

DAY 1

**Wear a clean disposable lab coat**

Clean bone pulverizing equipment:

Locate cylinder, end pieces, and impactor

Scrub equipment using 20% (in-house) bleach or 10% stabilized bleach, warm Tergazyme, and/or DNA-Off

Place equipment into 50mL Falcon tubes containing DNA-Off or bleach for 20 minutes on the rotator

Rinse parts thoroughly with dH<sub>2</sub>O

After rinsing, fill Falcon tubes with dH<sub>2</sub>O and place back on rotator for 20 minutes

Dry down equipment using isopropyl wipes

Stratalink equipment for at least 15 minutes (45 minutes is optimal)

Clean dead space hood:

Fresh 20% (in-house) bleach or 10% stabilized bleach

Isopropyl wipe 1 pipettor (p1000)

Parafilm strips

UV for at least 15 minutes

Clean Air Clean Hood (bone/tooth cutting):

Fresh 20% (in-house) bleach or 10% stabilized bleach

Bleach/isopropyl wipe Dremel

UV for at least 15 minutes

Pipette:

1.7mL EDTA (850µL x2, kept over sink) into labeled extraction tube

**Clean:**

Dremel bit, forceps, and scissors (if used) using bleach and isopropyl wipes

Stratalink for at least 15 minutes:

6 extraction tubes (2.0mL) labeled RB, Q, and 4 powder tubes

extraction tube (2.0mL) with 1.7mL EDTA

15mL Falcon tube for RB's

2 cotton swabs

*Approved by Director: Dr. Guy Vallaro*

Cutting disc  
2 sanding discs  
Dremel bit  
Screwdriver  
Scissors (if used)  
Forceps  
2 sheets of weigh paper folded diagonally

In dead space hood:

Move everything from the Stratalinker to the dead space hood  
Swab the inside of the cylinder, end pieces, and impactor  
Cut ~1/2-1/3 of swab into RB tube, place remainder in 15mL Falcon tube (snap off shaft)  
Add 1.6mL EDTA to RB (800µL x2)  
Parafilm tube, place on room temperature nutator overnight  
Repeat swabbing of parts with 2<sup>nd</sup> swab, place entire swab into the 15mL Falcon tube (snap off shaft)  
Store tube of swabs at 4°C (short term) or -20°C (long term)  
Assemble the cylinder, end pieces, and impactor

Transfer:

Dremel bit, screwdriver, and sanding/cutting discs to Air Clean Hood

**Take out evidence:**

Photograph evidence  
Document a description on Worksheets

In Air Clean Hood:

**Wear goggles and change gloves immediately after cutting**

Place bone/tooth in hood  
Screw sanding disc onto Dremel bit  
Sand the exterior surface of the bone/tooth (use the second disc if necessary)  
Screw the cutting disc on

For a tooth: Remove the root(s) (cut at the dentin)

For a bone: Remove ~1-2cm X 1-2cm section (make a wedge-shaped cutting)

\*Remember to take a photograph of the evidence after you take a cutting- this includes any soft tissue and/or bone marrow that is removed from the evidence.

Pulverize sample:

Transfer cylinder, end pieces, and impactor (already assembled) to the counter

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**State of Connecticut Department of Emergency Services and Public Protection  
Division of Scientific Services**

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Place excised portion of sample into cylinder under the impactor  
Place the cylinder into the freezer/mill

**Grab the Cryogenic gloves, and keep on the goggles**

(Liquid Nitrogen located downstairs in the Chemical room)

Fill the freezer/mill with liquid Nitrogen to the “fill line”  
Close the freezer/mill and allow the liquid Nitrogen to freeze the sample for 10 minutes  
After 10 minutes, open the freezer/mill and re-fill with liquid Nitrogen to the “fill line”

Using the touch LED screen, set the program as follows: Pre cooling period- 1 minute, Running period- 5 minutes, Cooling period- 2 minutes, Rate 15 cps, Cycle- 1

Press anywhere on the main screen

Press on the “Settings” tab

Move the bar along the time line to the desired settings

Once done, press “Return” tab

Press “Start” tab

When the freezer/mill is done with the cycle, remove the cylinder (use the cryogenic gloves)

Open the cylinder with the extractor and transfer the powder onto the weigh paper

Place enough powder for a single extraction into each tube (~1/8- 1/4 of a 2.0mL tube)

Store powder tubes at 4°C (short term) or -20°C (long term)

Cleanup:

Bleach/isopropyl wipe Dremel

Place discs, plastic cylinder, and weigh papers into biohazard waste

Scrub Dremel bit, impactor, and end pieces with warm Tergazyme and/or bleach

Repeat initial DNA-Off or bleach / dH<sub>2</sub>O cleaning of end pieces and impactor

Change pre-filter in Air Clean Hood

Bleach down Air Clean Hood and counters

Place disposable lab coat, pre-filter, and gloves in biohazard box

Bleach and clean out dead space hood

Perform at **END OF DAY** to avoid over-decalcification:

Clean dead space hood using 20% (in-house) bleach or 10% stabilized bleach

Isopropyl wipe pipettor (p1000)

Pipette 1.7mL EDTA into a labeled extraction tube (2.0mL)

Stratalink tube of EDTA and 1 piece of parafilm

UV hood for at least 15 minutes

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Add 1.6mL EDTA to powder tube (800 $\mu$ L x2)  
Parafilm tube and invert to thoroughly mix the liquid and powder  
Place on room temperature nutator for 8-16hrs (up to 24hrs acceptable)

## **DAY 2- Morning**

Clean Laminar Flow Hood: Fresh 20% (in-house) bleach or 10% stabilized bleach  
Isopropyl wipe 3 pipettors (p2, p200, p1000)  
UV at least 15 minutes

Make SEB/DTT: 5mL SEB (pre-aliquoted) + 0.03g DTT (refrigerator) in 15mL Falcon tube

Stratalink: SEB/DTT, dH<sub>2</sub>O in 15mL Flacon tube, 1.5mL rack  
Stratalink for at least 15 minutes

Extraction: Remove parafilm from RB, place in centrifuge  
Repeat for Q  
Spin for 1 minute at 8000 rcf

### **For the remainder of the procedure:**

**Manipulate RB tube and place into centrifuge before touching Q tube**  
**Change gloves each time after handling Q tube**  
**Apply UV to Laminar Hood during spins**

Pipette off EDTA from RB using p1000/p200  
Add 1mL dH<sub>2</sub>O, place in centrifuge  
Repeat for Q, invert/flick to re-suspend (do not vortex)  
Repeat spin

Pipette off dH<sub>2</sub>O from RB using p1000/p200  
Add 1mL dH<sub>2</sub>O, place in centrifuge  
Repeat for Q, invert/flick to re-suspend (do not vortex)  
Repeat spin

Pipette off dH<sub>2</sub>O from RB using p1000/p200  
Add 1mL dH<sub>2</sub>O, place in centrifuge  
Repeat for Q, invert/flick to re-suspend (do not vortex)  
Repeat spin

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Pipette off dH<sub>2</sub>O from RB using p1000, p200  
Add 300µL SEB/DTT, 2µL proK to RB  
Repeat for Q, invert/flick to re-suspend  
Place on nutator in 56°C incubator w/ FAN ON 6-8 hrs (up to overnight)

**DAY 2- Afternoon (or DAY 3- Morning)**

Turn on 70°C heat block

Clean Laminar Flow Hood: Fresh 20% (in-house) bleach or 10% stabilized bleach  
Isopropyl wipe 2 pipettors (p200, p1000)  
UV at least 15 minutes

Ensure AW1 and AW2 have been properly prepared (add 125mL EtOH to AW1 and 160mL EtOH to AW2)

Prepare rack of: 7 extraction tubes labeled AL, EtOH, AW1, AW2, AE, RB, and Q  
Spin basket in an extraction tube  
8 waste tubes  
2 columns labeled RB, Q  
Aliquot 1.2mL EtOH, AW1, AW2; 750µL AL; 150µL AE

Stratalink: Rack with aliquoted reagents, tube w/ basket, waste tubes, columns for at least 15 minutes

Extraction: **Manipulate the RB tube and place into centrifuge before touching Q tube**  
**Change gloves each time after handling Q tube**  
**Apply UV to laminar hood during spins**

Pulse spin tubes

Transfer RB swab into basket using a pipette tip, place basket back into original tube, and spin for 3 minutes at 10,000rcf

Discard basket into biohazard bin

Add 300µL AL to each tube, invert mix  
Place into 70°C heat block for 10 minutes

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Add 400 $\mu$ L EtOH to each tube

Transfer 500 $\mu$ L EtOH/sample mix to column, place in centrifuge

Spin for 1 minute at 8000 rcf

Transfer to a new waste tube

Add remainder of sample to the column

Repeat spin

Transfer to new waste tube

Add 500 $\mu$ L AW1

Repeat spin

Transfer to new waste tube

Add 500 $\mu$ L AW2

Spin for 3 minutes at full speed (13,000 rcf)

Transfer to new waste tube

Spin for an additional 1 minute at full speed (13,000 rcf)

Transfer column to an extraction tube

Add 60 $\mu$ L AE\*

Allow to sit at room temperature for 5 minutes

Spin for 1 minute at 8000 rcf

Using a sterile pipette tip, determine the volumes of the RB and the sample extracts. The elution volumes shall be documented manually on QRM-5. The volume of the RB must not exceed the volume of the sample. If necessary, add AE buffer to bring the sample up to the volume of the RB.

\*Note: If you add 66 $\mu$ L AE instead of 60 $\mu$ L, you usually do not need to adjust the final volume.