PART 1

Turn on 56°C heat block

Make Tergazyme: 10mL dH_20 + 0.5g Tergazyme (drawer) in 15mL Falcon tube

Clean grinder: Pour ~5mL Tergazyme into beaker, warm it on heat table (do not boil)
Clean grinder with swab/ warm Tergazyme, simulate grinding, rinse with dH_20
Repeat at least 2 times
Add 400µL 1 N H_2S0_4 and let sit for 20 minutes (in acid hood)

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

Stratalink: Falcon tube of- Tergazyme, Histoclear, 100% Ethanol, dH_20, SEB/DTT
10 screw-top tubes labeled- Tergazyme (2 tubes), Histoclear,
EtOH (2 tubes), dH_20 (3 tubes), Q1, RBQ1
Stratalink for at least 15 minutes

Clean Dead Space Hood: Fresh 20% (in-house) bleach or 10% stabilized bleach, Kimwipe,
Falcon tube rack
Isopropyl wipe- tweezers, ruler, scissors/scalpel, 3 pipettors (p10, p200, p1000)
UV at least 15minutes

Dump out acid from grinder, rinse with dH_20
Pulse spin, pipette off excess liquid in hood
Stratalink grinder for at least 15 minutes

In dead space hood: Aliquot 1mL Terg., Histoclear, EtOH, and dH_20 into labeled screw-top tubes
Add 200µL SEB/DTT to grinder, simulate grinding, transfer to RBQ1 tube
Add 1µL proK, place tube into 56°C heat block

Take out evidence and fill out Worksheets

In dead space hood: Measure hair in hood

Remove ~2cm from root end (if apparent) or more if needed (at the discretion of the analyst)

Transfer to 1mL Histoclear screw-top tube and sonicate for 20 minutes

Transfer hair to EtOH screw-top tube, invert 3-4 times

Transfer hair to dH_20 screw-top tube, invert 3-4 times

Transfer hair to 1mL Tergazyme screw-top tube and sonicate for 20 minutes

Transfer hair to dH_20 screw-top tube, invert 3-4 times

Transfer hair to new 1mL Tergazyme screw-top tube and sonicate for 20 minutes

Transfer hair to EtOH screw-top tube, invert 3-4 times

Transfer hair to dH_20 screw-top tube, invert 3-4 times

Add 200µL SEB/DTT to grinder, transfer hair to grinder

Grind, pipette fluid to tube, add 1µL proK

Place into 56°C heat block for 2hrs up to Overnight

PART 2

Stratalink: Rack with dH_20, 2 Microcon sets (filter and tube), 4 additional Microcon tubes

Clean Laminar Hood: Fresh 20% (in-house) bleach or 10% stabilized bleach, Kimwipe, Falcon tube rack
Isopropyl wipe- 2 pipettors (p200, p1000)
Autoclaved beaker to heat dH_20
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 at 56°C to collect condensate

Add 200µL PCIA to each tube, vortex, and spin for 3 minutes at 10,000g (rcf)

Add 200µL of dH_20 to Microcon set while waiting for tubes to spin

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

Transfer filter to new Microcon tube, add 400µL of dH_20, and spin for 5 minutes at 3,000g(rcf)

Pour ~1mL dH_20 into the beaker and heat until boiled (~3-5 minutes)

Add 60µL of the heated dH_20 to the filter, invert into a new Microcon tube, vortex, and spin for 3 minutes at 10,000g (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-4. 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.