1 Organic Extractions of Blood, Saliva, and Cigarette Butts

NOTE: All knowns must be extracted separately from unknowns.

1.1 Aseptically place the stain (approximately 3 mm by 3 mm) in a labeled microcentrifuge tube. In the case of cigarette butts, cut a small area of the filter-tip end of the paper or a small piece of filter tip plus paper and add to a labeled microcentrifuge tube.

1.2 To the sample add 300-600 µl stain extraction buffer and 10-15 µl of proteinase K solution. Vortex briefly on low speed and spin briefly in a microcentrifuge to force the cutting into the extraction fluid. (Note: Volumes may be increased if working with larger sample sizes.

1.3 Incubate the samples from 2 hours to overnight at 56°C .

1.4 Spin briefly in a microcentrifuge to force condensate into the bottom of the tube.

1.5 Using a sterile wooden applicator stick or sterile forceps, transfer the cutting into a basket insert. Place the basket in the tube containing the stain extract. Cap the spin-ease tube. Spin in a microcentrifuge at high speed for 5 minutes. (NOTE: The DNA Database does not save samples as described in steps 1.5 and 1.6. Case analysts need not save the cuttings from known samples either for which additional stain material remains).

1.6 Remove the sample from the basket insert and place into a new sterile labeled tube and allow to air dry or leave it to dry in the basket.

1.7 Add 300 - 500µl phenol/chloroform/isoamyl alcohol to the stain extract. Vortex or hand shake the mixture briefly to attain a milky emulsion. Spin the tube in a microcentrifuge at high speed for at least 3 minutes.

1.8 To a microcon 100 concentrator add 100 µl TE. Transfer the aqueous phase (top phase) from the tube in step 1.7 to the concentrator. Avoid pipetting organic solvent (bottom phase) or protein interface from the tube into the concentrator.

1.9 Place a spin cap on the concentrator and spin in a microcentrifuge for approximately 10 minutes (Sorvall MC12V centrifuge at no more than 4000 rpm (low speed) and the Biofuge 15 at 510 rcf) .

1.10 Carefully remove the concentrator unit from the assembly and discard the fluid from the filtrate cup. Return the concentrator to the top of the filtrate cup.

1.11 Remove the spin cap and add 200 µl TE to the concentrator. Replace the spin cap and spin assembly in a microcentrifuge (Sorvall MC12V centrifuge at no more than 4000 rpm (low speed) and the Biofuge 15 at 510 rcf) for approximately 10 minutes or until all liquid has gone through concentrator. Note: After thorough centifugation, some liquid may remain in the concentrator. Adjust for this volume when performing the next step.

1.12 Remove the spin cap and add a measured volume of TE that is between 20 µl and 200 µl to the concentrator. (Volume of TE is dependent on the estimated concentration of the stain. If the concentration is low, bring to volume with lower amounts of TE. If the concentration is heavy, bring to volume in a higher volume of TE). Remove the concentrator from the filtrate cup and carefully invert the concentrator onto a labeled retentate cup. Discard the filtrate cup

1.13 Centrifuge the assembly in a microcentrifuge (Sorvall MC12V centrifuge at no more than 4000 rpm (low speed) and the Biofuge 15 at 510 rcf) for approximately 5 minutes.

1.14 Discard the concentrator. Cap the retentate cup.

1.15 Estimate the quantity of DNA in the sample by slot blot hybridization. The DNA Database Unit does not quantitate samples, but uses an indirect method based on size of the sample. The known forensic blood standards are not required to be quantitated, it is good practice to do so.

1.16 After quantitation, the sample can be amplified.

1.17 The samples can be stored at 4°C or frozen. Prior to use of samples after storage, they should be vortexed, and spun in a microcentrifuge for 5 seconds.

2 Organic Extraction of Hairs Roots

2.1 If a hair with roots has not been previously washed , rinse it thoroughly in 100% ethanol holding the hair with sterile forceps. Follow the ethanol rinse with a thorough rinse in sterile dH2O.

2.2 Place at least 1 cm of the hair containing the root end into a labeled microcentrifuge tube. STR analysis will only be performed on the hair root, not the shaft.

2.3 Follow the same protocol as for saliva and blood.

3 Vaginal and Seminal Stains

3.1 Using a clean surface for each sample, cut the semen stain or cut the swab from the applicator stick and place it into a labeled microcentrifuge tube.

3.2 To the sample add:

at least 400 µl Stain Extraction Buffer
5 - 10 µl proteinase K

Note: volume may vary due to sample size.

Vortex briefly and spin briefly in a microcentrifuge to force the material into the extraction fluid.

3.3 Incubate at 37°C for at least 2 hours to overnight.

3.4 Using a sterile wooden applicator stick or sterile forceps, transfer the material to a basket insert. Place the basket insert into the spin-ease tube containing the stain extract. Spin in a microcentrifuge at maximum speed for approximately 5 minutes.

3.5 Remove the case sample and basket insert and place into a new sterile labeled

tube and allow to air dry.

3.6 While being very careful not to disturb the pelleted material, remove the supernatant fluid from the original tube and place it into a new labeled tube. THIS SUPERNATANT IS THE FEMALE FRACTION. ANALYSIS OF THE FEMALE FRACTION RESUMES AT STEP 3.11. THE PELLET REMAINING IN THE TUBE IS THE CELL PELLET.

3.7 Wash the cell pellet by adding 1000 µl Tris/EDTA/NaCl, vortex the suspension briefly, and spin the tube in a microcentrifuge at maximum speed for at least 5 minutes. Remove and discard the supernatant fluid, being careful not to disturb the cell pellet.

3.8 Repeat step 3.3.7 two to four (2-4) additional times for a maximum of five washes to the cell pellet. The number of washes may depend on the estimated quantity of sperm by the Body Fluid analysis.

3.9 To the tube containing the washed pellet, add:

350 µl Stain Extraction Buffer
40 µl .39M DTT
10 µl proteinase K

Close the tube caps, vortex briefly, and spin briefly in a microcentrifuge to force all the fluid and material to the bottom of the tubes.

3.10 Incubate at 37°C for at least 2 hours.

3.11 To the tube containing the cell pellet and to the tube containing the female fraction, add 300 - 500 µl phenol/chloroform/isoamyl alcohol. Vortex (low speed) or hand shake the mixture briefly to attain a milky emulsion. Spin the tube in a microcentrifuge at maximum speed for at least 3 minutes.

3.12 Assemble and label a microcon 100 unit for each of the above samples. (NOTE: From this point on, the steps are done in duplicate, once for the female fraction and once for the cell pellet.) To the top of the concentrator, add 100 µl TE.

Transfer the aqueous phase (top phase) from the appropriate tube to the top of the concentrator. Avoid pipetting organic solvent (bottom phase) or the protein interface from the tube into the concentrator.

3.13 Place a spin cap on the concentrator and spin in a microcentrifuge (Sorvall MC12V centrifuge at no more than 4000 rpm (low speed) and the Biofuge 15 at 510 rcf) for approximately 10 minutes.

3.14 Carefully remove the concentrator unit from the assembly and discard the filtrate fluid from the filtrate cup. Return the concentrator to the top of the filtrate cup.

3.15 Remove the spin cap and add 200 µl TE to the concentrator. Replace the spin cap and spin in a microcentrifuge (Sorvall MC12V centrifuge at no more than 4000 rpm (low speed) and the Biofuge 15 at 510 rcf) for approximately 10 minutes

3.16 Remove the spin cap and add a measured volume of TE that is between 20 µl and 200 µl to the concentrator. (Volume of TE is dependent on the estimated concentration of the stain. If the concentration is low, bring to volume with lower amounts of TE. If the concentration is heavy, bring to volume in higher volume of TE). Remove the concentrator from the filtrate cup and carefully invert the concentrator onto a labeled retentate cup. Discard the filtrate cup.

3.17 Spin the assembly in a microcentrifuge (Sorvall MC12V centrifuge at no more than 4000 rpm (low speed) and the Biofuge 15 at 510 rcf) for approximately 5 minutes.

3.18 Discard the concentrator. Cap the retentate cup.

3.19 Estimate the quantity of DNA in the sample by slot blot hybridization.

3.20 After quantitation, the sample can be amplified.

3.21 The samples can be stored at 4°C. Prior to use of samples after storage, they should be vortexed briefly and spun briefly in a microcentrifuge.

4 <u>Vaginal and Seminal Slides</u>

Note: Analysts should clean the slide before removal of the coverslip to avoid identification of extraneous DNA. Use an isopropyl alcohol pad to thoroughly clean the slide and coverslip.

4.1 Soak the slides in Xylene (enough to cover to slides) for approximately 20 minutes at room temperature.

4.2 Gently slide the coverslip off the slide. Using a sterile scalpel blade, scrap material off the coverslip and onto a clean piece of weigh paper or equivalent.

4.3 Moisten a sterile swab with xylene and remove any material on the slide with the swab. Use this swab or a new one to collect the material scraped from the coverslip. Aseptically cut (or pull) the swab material from the applicator stick and place it into a labeled microcentrifuge tube.

4.4 Add 500 µl xylene to each tube. Vortex. Spin in a centrifuge at top speed for approximately 5 minutes.

4.5 Remove xylene and add 500 µl sterile dH_2O to each tube. Vortex. Spin in a centrifuge at top speed for approximately 5 minutes. Remove water.

4.6 To the sample add:

500 µl Stain Extraction Buffer
5 µl proteinase K

Vortex briefly and spin briefly in a microcentrifuge to force the material into the extraction fluid.

4.7 Incubate at 37°C for at least 2 hours.

4.8 Analysis of DNA from vaginal and seminal slides resumes at 3.3.4 above.

5 DNA Extraction from Bones and Teeth

NOTES:
1) All extraction steps and procedures must be performed in the extraction work area.
2) WARNING!!! Wear eye and hand protection when handling liquid Nitrogen.
3) WARNING!!! Always wear safety glasses and gloves when handling phenol/chloroform.
4) Bone specimens shall be stored refrigerated or frozen until processed. The bone preparation procedure should be performed in a Biological Safety Hood.

5.1 Sample Preparation

5.1.1 Large Dried Bones (no marrow or associated tissue)

5.1.1.1 A photograph of the bone may be made before processing.
5.1.1.2 The area of the bone to be cut and used in analysis should be ground off mechanically (e.g. using a grinding bit and a Dremel tool) to remove soil and dirt.
5.1.1.3 Remove a cross-sectional wedge of bone using a cutting tool (e.g. a Dremel tool with a cut-off wheel). Do not cut the bone in half; this preserves the bone for further anthropological study if necessary.
5.1.1.4 The bone section should be placed in a 50 ml conical tube and thoroughly washed with freshly prepared 5-10% bleach solution. Drain off the bleach and wash at least 3 times in sterile dH_20 followed by a rinse in 100% Ethanol. Allow the bone to dry.

5.1.2 Small Dried Bones (no marrow or associated tissue)

5.1.2.1 A photograph of the bone may be made before processing.
5.1.2.2 Wash the bone. It may be necessary to remove any debris or associated dirt from the bone prior to washing. Use sterile swabs and sterile dH_20 to physically remove excess dirt. The bone should then be placed in a 50 ml conical tube and thoroughly washed with

freshly prepared 5-10% bleach solution. Drain off the bleach and wash at least 3 times in sterile dH_20 followed by a rinse in 100% Ethanol. Allow the bone to dry.

5.1.3 Large and Small "Fresh" Bones (bones with associated tissue and marrow)

5.1.3.1 A photograph of the bone may be made before processing.
5.1.3.2 If the tissue and marrow is not too degraded, take a sample of tissue and marrow and place in separate labeled microcentrifuge tubes.
5.1.3.3 Preparation of bone

5.1.3.3.1 Using a sterile scalpel blade remove any associated tissue of the bone to be processed. Note: It is helpful to remove the tough fibrous membrane, the periosteum prior to processing because it will aid in the organic extraction (the periosteum is a large source of protein and it is difficult to remove it all during the P/C/I portion of the extraction).
5.1.3.3.2 Removal of bone section for DNA extraction using one of the following methods.
5.1.3.3.3 Remove a cross-sectional wedge of bone using a cutting tool (e.g. a Dremel tool with a cut-off wheel).
5.1.3.3.4 Using a decontaminated drill bit, drill 4-5 several holes though the bone. Collect the powder for analysis.

5.1.4 Tooth

5.1.4.1 A photograph of the tooth should be made before processing.
5.1.4.2 Wash the tooth. It may be necessary to remove any debris or associated dirt from the tooth prior to washing. The tooth should be placed in a 50 ml conical tube and thoroughly washed with freshly prepared 5-10% bleach solution. Drain off the bleach and wash at least 3 times in sterile dH_20 followed by a rinse in 100% Ethanol. Allow the tooth to dry.

5.2 Produce a fine powder from the bone or tooth using one of the following methods:

5.2.1 Liquid Nitrogen: NOTE! Clean container such as an ice bucket before use. Wash the container thoroughly with 10% Chlorox. Rinse bucket five (5) times with dH_20 and three (3) times with sterile dH_20 rinses.

5.2.1.1 Transfer the sample into a decontaminated mortar (autoclaved or washed with 5% bleach, rinsed 3X with sterile dH_20 and a final 100% alcohol wash), add liquid nitrogen to the mortar and allow the tissue to freeze (approximately 30 seconds to 1 minute or until liquid nitrogen has evaporated). Grind the sample with a sterile pestle (autoclaved) to a fine powder. Sample may have to be refrozen several times with additional liquid nitrogen while grinding to a powder.

5.2.1.2 Using a sterile scalpel blade, separate the crushed bone into small pieces and place approximately 0.02 - 0.05 g into a 1.5 ml microcentrifuge tube.

5.2.2 Grinding Method:

5.2.2.1 Using a cleaned Laboratory Grinder (washed with 5% bleach, rinsed 3X with sterile dH_20, a final 100% alcohol wash, and dried), grind the sample to a fine powder.

5.2.2.2 Using a sterile scalpel blade, place approximately 0.05 - 0.15 g of the powdered bone into a 15 ml microcentrifuge tube.

5.3 DNA Extraction (Organic)

5.3.1 To .5-3 gm of sample (depends on quality of bone/tooth) add 3 ml stain extraction buffer and 75 µl Proteinase K solution to the sample. Vortex low speed for approximately 15-20 seconds and briefly centrifuge to force the cutting into the extraction fluid. Briefly centrifuge the tube to force the liquid and bone into the bottom of the tube.

5.3.2 Incubate the tube containing the sample at 56°C overnight (18 hour minimum/24 hours maximum).

5.3.3 Centrifuge the tube briefly to force condensate into the bottom of the tube.

5.3.4 Add 3 ml phenol/chloroform/isoamyl alcohol (PCI) to the stain extract. Recap and vortex (low speed) the mixture briefly to attain a milky emulsion. Centrifuge at high speed for 3 minutes. Repeated PCI extractions may be necessary if a large protein interface is present following the first extraction.

5.3.5 Prepare a Centricon 100 concentrator by labeling and adding 100 µl TE^{-4}. Transfer the aqueous phase (top phase) from the tube to the concentrator. *Avoid pipetting the organic solvent (bottom layer) into the concentrator.*

5.3.6 Place a spin cap on the concentrator and spin in a centrifuge at 500 x g for 15 minutes, or until all of the solution has passed through the membrane. *Note: 500 x g is equivalent to 500 RCF on the BIOFUGE 15, 3000 rpm for the Sorval MC12V/FA micro rotor, or must be calculated or read from a nomograph prepared for the centrifuge and rotor being used.*

5.3.7 Carefully remove the concentrator unit from the assembly and discard the filtrate from the filter cup. Return the concentrator to the top of the filter cup.

5.3.8 Add 500 µl TE^{-4} to the concentrator, replace spin cap, and centrifuge at 500 x g for 15 minutes, or until all of the solution has passed through the membrane.

5.3.9 Remove the spin cap and add 50 µl TE^{-4} to the concentrator. Remove the concentrator from the filter cup and carefully invert the concentrator on to a new labeled retentate cup. Discard the used filtrate cup.

5.3.10 Centrifuge the assembly at 500 x g for 5 minutes.

5.3.11 Remove and discard the concentrator. Transfer to a new labeled microcentrifuge tube and cap.

5.3.12 Estimate the quantity of DNA in the sample by slot blot hybridization.

5.3.13 After quantification, the sample can be amplified.

5.3.14 Store samples at a temperature of 4°C (short term) or frozen (long term). Prior to use, the samples should be vortexed briefly and centrifuged for 5 seconds.

5.4 DNA Extraction (Decalcification Mathod: For extracting DNA from very old or degraded samples)

Modified from M.N. Hochmeister et. al, *"Typing of Deoxyribonucleic Acid (DNA) Extracted from Compact Bone from Human Remains"* J. For. Sci., JFSCA, Vol. 36, No. 6, Nov. 1991, pp.1649-1661).

5.4.1 Split and transfer 15 g of the powder (or maximum amount of sample) into 3 sterile 50-ml polypropylene tubes (approximately 5 g/tube) and add 40 ml of 0.5M EDTA, pH 7.5 and place the tubes on a rotator at 4°C for approximately 24 hr.

5.4.2 Centrifuge the tubes at 2000 g for 15 minutes. Remove supernatant, add 40 ml of 0.5M EDTA, pH 7.5. and place the tubes on a rotator at 4°C for 24 hr as above. Repeat the decalcification process for 5 days.

5.4.3 The decalcification process will take approximately 5 days. The process may be monitored with addition of saturated solution of ammonium oxalate, at pH 3.0, to the decanted supernatant. When the solution remains clear after the addition of ammonium oxalate, the decalcification process may be stopped.

5.4.4 To remove accumulated ions following the decalcification, wash the pellets each in 40 ml of sterile dH_2O. Add dH_2O, recap and shake the tube for 2 minutes, centrifuge at 2000 g for 15 minutes and discard supernatant. Repeat for a total of three washes.

5.4.5 Add 2 ml of prewarmed (56°C) extraction buffer to the pellets, vortex, and incubate at 56°C for 2 h with intermediate shaking. Extract for an additional 10 h without agitation.

5.4.6 Add 100 µl Proteinase K and incubate 3 h at 56°C with intermediate shaking.

5.4.7 Extract three times with equal volumes of phenol/chloroform/isoamyl alcohol (25:24:1). Combine the aqueous phase from the three tubes into a single 15 ml conical tube after the first phenol extraction.

5.4.8 Concentrate the sample using a Centricon microconcentrator.

5.4.9 Assemble the concentrator and place 1 ml of TE in the filter cup. Place the sample extract into the filter cup (containing TE) and wash the retentate three times with 2 ml of TE buffer.

5.4.9.1 First wash (same wash above): 1 ml TE followed by centrifugation at 6,000 rpm using the Sorvall Centrifuge with the SS-34 rotor (or equivalent).

5.4.9.2 Second wash: 500 µl TE followed by centrifugation at 6,000 rpm using the Sorvall Centrifuge with the SS-34 (or equivalent).

5.4.9.3 Third wash: 500 µl TE followed by centrifugation at 6,000 rpm using the Sorvall Centrifuge with the SS-34 (or equivalent).

5.4.10 Place 100 µl of TE into the filter cup and place the inverted filter cup into the retentate cup.

5.4.11 Centrifuge at 2,500 rpm using the SS-3 (or equivalent).

5.4.12 Estimate the quantity of DNA in the sample by slot blot hybridization.

5.4.13 After quantification, the sample can be amplified.

5.4.14 Store samples at a temperature of 4°C or frozen. Prior to use, the

samples should be vortexed briefly and centrifuged for 5 seconds.

6 Concentration/Clean Up of Extracted DNA

NOTE: This protocol may be used if 1) there are particulates in the extract that might effect the amplification, 2) organically extracted DNA amplifies poorly and inhibitors are suspected in the extract.

6.1 Thoroughly vortex the extracted DNA and centrifuge briefly at high speed (approximately 5 seconds).

6.2 Using a pipette, carefully remove as much of the DNA extract possible without disturbing the pellet. If less than 100 µl of DNA extract is obtained, bring the total volume to at least 200 µl using TE.

6.3 If working with a sample which has been organically extracted, bring the final volume up to at least 200 µl with TE.

6.4 Add an equal volume of phenol/chloroform/isoamyl alcohol to the DNA extract. Vortex (low speed) or hand-shake the mixture briefly to attain a milky emulsion. Spin the tube in a microcentrifuge at high speed for at least 3 minutes

6.5 To a microcon 100 concentrator, add 100 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.

6.6 Place a spin cap on the concentrator and spin in a microcentrifuge at 3000 rpm for approximately 10 minutes.

6.7 (Optional if just concentrating sample) Carefully remove the concentrator unit from the assembly and discard the fluid from the filtrate cup. Return the concentrator to the top of the filtrate cup.

6.8 (Optional if just concentrating sample) Remove the spin cap and add 200 µl TE to the concentrator. Replace the spin cap and spin assembly in a microcentrifuge at 3,000 rpm for at least 10 minutes.

6.9 Remove the spin cap and add 50 µl TE that to the concentrator (more or less TE

may be added depending on the results of the previous quantitation). Remove the concentrator from the filtrate cup and carefully invert the concentrator onto a labeled retentate cup. Discard the filtrate cup.

6.10 Spin the assembly in a microcentrifuge at 3000 rpm for 5 minutes.

6.11 Discard the concentrator. Cap the retentate cup.

6.12 DNA quantity may be estimated by slot blot hybridization. If the sample is being "cleaned up" and is brought up in the same volume as the original sample, the slot blot may not be necessary.

6.13 After quantification, the sample can be amplified.

6.14 Store the samples at 4°C (short term) or frozen (long term). Prior to use of samples after storage, they should be vortexed, and then centrifuged for 5 seconds.

Revision History		
Effective Date	Revision Number	Reason
Unknown	00	Original Document for DNA Tech Procedures
January 4, 2002	01	Addition of Speed-Vac Procedure (Change in DNA Technical Procedures)
November 26, 2002	02	Procedure Updates; Collation of Procedures and use of WP Outline; change in page numbering (Change in DNA Technical Procedures)
August 7, 2003	03	Addition of Table of Contents; no substantive changes in any procedure. (Change in DNA Technical Procedures)
July 19, 2004	04	Combined “Organic DNA Extraction”, “DNA Extracted from Bones and Teeth” and “Concentration/Clean Up of Extracted DNA” Procedures to make “Organic DNA Extractions” SOP a separate document. No technical changes in SOPs.

APPROVAL SIGNATURES	Date
Author/Title (Print)	
(Signature)	
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