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_20
After rinsing, fill Falcon tubes with dH_20 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(s)

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
*adjust the number of labeled tubes if more than one RB tube will be extracted
Extraction tube(s) (2.0mL) with 1.7mL EDTA
15mL Falcon tube for RB's
2 cotton swabs

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

<u>In dead space hood:</u>

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 swab (800µL x2)
Add 1.6mL EDTA to additional RB tubes as necessary (800µL x2)
Parafilm tube(s), place on room temperature nutator overnight
Repeat swabbing of parts with 2nd swab, place entire swab into the 15mL Falcon tube (snap off shaft)
Store tube of swabs at 4°C (short term) or -20ºC (long term)
Assemble the cylinder, end pieces, and impactor

<u>Transfer:</u>

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

Take out evidence:

Photograph evidence
Document a description on Worksheets

<u>In Air Clean Hood:</u>

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
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 -20ºC (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_20 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 labeled extraction tube(s) (2.0mL)
Stratalink tube(s) of EDTA and piece(s) of parafilm
UV hood for at least 15 minutes

Add 1.6mL EDTA to powder tube(s) (800µL x2)
Parafilm tube(s) 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_20 in 15mL Flacon tube, 1.5mL rack for at least 15 minutes

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

For the remainder of the procedure:
Manipulate RB tube(s) and place into centrifuge before touching Q tube(s)
Change gloves each time after handling Q tube(s)
Apply UV to Laminar Hood during spins

Pipette off EDTA from RB(s) using p1000/p200
Add 1mL dH_20, place in centrifuge
Repeat for Q(s), invert/flick to re-suspend (do not vortex)
Repeat spin

Pipette off dH_20 from RB(s) using p1000/p200
Add 1mL dH_20, place in centrifuge
Repeat for Q(s), invert/flick to re-suspend (do not vortex)
Repeat spin

Pipette off dH_20 from RB(s) using p1000/p200
Add 1mL dH_20, place in centrifuge
Repeat for Q(s), invert/flick to re-suspend (do not vortex)
Repeat spin

Pipette off dH_20 from RB(s) using p1000, p200
Add 300µL SEB/DTT, 2µL proK to RB(s)
Repeat for Q(s), 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 (more will be needed if multiple tubes of RB and powder are used)
2 columns labeled RB, Q
Aliquot 1.2mL EtOH, AW1, AW2; 750µL AL; 150µL AE

*Adjust number of AL and EtOH tubes if utilizing multiple RB and powder tubes.

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

Extraction: **Manipulate the RB tube(s) and place into centrifuge before touching Q tube(s)**
Change gloves each time after handling Q tube(s)
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

Add 400µL EtOH to each tube
Transfer 500µL 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
*Repeat above as needed until all EtOH/sample mix have been added to the column

Transfer to new waste tube
Add 500µL AW1
Repeat spin

Transfer to new waste tube
Add 500µL 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 60µL 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 66µL AE instead of 60µL, you usually do not need to adjust the final volume.