

*Approved by Director: Dr. Guy Vallaro*

## **PART 1**

Turn on 56°C heat block

Make Tergazyme: 10mL dH<sub>2</sub>O + 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, rinse with dH<sub>2</sub>O

Repeat at least 2 times

Add 400µL 1 N H<sub>2</sub>SO<sub>4</sub> 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- 100% Ethanol, Tergazyme, dH<sub>2</sub>O, SEB/DTT

5 screw-top tubes labeled- Tergazyme, EtOH, dH<sub>2</sub>O, Q1, RBQ1

Stratalink for at least 15 minutes

Clean Dead Space Hood: Fresh 20% (in-house) bleach or 10% stabilized bleach, Kimwipe,

Falcon tubetrack

Isopropyl wipe- tweezers, ruler, scissors/scalpel, 3 pipettors (p10, p200, p1000)

UV at least 15minutes

Dump out acid from grinder, rinse with dH<sub>2</sub>O

Pulse spin, pipet off excess liquid in hood

Stratalink grinder for at least 15 minutes

In dead space hood: Aliquot 1mL Terg., EtOH, and dH<sub>2</sub>O 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 Tergazyme tube

Sonicate 20 minutes (additional sonications may be required)

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Transfer hair to EtOH screw-top tube, invert 3-4 times  
Transfer hair to dH<sub>2</sub>O 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<sub>2</sub>O

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<sub>2</sub>O  
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 200µL PCIA to each tube, vortex, and spin for 3 minutes at 10,000g (rcf)

Add 200µL of dH<sub>2</sub>O to Microcon set while waiting for tubes to spin

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

Transfer filter to new Microcon tube, add 400µL of dH<sub>2</sub>O, and spin for 5 minutes at 500-3,000g (rcf)

Pour some dH<sub>2</sub>O into the beaker and heat until boiled (~3-5 minutes)

Add 60µL of the heated dH<sub>2</sub>O 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<sub>2</sub>O to bring the sample up to the volume of the RB