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

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 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

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

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 pipettes (p2, p200, p1000)
UV at least 15 minutes

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

Stratalink:
Extraction Buffer/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 Extraction Buffer/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)

Stratalink: Rack with dH_20
Microcon sets (filter and tube)
Additional Microcon tubes (according to how many RB and sample tubes you have).

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

For the remainder of the procedure manipulate the RB tube and place into centrifuge before touching Q tube. Change gloves each time after handling Q tube. Apply UV to hood during spins.

Pulse spin tubes that have been incubating to collect condensate.

Add 300 µL PCIA to each tube, vortex, and spin for 3 minutes at 10,000 g (rcf).

Add 100 µL of dH_2O to Microcon set while waiting for tubes to spin.

Pipette off supernatant and add to Microcon set, spin for 5 minutes at 500-3,000 g (rcf).

Transfer filter to new Microcon tube, add 400 µL of dH_2O, and spin for 5 minutes at 500-3,000 g (rcf).

Add 60 µL of dH_2O to the filter, invert into a new Microcon tube, vortex, and spin for 3 minutes at 10,000 g (rcf).

Using a sterile pipette tip, determine the volumes of the RB and the sample extracts. The elution volumes shall be documented manually on DNA QR-341. The volume of the RB must not exceed the volume of the sample. If necessary, add dH_2O to bring the sample up to the volume of the RB.

Transfer RB extracts into one new Microcon set. Transfer the sample extracts into another Microcon set. Spin for 5 minutes at 500-3,000 g (rcf).

Add 60 µL of dH_2O to the filter, invert into a new Microcon tube, vortex, and spin for 3 minutes at 10,000 g (rcf).

Using a sterile pipette tip, determine the volumes of the RB and the sample extracts. The elution volumes shall be documented manually on DNA QR-341. The volume of the RB must not exceed the volume of the sample. If necessary, add dH_2O to bring the sample up to the volume of the RB.