Blood/Buccal Extraction

PART 1

Turn on 56°C heat block

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

Stratalink: SEB/DTT, 2 extraction tubes labeled RBK1, K1

Clean Laminar Hood: Fresh 20% bleach (in house) or 10% stabilized bleach, Kimwipe, Falcon tube rack
Isopropyl wipe: tweezers, ruler, scissors/scalpel, 2 pipettors (p2, p1000)
UV at least 15 minutes

In Laminar Hood: Add 300µL SEB/DTT, 2µL proK to RBK1, K1 tubes
Place RBK1 tube into 56°C heat block

Take out evidence and fill out Worksheets
Bring evidence into Hood

In Laminar Hood: Add ~3mm x 3mm section of bloodstain or ~1/3- 1/2 of buccal swab to K1 tube
Vortex and spin down
Place K1 tube into 56°C heat block for 2hrs up to Overnight

PART 2

Stratalink: A rack with dH_2O
Spinease basket, extraction tube to hold basket
2 Microcon sets (filter and tube)
4 additional Microcon tubes

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

Pulse-spin tubes that have been incubating to collect condensate

Using a sterile pipette tip, transfer cutting to basket, place basket into original extraction tube, spin for 30 seconds at full speed

Discard basket into biohazard bin

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

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

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

Pipet 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_2O, and spin for 5 minutes at 500-3,000g (rcf)

Add 60µL of dH_2O to the filter, invert into 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.