

3.1 PURPOSE:

- 3.1.1 To isolate and purify DNA from body fluids and tissue samples.

3.2 RESPONSIBILITY:

- 3.2.1 Forensic Science Examiners from the CT DESPP Division of Scientific Services who have been trained in the discipline of Mitochondrial DNA Analysis according to the Mitochondrial DNA Section Training Manual.

3.3 SAFETY:

- 3.3.1 Use appropriate measures for the proper handling of physical evidence following the Mitochondrial DNA General Evidence Procedures (mtDNA SOP-01) and according to the Laboratory Safety Manual.

3.4 Extraction from Body Fluids and Tissues

- 3.4.1 Body fluid samples can be transferred onto swabs, cotton swatches, FTA paper, or filter paper. Alternatively, liquid blood may be extracted directly. Tissue samples may be rinsed briefly in 1% SDS prior to extraction based on the nature of the evidence. This is done by placing the tissue in a 15 ml capped, sterile, disposable tube and inverting several times. As an alternative to PCIA extraction and MicroconTM 100 filtration, the DNA extract may be purified using the QIAamp procedure described in mtDNA SOP-04, section 11.
- 3.4.2 Prepare a reagent blank for each extraction procedure by adding ~ 300 µL of SEB/DTT and 2 µL of proteinase-K [600 U/mL] to a 2 mL microcentrifuge tube. Briefly vortex and place in designated heat block and incubate at 56°C for 2 hours to overnight.
- 3.4.3 Add ~ 300 µL of SEB/DTT to a 2 mL microcentrifuge tube. Add sample to tube as follows. For stains or tissue samples, use a small portion (approx. 3 mm x 3 mm). For buccal swabs, use approximately 1/3 of the swab. For liquid blood, use 5 µL.
- 3.4.4 Add 2 µL of proteinase-K [600 U/mL]. Briefly vortex and spin in a microcentrifuge to force the sample into the liquid.
- 3.4.5 Incubate the tube at 56 °C for 2 hours to overnight. Briefly spin the tubes in a microcentrifuge.

- 3.4.6 Transfer the cutting, swab, or remaining undigested tissue into an extraction tube filter basket insert. Place the filter insert into the same tube containing the stain extract. Spin at full speed for 30 seconds. Remove and discard the cutting and the filter.
- 3.4.7 Add ~ 300 µL PCIA to the reagent blank and sample tubes.
- 3.4.8 Vortex briefly and centrifuge for 3 minutes at 10,000 X g. Perform additional spins as needed. Repeat PCIA step as needed.
- 3.4.9 Transfer the aqueous phase to a Microcon™ 100, add dH₂O to bring the volume in the Microcon™ 100 to 400 µL.
- 3.4.10 Spin the Microcon™ 100 concentrators for 5 minutes at a setting of 500-3000 X g. (Note: additional spin time and/or higher speed may be required to filter the entire volume.)
- 3.4.11 Discard the wash tube and move the Microcon™ 100 to a new tube.
- 3.4.12 Add ~ 400 µL of dH₂O to the Microcon™ 100 concentrator.
- 3.4.13 Spin again at a setting of 500-3000 X g for 5 minutes. Discard the wash tube and move the Microcon™ 100 to a new tube. (Note: additional spin time and/or higher speed may be required to filter the entire volume.) Perform additional washes as needed.
- 3.4.14 Add ~ 60 µL dH₂O to the Microcon™ 100.
- 3.4.15 Invert the Microcon™ 100 into a new tube, vortex briefly and spin in a microcentrifuge at a setting of 10,000 X g for 3 minutes.
- 3.4.16 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 dH₂O to bring the sample up to the volume of the RB.
- 3.4.17 Store extracted DNA at 4 °C.
- 3.4.18 The samples can be amplified using the following dilutions in dH₂O as a general guide:
- 3.4.18.1 Heavy blood stains on cotton sheeting, FTA paper, or filter paper: 1:50
- 3.4.18.2 Light blood stains on cotton sheeting, FTA paper, or filter paper: 1:10

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- 3.4.18.3 Liquid blood: 1:50
- 3.4.18.4 Any blood stain on gauze: 1:10
- 3.4.18.5 1/3 of a buccal swab: 1:50
- 3.4.18.5 Buccal sample on FTA card 1:50

- 3.4.19 See mtDNA WI-01 for further instructions on DNA extraction of blood and buccal samples.

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