules.

*Chemicals* – 0.5 N HCl, Chloroform (CHCl₃), Methanol (CH₃OH), DI water

Sample Preparation

1. Weigh out approximately 50 mg of bone or dentine from each specimen for prep, removing all surface tissue using a scalpel. Since bone and dentine are ~30% organic, this starting mass should generate ~15 mg of collagen for analysis, which is more than enough for a couple analyses on the mass spectrometer.

2. Place your samples in individually labeled, 10 ml glass vials. You should use Al foil lined lids for capping vials because glues and other organic residues on the inside of other caps may contaminate samples during the later stages of the extraction procedure. If foil-lined caps are unavailable, you can improvise by first covering the opening of the vial with piece of Al foil and then screwing on the lid.

3. The first stage in the extraction procedure is **decalcification** (i.e., removal of the inorganic mineral phase of the bone/dentine). We do this step so that we can get an accurate mass for the collagen present in the sample and also to remove any potential C contamination from carbonate preserved within the hydroxyapatite mineral of the bone. Add ~5 ml of 0.5 N HCl to the sample, agitate for ~10 minutes in a sonicator or shaker-table, then refrigerate for 2-3 days. To assess whether decalcification is complete, pull the sample from the vial using a pair of tweezers and check how flexible the sample is – when you can bend or squish your sample easily, decalcification is complete. If your sample remains rigid, change the acid and let it sit for an additional day or 2.

4. Once decalcification is complete, use a pipette to remove the HCl from the vial and rinse your sample 5 times with deionized (DI) water. When finished rinsing, pipette off as much water as possible from the sample in preparation for the next step.

5. The next stage of the extraction procedure is **lipid extraction**. Since lipids tend to yield δ¹³C values that are significantly lower than other tissues, it is VERY important to remove all lipids from samples prior to stable isotope analysis. To begin the lipid extraction, make an extraction mixture of chloroform, methanol, and water in the ratio of 1.0:2.0:0.8, respectively. You should plan on adding ~6 ml of extraction mixture to each sample, so calculate how much total mixture you will require for all samples and then mix the proper proportions of each liquid.

6. After adding solution to sample, sonicate for ~30 minutes.
7. Add ~2 ml of water to solution. The sample should separate into two distinct phases: an upper layer of methanol and water and a bottom layer of chloroform, which contains the lipids. A large amount of lipid in the chloroform phase will give it a milky appearance.

8. Decant solution from sample. Repeat lipid extraction until the chloroform phase is clear; for extremely fatty materials (i.e., marine mammal bones), it may be necessary to repeat this step 3 or 4 times. NOTE: During this portion of the extraction, samples may be allowed to sit overnight in the extraction mixture.

9. Once all lipids have been removed, rinse the sample 5 times with DI water and freeze-dry samples overnight. Samples are now ready to be analyzed on the Elemental Analyzer (EA) and mass spectrometer.

Sample Analysis
1. First, tare a tin capsule on the balance.
2. Carefully use the scalpel to cut small pieces from each specimen and weigh out 1.5±0.1 mg of the sample into the tin capsule.
3. While still on the balance, fold-over and crimp the opening of the tin capsule.
4. Place the tin capsule on a sheet of weigh paper or Al foil and using either tweezers or the special crimping tool, compress the sample into a compact ball, trying to remove as much air from the sample as possible. NOTE: We don’t want any air trapped in the tin capsule because the N\textsubscript{2} contamination would alter the δ\textsuperscript{15}N value obtained for the specimen.
5. If during compressing, the tin capsule tears, place the specimen (tin capsule and all) into a new tin capsule and repeat the process.
6. Once compressed, place the tin capsule into the sample tray and proceed to the next sample. Record the position of each sample in the tray on your sample data sheet.
7. When finished, place the tray in a desiccator cabinet until you are able to run your samples on the EA and mass spectrometer.