# Procedure for Organic DNA Extractions

**1.0 Purpose -** To specify the steps for performing organic DNA extractions.

**2.0 Scope** - This procedure applies to DNA Database Forensic Scientists in the DNA Database Section who perform organic DNA extractions.

**3.0 Definitions**

- **Ethylenediaminetetraacetic acid (EDTA):** A component of the reactions used in the lysis process, which inhibits nuclease activity.
- **Known:** Biological material whose identity is established.
- **Organic DNA Extraction:** A method that uses organic chemicals (phenol/chloroform/isoamyl alcohol) to isolate genomic DNA.
- **Proteinase K (Pro K):** A proteolytic enzyme that reduces proteins to their constituent amino acids. In particular, ProK removes the histone groups that keep the DNA tightly bound within the cell. The enzymatic activity of ProK lasts for approximately two hours and eventually self-digests.
- **Sodium dodecyl sulfate (SDS):** Serves to rupture the cell nuclear membrane to expose the nucleic acids. It also assists in the denaturation of the nuclear proteins that are attached to the DNA.

**4.0 Equipment, Materials, and Reagents**

- Stain Extraction Buffer (SEB)
- Tris/EDTA Solution (TE)
- Proteinase K Solution (ProK)
- Phenol/Chloroform/Isoamyl Alcohol (PCI)
- Sterile scissors and hand-held hole punchers
- Centrifuge
- Heat Block with calibrated thermometer
- Calibrated pipettes (various sizes)
- ART Pipette Tips (or equivalent, various sizes)
- Autoclaved 1.5 mL microcentrifuge tubes
- Microcon 100 Filters and corresponding centrifuge tubes (or equivalent)
- Vortex mixer
- Certified biosafety cabinet
- Various lab equipment (various disposable conical tubes, lab tape, lab coat, microcentrifuge tubes and racks, wipes, etc)
- 10 % Bleach solution
- 70% Isopropyl Alcohol

**5.0 Procedure**

**5.1 Overview**

**5.1.1** Negative extraction control

**5.1.1.1** For each extraction, a reagent blank shall be prepared. This blank will consist of the reagents used in the extraction process and be treated the same as any other sample throughout the entire process. Also, the final volume of this control shall be the same as

the sample(s) brought up in the most minimal volume and amplified using the maximum volume.

**5.1.1.2** If a dilution is made of a sample prior to amplification, a dilution of the corresponding negative control does not have to be made if the same lot of TE is used for the Negative Amplification Control.

**5.1.1.3** It is acceptable to run more than one reagent blank..

### 5.2 Organic Extractions of Blood and Epithelial Cells

**5.2.1** All tubes shall be labeled with a unique identifier, date of extraction, and analyst initials.

**5.2.2** Aseptically place the sample in a labeled microcentrifuge tube.

**5.2.3** To the sample, add 500 µL SEB and 10 µL of Proteinase K solution. Vortex on low speed and spin in a microcentrifuge at high speed for 5 seconds to force the cutting into the extraction fluid.

**5.2.4** Incubate the samples from 2 hours to overnight at 56 °C. If samples cannot be extracted immediately after incubation, then freeze and heat back to 56 °C before proceeding.

**5.2.5** Additional Proteinase K may be added to samples after the initial incubation if a high amount of protein is suspected of being in the sample. Add an additional 10 µL of Proteinase K solution. Vortex on low speed,and spinin a microcentrifuge at high speed for 5 seconds to force the cutting into the extraction fluid and incubate at least an additional hour at 56 °C.

**5.2.6** Spin in a microcentrifuge to force condensate into the bottom of the tube.

**5.2.7** In a certified chemical fume hood or cabinet, add 500 µL phenol/chloroform/isoamyl alcohol (PCI) to the stain extract. Mix with the pipettor by drawing the solution up and down slowly several times. Cap the tube and vortex or hand shake the mixture to attain a milky emulsion. Spin the tube in a microcentrifuge at high speed for 3 minutes.

**5.2.8** Label the Microcon 100 filter or equivalent (concentrator) and corresponding centrifuge tube. The label shall be a unique identifier (i.e., an identifiable portion of the database specimen number). Wet the membrane of the concentrator with approximately 20 µL TE. Transfer the aqueous phase (top phase) from the tube into the concentrator. Avoid pipetting organic solvent (bottom phase) or protein interface from the tube into the concentrator.

**5.2.9** Cap the centrifuge tube containing the concentrator and spin in a microcentrifuge for 10 minutes at no greater than 4000 rpm. If fluid remains on the concentrator, spin for an additional 10 minutes.

**5.2.10** Remove the tube cap and add 200 µL TE to the concentrator. Replace the tube cap and spin the unit in a microcentrifuge at no greater than 4000 rpm for 10 minutes. If fluid remains in the concentrator, spin for an additional 10 minutes. **Note:** Residual protein can cause the concentrator filter to become clogged, resulting in some liquid remaining in the concentrator after centrifugation. Adjust for this volume when performing the next step.

**5.2.11** Remove the spin cap and add a measured volume of TE. Buccal samples are brought up in 100 ul of TE and blood samples in 200 ul. Samples may be brought up in lower volumes based on the

analyst's discretion. Remove the concentrator from the centrifuge tube and invert the concentrator onto a labeled microcentrifuge tube. Discard the centrifuge tube.

**5.2.12** Centrifuge the assembly in a microcentrifuge at approximately 4000 rpm for 5 minutes.

**5.2.13** Discard the concentrator. Cap the microcentrifuge tube.

### 5.6 Clean-Up of Extracted DNA

**NOTE:** This procedure may be used if there are particulates in the extract that might affect the amplification, or inhibitors are suspected in the extract. The associated negative extraction control must also be subjected to this procedure in the event that any reagents used in the initial extraction have changed in the interim.

**5.6.1** Vortex the extracted DNA and centrifuge at high speed for 5 seconds.

**5.6.2** Bring the total volume of the DNA extract to 200 µL using TE.

**5.6.3** Add an equal volume of PCI to the DNA extract. Mix with the pipettor by drawing the solution up and down slowly several times. Vortex (low speed) or hand-shake the mixture to attain a milky emulsion. Spin the tube in a microcentrifuge at high speed for 5 minutes.

**5.6.4** Wet the membrane of a new, labeled Microcon 100 concentrator (or equivalent) with 20 µL TE. Transfer the aqueous phase (top phase) from the tube to the concentrator. Avoid pipetting organic solvent (bottom phase) from the tube into the concentrator.

**5.6.5** Cap the concentrator and spin in a microcentrifuge at 4000 rpm for 10 minutes.

**5.6.6** Remove the spin cap and add 200 µL of TE to the concentrator. Replace the spin cap and spin the assembly in a microcentrifuge at 4000 rpm for 10 minutes.

**5.6.7** Remove the spin cap and add a measured volume of TE. (100 for buccal and 200 for blood). Remove the concentrator from the corresponding centrifuge tube and invert the concentrator onto a labeled microcentrifuge tube. Discard the corresponding centrifuge tube.

**5.6.8** Spin the assembly in a microcentrifuge at 4000 rpm for 5 minutes.

**5.6.9** Discard the concentrator. Cap the microcentrifuge tube.

### 5.7 Concentration of Extracted DNA

**NOTE:** This procedure may be used if the original final volume of the DNA extract leaves the extract too diluted to obtain a usable DNA profile. If the final volume used in **5.7.4** is less than the final volume of the associated negative extraction control, the control must also be concentrated using the steps below.

**5.7.1** Vortex the extracted DNA and centrifuge at high speed for 5 seconds.

**5.7.2** Wet the membrane of a new, labeled Microcon 100 concentrator (or equivalent) with TE. Transfer the extracted DNA to the concentrator.

**5.7.3** Cap the concentrator and spin in a microcentrifuge at 4000 rpm for approximately 10 minutes.

**5.7.4** Remove the spin cap and add a measured volume of TE. The TE amount cannot be less than 20 µL. Remove the concentrator from the corresponding microcentrifuge tube and invert the concentrator onto a labeled microcentrifuge tube. Discard the corresponding centrifuge tube.

**5.7.5** Spin the assembly in a microcentrifuge at 4000 rpm for 5 minutes.

**5.7.6** Discard the concentrator. Cap the microcentrifuge tube.

**5.8 Storage of DNA Extracts -** Store the samples at 4 °C (short term) or frozen (long term). Prior to use of samples after storage, they shall be vortexed, and then centrifuged for 5 seconds.

**6.0 Limitations-** N/A

**7.0 Safety**

**7.1** Phenol/Chloroform/Isoamyl alcohol is a known irritant, inhalation hazard and a suspect carcinogen. Nitrile gloves, fume hood and eye protection is required during use.

**7.2** Pregnancy – those who are currently pregnant should avoid performing this procedure.

**8.0 References**

DNA Database Section Procedure for DNA Database Training

DNA Database Section Procedure

DNA Database Section Procedure for Calibration and Equipment Maintenance

**9.0 Records**

- DNA Database Section Extraction worksheet (to be used in in-house analysis, QC, and training)

**10.0 Attachments** – N/A

| **Revision History** | | |
|---|---|---|
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