

1 Aseptic Technique/Quality Control

- 1.1 Aseptic Technique and Contamination Control
 - 1.1.1 All items used in the identification, transfer and isolation of forensic DNA must be sterile and/or free of contaminate DNA. Items that require sterilization may either be flamed, autoclaved, or dry heat sterilized.
 - 1.1.2 A fresh, sterile ART (aerosol resistant tips) pipet tip must be used for each transfer of DNA or chemical (when appropriate) to be used for DNA analysis.
 - 1.1.3 Benches and work areas in hoods should be cleaned with an appropriate disinfectant (freshly made 10 percent bleach or Amphyl, for instance) prior to and after use, and in between cases or Known vs Question samples.
 - 1.1.4 When samples are opened in the laboratory, traffic of unnecessary laboratory personnel should be minimized to avoid the possibility of contamination. All isolations, extractions, and amplifications should be performed in a clean, decontaminated area.
 - 1.1.5 Handling of Known and Question samples

The extraction of the Known and Question samples shall be separated by time. Between extraction of the Known and Question samples, the work space and instruments shall be decontaminated as per the "DECONTAMINATION AND CLEAN-UP PROTOCOL".

- 1.1.6 Gloves and lab coats of the appropriate color must be worn at all times while handling samples. Gloves should be changed before moving from one work area to the other or when a glove is soiled (e.g. material spilled onto glove, a non-clean item is touched).
- 1.1.7 In order to avoid contamination, special attention must be paid to the work area(s) where samples will be examined, extracted, and amplified.
 - The Examination Work area(s) must be separated in time or space from the amplification setup areas.



- The Extraction Work area(s) must be physically separated from the amplified DNA work area. Known and Unknown samples shall be extracted separately (at different times, see 1.5 above).
- The PCR Setup Work area must be physically separated from the amplified DNA work area.
- The Amplified DNA Work area must be physically separated from all other areas to contain the amplified DNA product. All equipment and reagents used in this area shall be dedicated and must not be used in either extraction or PCR setup.
- 1.2 PCR Controls

The use of positive and negative controls shall be used for every test conducted, when appropriate. Negative controls that shall be included, when appropriate, with each sample set are: (A) an extraction reagent blank, (B) a reagent blank for amplification, and (C) a positive control (human known DNA) for amplification, and (D) a positive extraction control (MJB).

The DNA Database Unit will use a negative extraction control, a positive amplification control (K562), and a negative amplification control.

- 1.3 Interpretation of Positive Controls
 - 1.3.1 After processing the positive control(s), each positive control must exhibit a profile consistent with its known type. However, it is not essential that the positive extraction control (MJB) produce a profile.
 - 1.3.2 If a positive control does not exhibit any bands or does not exhibit a profile consistent with its known type, then the locus(loci) must be considered uninterpretable or the test must be repeated.
- 1.4 Interpretation of Negative Controls



- 1.4.1 <u>Extraction Blanks</u>: After extracting the extraction blank and quantitating the DNA, there should be no human-specific DNA present. If human-specific DNA is present, then there is contamination present and the extraction, quantitation and/or amplification must be repeated.
- 1.4.2 <u>Amplification Reagent Blanks</u>: The reagent blank(s) shall not exhibit bands following amplification and scanning. If bands are present, then there may have been contamination or there was bleed over in the wells. In either case, the presence of bands in the negative lane(s) must be fully explained and reported to the DNA technical manager/SAC and/or the test is invalid and must be repeated (the gel must be re-run or the entire case be re-amplified).



2 Decontamination and Clean-up Protocol

NOTE: Areas should be decontaminated in between each set of casework and between extracting/amplifying known vs question samples for an individual case.

- 2.1 <u>Bench areas and hoods</u>:
 - 2.1.1 If present, remove paper lab bench covering and discard.
- 2.1.2 Wash areas (including hood) with an appropriate disinfecting solution (such as fresh 10% bleach solution or Amphyl) to remove DNA contamination or chemical residue and/or dust.

SAFETY NOTE: Wear gloves and safety glasses when using bleach solution.

- 2.1.3 Replace paper lab bench covering (if applicable).
- 2.1.4 After usage of Biosafety Hoods or bench top, clean as instructed above.
- 2.2 <u>Convertible manifold system for chemiluminescence</u>:

Wash the manifold system after use with a detergent solution and rinse thoroughly with water. Shake manifold to remove excess water and allow to air dry or blot dry with Kimwipes or other low lint cloth.

NOTE: Be very careful not to damage the gasket on the bottom assembly.

2.3 <u>Sterilization of scissors and tweezers</u>:

Sterilize scissors and tweezers at the beginning of each procedure and in between every sample. This can be accomplished by flaming or dry heat sterilization.

- 2.4 <u>Glass Plates</u>:
 - 2.4.1 After polyacrylamide gels have been scanned: Scrape glass plates after usage then clean with lab detergent (a non-metallic scrub brush may be used if necessary). Rinse plates thoroughly with water to remove all traces of detergent.



2.4.2 As needed, clean glass plates in 2M NaOH solution to remove excess chemicals.

2.5 <u>Electrophoresis Tanks</u>:

Periodically, upper and lower chambers of the electrophoresis tanks should be rinsed with water to remove excess buffer solution and polyacryamide.

2.6 <u>Decontamination of Pipettors</u>:

Since aerosol resistant tips are used, it is not necessary to decontaminate pipettors regularly. Between cases, and known and questioned samples, pipettors can be cleaned as noted below.

- 2.6.1 <u>Cleaning</u>: Clean pipettors by wiping down with a clean tissue or cloth soaked in a disinfecting solution. Be sure to thoroughly wipe gnarled surfaces and tip. To clean the tip, pull out and remove the pipet tip ejector and wipe with a disinfecting solution.
- 2.6.2 <u>Decontamination</u>: If the inside of a pipettor (shaft) is suspect of being contaminated, disassemble the pipettor for cleaning:
- 2.6.3 Remove the pipettor tip ejector.
- 2.6.4 Remove the entire tip assemble by unscrewing the coupling ring. Carefully remove the piston assembly.

NOTE: BE VERY CAREFUL WHEN REMOVING THE PISTON ASSEMBLY FROM THE SHAFT NOT TO LOOSE THE O-RING AND SEAL LOCATED AT THE BASE OF THE ASSEMBLY (SOMETIMES THE O-RING AND SEAL WILL STAY IN THE SHAFT)!

- 2.6.5 Look into the shaft and remove the O-ring and seal if present.
- 2.6.6 Flush shaft (inside and outside surfaces) with 10% bleach solution. Rinse thoroughly with water to remove residual bleach.
- 2.6.7 Shake dry to remove excess water, allow to air dry, and reassemble. Make



sure pipettor is functioning properly before use in casework.

2.6.8 If there are any questions regarding disassembly or reassembly, consult the instruction manual located in the STR files.



3 Organic Extractions

NOTE: All knowns must be extracted separately from unknowns.

- 3.1 Blood, Saliva, and Cigarette Butts
 - 3.1.1 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.
 - 3.1.2 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.
 - 3.1.3 Incubate the samples from 2 hours to overnight at 56°C.
 - 3.1.4 Spin briefly in a microcentrifuge to force condensate into the bottom of the tube.
 - 3.1.5 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).
 - 3.1.6 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.
 - 3.1.7 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.
 - 3.1.8 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.



- 3.1.9 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).
- 3.1.10 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.
- 3.1.11 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.
- 3.1.12 Remove the spin cap and add a measured volume of TE that is between 20 μ l and 200 μ l 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
- 3.1.13 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.
- 3.1.14 Discard the concentrator. Cap the retentate cup.
- 3.1.15 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.
- 3.1.16 After quantitation, the sample can be amplified.
- 3.1.17 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.

3.2 <u>Hairs</u>

- 3.2.1 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.
- 3.2.2 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.
- 3.2.3 Follow the same protocol as for saliva and blood.

3.3 <u>Vaginal and Seminal Stains</u>

- 3.3.1 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.
- 3.3.2 To the sample add:

at least 400 μ l Stain Extraction Buffer 5 - 10 μ l 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.

- 3.3.3 Incubate at 37°C for at least 2 hours to overnight.
- 3.3.4 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.
- 3.3.5 Remove the case sample and basket insert and place into a new sterile labeled tube and allow to air dry.



- 3.3.6 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.
- 3.3.7 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.
- 3.3.8 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.
- 3.3.9 To the tube containing the washed pellet, add:

350 μl Stain Extraction Buffer40 μl .39M DTT10 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.

- 3.3.10 Incubate at 37°C for at least 2 hours.
- 3.3.11 To the tube containing the cell pellet and to the tube containing the female fraction, add 300 500 μ l 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.
- 3.3.12 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 μ l 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.



- 3.3.13 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.
- 3.3.14 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.
- 3.3.15 Remove the spin cap and add 200 μ l 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
- 3.3.16 Remove the spin cap and add a measured volume of TE that is between 20 μ l and 200 μ l 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.
- 3.3.17 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.
- 3.3.18 Discard the concentrator. Cap the retentate cup.
- 3.3.19 Estimate the quantity of DNA in the sample by slot blot hybridization.
- 3.3.20 After quantitation, the sample can be amplified.
- 3.3.21 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.
- 3.4 Vaginal and Seminal Slides
 - 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.



- 3.4.1 Soak the slides in Xylene (enough to cover to slides) for approximately 20 minutes at room temperature.
- 3.4.2 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.
- 3.4.3 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.
- 3.4.4 Add 500 µl xylene to each tube. Vortex. Spin in a centrifuge at top speed for approximately 5 minutes.
- 3.4.5 Remove xylene and add 500 μ l sterile dH₂O to each tube. Vortex. Spin in a centrifuge at top speed for approximately 5 minutes. Remove water.
- 3.4.6 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.

- 3.4.7 Incubate at 37°C for at least 2 hours.
- 3.4.8 Analysis of DNA from vaginal and seminal slides resumes at 3.3.4 above.



4 DNA EXTRACTION FROM BONE 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.

Bone specimens shall be stored refrigerated or frozen until processed. The bone preparation procedure should be performed in a Biological Safety Hood.

- 4.1 Sample Preparation
 - 4.1.1 Large Dried Bones (no marrow or associated tissue)
 - 4.1.1.1 A photograph of the bone may be made before processing.
 - 4.1.1.2 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.
 - 4.1.1.3 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.
 - 4.1.1.4 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.
 - 4.1.2 Small Dried Bones (no marrow or associated tissue)
 - 4.1.2.1 A photograph of the bone may be made before processing.
 - 4.1.2.2 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.



- 4.1.3 Large and Small "Fresh" Bones (bones with associated tissue and marrow)
 - 4.1.3.1 A photograph of the bone may be made before processing.
 - 4.1.3.2 If the tissue and marrow is not too degraded, take a sample of tissue and marrow and place in separate labeled microcentrifuge tubes.
 - 4.1.3.3 Preparation of bone
 - 4.1.3.3.1 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).
 - 4.1.3.3.2 Removal of bone section for DNA extraction using one of the following methods.
 - 4.1.3.3.3 Remove a cross-sectional wedge of bone using a cutting tool (e.g. a Dremel tool with a cut-off wheel).
 - 4.1.3.3.4 Using a decontaminated drill bit, drill 4-5 several holes though the bone. Collect the powder for analysis.
- 4.1.4 Tooth
 - 4.1.4.1 A photograph of the tooth should be made before processing.
 - 4.1.4.2 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.
- 4.2 Produce a fine powder from the bone or tooth using one of the following methods:
 - 4.2.1 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 dH₂0 and three (3) times with sterile dH₂0 rinses.



- 4.2.1.1 Transfer the sample into a decontaminated mortar (autoclaved or washed with 5% bleach, rinsed 3X with sterile dH₂0 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.
- 4.2.1.2 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.
- 4.2.2 Grinding Method:
 - 4.2.2.1 Using a cleaned Laboratory Grinder (washed with 5% bleach, rinsed 3X with sterile dH₂0, a final 100% alcohol wash, and dried), grind the sample to a fine powder.
 - 4.2.2.2 Using a sterile scalpel blade, place approximately 0.05
 0.15 g of the powdered bone into a 15 ml microcentrifuge tube.
- 4.3 DNA Extraction (Organic)
 - 4.3.1 To .5-3 gm of sample (depends on quality of bone/tooth) add 3 ml stain extraction buffer and 75 μ l 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.
 - 4.3.2 Incubate the tube containing the sample at 56°C overnight (18 hour minimum/24 hours maximum).
 - 4.3.3 Centrifuge the tube briefly to force condensate into the bottom of the tube.
 - 4.3.4 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.

- 4.3.5 Prepare a Centricon 100 concentrator by labeling and adding $100 \ \mu l \ TE^4$. Transfer the aqueous phase (top phase) from the tube to the concentrator. *Avoid pipetting the organic solvent (bottom layer) into the concentrator.*
- 4.3.6 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.
- 4.3.7 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.
- 4.3.8 Add 500 μ l 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.
- 4.3.9 Remove the spin cap and add 50 μ l 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.
- 4.3.10 Centrifuge the assembly at 500 x g for 5 minutes.
- 4.3.11 Remove and discard the concentrator. Transfer to a new labeled microcentrifuge tube and cap.
- 4.3.12 Estimate the quantity of DNA in the sample by slot blot hybridization.
- 4.3.13 After quantification, the sample can be amplified.
- 4.3.14 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.
- 4.4 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).

- 4.4.1 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.
- 4.4.2 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.
- 4.4.3 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.
- 4.4.4 To remove accumulated ions following the decalcification, wash the pellets each in 40 ml of sterile dH₂O. Add dH₂O, 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.
- 4.4.5 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.
- 4.4.6 Add 100 μ l Proteinase K and incubate 3 h at 56°C with intermediate shaking.
- 4.4.7 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.
- 4.4.8 Concentrate the sample using a Centricon microconcentrator.
- 4.4.9 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.

- 4.4.9.1 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).
- 4.4.9.2 Second wash: 500 μl TE followed by centrifugation at 6,000 rpm using the Sorvall Centrifuge with the SS-34 (or equivalent).
- 4.4.9.3 Third wash: 500 μl TE followed by centrifugation at 6,000 rpm using the Sorvall Centrifuge with the SS-34 (or equivalent).
- 4.4.10 Place 100 μ l of TE into the filter cup and place the inverted filter cup into the retentate cup.
- 4.4.11 Centrifuge at 2,500 rpm using the SS-3 (or equivalent).
- 4.4.12 Estimate the quantity of DNA in the sample by slot blot hybridization.
- 4.4.13 After quantification, the sample can be amplified.
- 4.4.14 Store samples at a temperature of 4°C or frozen. Prior to use, the samples should be vortexed briefly and centrifuged for 5 seconds.



5 FTA Extraction and Amplification

- 5.1 Place sample(s) into an microcentrifuge tube.
- 5.2 Add 200 µl of FTA Purification Reagent to each tube. Cap each tube and vortex 1 to 2 seconds at low speed.
- 5.3 Allow the tube(s) to sit for 5 minutes at room temperature with a second brief vortex halfway through the incubation.
- 5.4 After the 5 minute incubation, vortex for a third time and then carefully remove as much of the reagent as possible.
- 5.5 Repeat steps 2 through 4 an additional two times for a total of three washes with the FTA Purification Reagent.
- 5.6 After FTA Purification Reagent has been removed for the third time, add 200 µl of TE. Cap each tube and vortex 1 to 2 seconds at low speed.
- 5.7 Allow the tubes to sit for 5 minutes at room temperature with a brief vortex half way through the incubation.
- 5.8 Draw off the TE and replace with an additional 200 µl of TE. Cap each tube and vortex 1 to 2 seconds at low speed.
- 5.9 Allow the tubes to sit for 5 minutes at room temperature with a brief vortex half way through the incubation.
- 5.10 Draw off the TE and allow the FTA paper punch to completely air dry. This will require approximately 1 hour at room temperature. Alternatively, the drying can be accelerated by placing the tube with the punch at 60°C for 30 minutes.
- 5.11 The complete PCR amplification mix is directly added to the punch containing the purified immobilized DNA.
- NOTE: The DNA extracted using the FTA protocol doesn't allow for quantitation prior to amplification. Analysts shall use a 1 mm Harris punch to sample known blood stains, since the approximate yield of DNA is 1 ng, our target level of template.



6 CONCENTRATION/CLEAN UP OF EXTRACTED DNA

NOTES: WARNING!!! Always wear safety glasses and gloves when handling phenol/chloroform.

This protocol may be used if 1) Chelex extracted DNA amplifies poorly, 2) the Chelex method produces low yields of DNA, or 3) 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 μ l of DNA extract is obtained, bring the total volume to 100 μ l using TE.
- 6.3 If working with a sample which has been organically extracted, bring the final volume up to 100µl 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 μ l 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.



7 DNA Quantitation

7.1 Preparation of Human DNA Standards:

*This only needs to be done as needed

- 7.1.1 Label seven autoclaved screw cap tubes A-G.
- 7.1.2 Vortex the DNA standard A to mix it thoroughly.
- 7.1.3 Transfer 120 μ l of DNA standard A into the tube labeled A.
- 7.1.4 Aliquot 60 µl of TE buffer into the six remaining tubes labeled B-G.
- 7.1.5 Add 60 µl of DNA Standard A (tube A) to the 60 µl of TE buffer in tube B. Vortex to mix well.
- 7.1.6 Add 60 µl of DNA Standard B (tube B) to the 60 µl of TE buffer in tube C. Vortex to mix well.
- 7.1.7 Add 60 µl of DNA Standard C (tube C) to the 60 µl of TE buffer in tube D. Vortex to mix well.
- 7.1.8 Continue the serial dilution through tube G.
- 7.1.9 If the dilution steps are performed as described, the seven DNA standard tubes will have the following concentrations of human DNA:

DNA StandardConcentration (ng/µl) Quantity DNA per 5 µl (ng)

А	2	10
В	1	5
С	0.5	2.5
D	0.25	1.25
E	0.125	0.625
F	0.0625	0.3215
G	0.03125	0.15625



7.2 <u>Slot Blotting:</u>

***Prior to slot blotting pre-warm the Hybridization and Wash Solutions in a 50°C water bath or incubator until all solids have dissolved into solution.

- 7.2.1 Determine the number of samples to be analyzed including the seven Human DNA Standards, the DNA Calibrators 1 and 2 (in the kit), and the one blank (spotting solution only). Aliquot 150µl of spotting solution into a new labeled reaction tube for each sample.
- 7.2.2 Label eight of the tubes as follows: A, B, C, D, E, F, G, H and label two tubes as follows: DNA Calibrator 1, DNA Calibrator 2.
- 7.2.3 Vortex the seven standards and the two calibrators. Add 5 μ l of each solution to the corresponding tube of spotting solution.
- 7.2.4 Add 5 μ l of each test sample to the remaining tubes of spotting solution.
- 7.2.5 While wearing clean gloves, cut a piece of Biodyne B membrane approximately 11 x 8 cm. If desired, cut the right corner to mark the orientation of the membrane. Be sure to make any labeling markings on the membrane at this time. Place the membrane in the hybridization tray and cover with 50 ml of Pre-Wetting solution for a few minutes.
- 7.2.6 Begin with both knobs on the apparatus turned to the OFF positions. Place the top plate of apparatus over membrane and turn clamp knob to the ON position. Turn on the vacuum. Turn vacuum knob to the ON position to allow the vacuum to pull the samples slowly through the membrane. Test apparatus to be sure vacuum is functioning by gently pulling the top plate up. If plate separates from apparatus, turn vacuum off and repeat by gently pressing top plate down as the vacuum is turned on. When the plate is suctioned to the apparatus, continue.
- 7.2.7 Using a new pipette tip for each sample, load the entire volume of each sample into separate wells. Leave the vacuum on until all of the samples have been drawn through the membrane (~30 seconds). Inspect that each sample slot has a uniform blue band.
- 7.2.8 Turn off the clamp. Turn off the vacuum source. Disassemble the $\Pi \alpha \gamma \epsilon 23 \text{ o} \phi 56$



slot blot apparatus and remove the membrane. PROCEED DIRECTLY TO HYBRIDIZATION. DO NOT LET THE MEMBRANE DRY OUT.

- 7.3 <u>Hybridization</u>: (in 50°C rotating water bath)
 - 7.3.1 Pour off pre-wetting solution and rinse the hybriboat with dH_2O .
 - 7.3.2 Pre-hybridization: Transfer the membrane to a hybriboat with 100 ml pre-warmed hybridization solution. Add 5 ml of 30% hydrogen peroxide. Place the lid on the tray. Use a lead weight to keep the tray from floating in the water bath. Rotate in a 50° C water bath for 15 minutes. Pour off solution.
 - 7.3.3 Hybridization: Add 30 ml hybridization solution to the hybriboat containing the membrane. Tilt the tray to one side and add 20 μ l of Quantiblot D17Z1 probe to the solution. Place the lid on the tray. Rotate in 50°C water bath for 20 minutes. Pour off the solution.
 - 7.3.4 Rinse the membrane briefly in about 50 ml pre-warmed wash solution by rocking the tray for several seconds. Pour off the solution.
 - 7.3.5 Stringent Wash/Conjugation: Add 30 ml of the pre-warmed wash solution to the hybriboat. Tilt the tray to one side and add the Enzyme Conjugate:HRP-SA to the wash as follows:

For Chemiluminescent detection: Add 90 µl of the conjugate

Place the lid on the tray. Rotate in 50° C water bath for 10 minutes. Pour off solution.

- 7.3.6 Rinse the membrane thoroughly for approximately 30 seconds in about 50 ml pre-warmed wash solution by rocking the tray at room temperature. Pour off the solution. Rinse again for approximately 1 minute. Pour off solution.
- 7.3.7 Wash the membrane by adding 50 ml of pre-warmed wash solution to the tray. Place lid on the tray. Rotate at room temperature on an orbital shaker for 10 minutes. Pour off solution.



- 7.3.8 Rinse the membrane briefly in 50 ml of citrate buffer by rocking the tray. Pour off the solution.
- 7.4 Chemiluminescent Detection:
 - 7.4.1 To 5 ml ECL Reagent 2 add 5 ml ECL Reagent 1. Do not prepare this mixture more than 5 minutes before use. Add the 10 ml ECL Reagent to the membrane in the tray and shake for approximately 1 minute at room temperature. Pour off solution.
 - 7.4.2 Place membrane in a development folder, squeeze out the excess reagent, and heat seal the edges. Take to darkroom, place in a lumisette and put next to the Reflection file (the gray film). Tape in place (optional). Expose to film for at least 10 minutes at room temperature.
 - 7.4.3 In darkroom under the red lights, remove tape (if used) and membrane from film and feed film into machine when the machine indicator ready light is lit. As the film feeds the red light will go out (it may not if the film is small) when the red light comes back on, make sure that no film is out and the white lights can be turned on.
 - 7.4.4 When film is developed the signals can be compared and quantitated.



- 8 Sample Amplification Using PowerPlex 1.1TM and Amelogenin, or PowerPlex 2.1TM
 - 8.1 Thaw the STR 10X Buffer, Amelogenin Primers, PowerPlex[™] Primer Pair Mix, PowerPlex[™] D16 Add-In primers (if appropriate) and BSA if not already thawed. Vortex each tube thoroughly before using and keep cold.
 - 8.2 Prepare the PowerPlex[™] Master Mix Preparation Worksheet for 1.1 or 2.1. Determine the number of samples to be amplified. Be sure to include K562, the negative amplification control, and MJB. Add four (4) additional reactions to this number to compensate for pipetting variations.
 - 8.3 Label the appropriate number of PCR reaction tubes and place them in the amplification tray.
 - 8.4 Add the sufficient amount of sterile dH_20 to each tube.
 - 8.5 Pipette samples into tubes.
 - 8.5.1 PowerPlex 1.1
 - 8.5.1.1 Pipette DNA from each sample (0.5 to 1 ng) into each respective tube to bring to the appropriate volume of 13.5 μl.
 - 8.5.1.2 For the positive amplification control (K562), add 0.1 to 0.25 μ l of K562 DNA to an appropriate amount of sterile dH₂0 to equal 13.5 μ l.
 - 8.5.1.3 For the negative amplification control, add 13.5 μ l of sterile dH₂0.
 - 8.5.2 PowerPlex 2.1
 - 8.5.2.1 Pipette DNA from each sample (0.5 to 1 ng) into each respective tube to bring to the appropriate volume of 18.5 μl.
 - 8.5.2.2 For the positive amplification control (K562), add 0.1 to 0.25 μ l of K562 DNA to appropriate amount of sterile dH₂0 to equal 18.5 μ l.
 - 8.5.3 For the negative amplification control, add 18.5 μ l of sterile dH₂0.



****NOTE - The volumes above are based on a 25 μ l amplification volume. Analysts may elect to double the amplification volume if they feel that inhibitors may be present. Members of the DNA database may use 12.5 μ l reactions in which 2.75 μ l of master mix is added to 9.75 μ l of water/template. For the positive amplification control (K562), add 0.15 μ l of K562. ****

- 8.6 Calculate the required amount of each component of the PCR master mix.Multiply the volume (µl) per sample by the total number of reactions to obtain the final volume (µl) per the Powerplex Master Mix Preparation Worksheet.
- 8.7 Add the final volume of each reagent into a sterile tube and gently mix (do not vortex). Add *Taq* Gold polymerase last.
- 8.8 Add 11.5 μl of the PCR Master Mix to each sample tube for Powerplex 1.1 reactions, and 6.5 μl to each for Powerplex 2.1 reactions. Cap tubes and place rack containing tubes into the thermocycler. Members of the DNA Database use 2.75 μl for both.
- 8.9 Turn on the thermocycler. Select the appropriate cycle on the:

For Powerplex 1.1 and 2.1 reactions using Taq Gold, use :

95°C for 11 minutes 96°C for 2 minutes, then:

94°C for 60 seconds Ramp to 60°C, hold for 60 seconds Ramp to 70°C, hold for 90 seconds For 10 cycles, then:

90°C for 60 seconds Ramp to 60°C, hold for 60 seconds Ramp to 70°C, hold for 90 seconds For 20 cycles, then:

60°C for 30 minutes, then:

4°C for infinite hold (to refrigerate until analyst takes samples out of thermocycler).





9 Polyacrylamide Gel Preparation

- 9.1 Select glass plates of size appropriate for the test to be run. Make sure the plate surfaces are clean, since plates that are not clean will be difficult to pour without bubbles. The shorter glass plate will be treated with gel slick. This does not need to be repeated each run. Every three or four runs is usually sufficient. Place ~2 ml of gel slick in the center of a clean plate. Using kimwipes, spread the gel slick using a circular motion covering the entire surface.
- 9.2 Allow plate to dry.
- 9.3 Treat the longer plate with bind silane. Add 3 μ l bind silane to a spot of dH₂O (~1 ml) spread on the well end of the plate. Using kimwipes, spread the bind silane on the well end of the plate. Note: Spread silane to bottom of place if gel is to be reused later.
- 9.4 Assemble the glass plates by placing a 0.4 mm side spacer along both edges of the longer glass plate. Place the shorter plate on top of this with the treated side toward the longer plate. Hold the plates together using clamps on each side of the assembly. Make sure the clamps are placed over the spacer. Allow room for the final clamps at the end nearest the wells. A small pipette tip may be placed between the plates at the well end for aid in pouring the gel. This is added after the first pair of clamps has been added.
- 9.5 Measure the following solutions in to a squeeze bottle according to the chart below:

Solution	Long (42 cm) gels
5 % Acrylamide	50 ml
Temed	35 µl
10 % APS	250 µl

9.6 Gently but thoroughly swirl the bottle after adding each solution.

NOTE: Do not introduce bubbles into the solution by excessive shaking as air will inhibit polymerization of the acrylamide.



- 9.7 As quickly as possible, gently pour the solution between the glass plates just until it begins to run out the other end. The plates may be tipped for ease of pouring. Lay plates flat and gently remove the pipette tip. Check for bubbles and remove the bubbles if seen. Push the comb between the plates and anchor with the remaining clamps.
- 9.8 Allow to polymerize for approximately 1.5 hours. Gels may be stored overnight if both ends of the plate are wrapped in paper towels soaked in Tris Borate (tank running buffer) or deionized water and placed in ziplock bags or wrap in plastic wrap. An alternative is to set up the gel in a vertical electrophoresis apparatus and cover with tank buffer. Do not allow the polymerized acrylamide to dry out.



10 Polyacrylamide Gel Electrophoresis

- 10.1 Remove the clamps from the polymerized acrylamide gel and clean the plates with dH₂O and kimwipes.
- 10.2 Shave any excess acrylamide from the plates using a razor blade.
- 10.3 Place foam blocks on the top of the short plate (press sticky side against the longer plate) then place the gel in the SA32 tanks with the longer plate outside and the shorter plate towards the tank. Be sure that the foam spacers are flush with the shorter plate before tightening the top clamps and that the drain for the top tank is closed. (This is the knob on the top right side of the tank.)
- 10.4 Place ~300 ml of .5X Tris Borate buffer in the top and bottom chambers of the tank. Be sure the seal is tight and there is no buffer leaking between the tank and the shorter glass plate.
- 10.5 Remove the comb and pre-run the gel for 10-20 minutes using the same conditions as shown in step 11 below to achieve a gel surface temperature of approximately 50°C. Remove the comb and rinse the wells with tank buffer.
- 10.6 Sample Preparation
 - 10.6.1 Each sample must be bracketed by Allelic Ladders provided by the manufacturer of the kit. Amelogenin ladder does not have to be included in each ladder lane. However, Amelogenin ladder must bracket all forensic samples. Should an analyst run out of Amelogenin Ladder or inadvertently forget to include the amelogenin ladder on an analytical gel, cells could still be made from the K562 (female) and MJB (male) positive controls (this is not a standard practice).
 - 10.6.2 Two-Color Detection (not using Fluorescent Ladder, CXR)
 - 10.6.2.1 Prepare an electrophoresis worksheet.
 - 10.6.2.2 In a biosafety cabinet, label sterile PCR amplification tubes and place in a thermocycler tray
 - 10.6.2.3 Prepare the samples by adding 2.5 µl loading dye to 2.5 µl amplified sample to each respective tube.
 - **10.6.2.4** Prepare the ladder samples by adding 2.5µl prepared



loading cocktail, approximately 0.2-0.5 µl Amelogenin ladder and approximately 0.5-1.5 µl PowerPlex[™] allelic ladder to each respective tube.

Note: Ladder amounts may vary more depending on the quality/strength of the ladder. A tube of Ladder that has been through many freeze/thaw cycles may be "weaker" than a fresh tube.

- 10.6.3 Two-Color Detection (using Fluorescent Ladder, CXR)
 - 10.6.3.1 Prepare electrophoresis worksheet. In a biosafety cabinet, label sterile PCR amplification tubes and place in a thermocycler tray.
 - 10.6.3.2 Prepare a loading cocktail by combining and mixing the Fluorescent Ladder (CXR) and load dye as follows:
 - [(1 µl Fluorescent Ladder) x (# lanes)] + [3µl Load Dye) x (# lanes)]
 - 10.6.3.3 Prepare the samples by adding 2.5µl of the prepared loading cocktail and 2.5 µl amplified sample to each respective tube.
 - 10.6.3.4 Prepare the ladder samples by adding 2.5µl prepared loading cocktail, 0.3 µl Amelogenin ladder and 0.6 µl PowerPlex[™] allelic ladder to each respective tube.

Note: Ladder amounts may vary depending on the quality/strength of the ladder. A tube of Ladder that has been through many freeze/thaw cycles may be "weaker" than a fresh tube.

- 10.7 Denature the samples by heating to 96°C for 2 minutes in the thermocycler. Immediately remove and chill on an ice block.
- 10.8 While denaturing, flush the wells with tank buffer using a 10 ml syringe with a needle to remove the excess urea.
- 10.9 Immediately after completion of denaturation, place the samples on an ice block. Load approximately 2 - 5 μl of each sample/dye mixture into the respective wells



using a fresh flat tip pipette for each sample. Sample amount loaded can vary depending on sample strength

- 10.10 Load 3 µl of Gel Tracking Dye in the gel. This dye is used as a visual indicator of migration.
- 10.11 At the completion of loading, attach the tank to the power supply and run at the following conditions:

3,500 V	300 mA	45 - 60 W - 1 gel
3,500 V	300 mA	60 - 120 W - 2 gels

Run the gel for approximately 80 to 100 minutes or until the second dye front at least passes the bottom gel fastener.



- 11 Scanning Polyacrylamide Gels
 - 11.1 Turn on the scanner and computer.
 - 11.2 Allow the laser in the scanner to warm up. (A red light will come on if there is an error, usually due to the scanner lid not being properly closed).
 - 11.3 After completion of electrophoresis:
 - 11.3.1 Remove gel from tank and rinse both sides of the glass plates thoroughly with dH₂O.
 - 11.3.2 Dry completely with a low lint cloth (e.g. KimWip) so that there are as few marks, dust, or streaks on the plates as possible.
 - 11.3.3 If black spacers are not used, cover the white spacers by placing black electrical tape on the long plate.
 - 11.4 Place the gel into the scanner:
 - 11.4.1 By lifting the lid to the unit.
 - 11.4.2 Placing the gel so that the short plate is on top and the bottom part of the gel (where the dye front should be) is resting on the movable bar. Make sure that bar is as close to the end of the plate as possible so as not to interfere with the scanning.
 - 11.4.3 Close the lid properly to the unit.
 - 11.5 Once your computer is turned on:
 - 11.5.1 Desktop files as well as the Macintosh hard drive icon (located in the upper right hand portion of the screen) will appear.
 - 11.5.2 Double click on the hard drive icon. This will list all of the files on the hard drive.
 - 11.6 To open Read Image Software:

Click on the PowerPlex Scan icon. Choose the correct icon; that depends on whether black spacers were used or if black tape was used to cover the black spacers.

11.7 The screen will appear that looks similar to this:



		FM BIO 100*	FM BIO II
	Material Type	Fluorescent	acrylamide gel
	Orientation	Flip Horizontal	Normal
	Resolution: Horizontal	15Cdpi	150dpi
0	Vertical	150 dpi	150dpi
	Gray Level Correction Type	range	range
	Cuttoff Threshold:		-
	Low (background)	50%	50%
are	High Signal	1%	1%
	Reading Sensitivity	80%	100% (505nm channel)
(See			100% (650nm channel)
	Focusing Point	n/a	-1.6mm for disposable gets

make sure scanning parameters correct for Powerplex table below).

Check to

11.8



- 11.9 To adjust parameters:
 - 11.9.1 Click on the PowerPlex Scan icon on the desktop and then "Setting".
 - 11.9.2 To get to the Powerplex mode, click on MATERIAL TYPE, select POWERPLEX.
 - 11.9.3 Click on all Channels that you will be scanning on:

Channel 1-- 605 nm Channel 2 -- 505 nm -- DDDD (Gamma Str) Channel 3 -- 585nm -- CTTV Channel 4 -- 650nm -- CXR

*Note: 1) The FMBIO II Instruments have an Autofocus feature. Autofocusing occurs during the first scan after the instrument has been turned on. The 605 nm filter is provided for Autofocusing, however, the 585nm filter is also suitable. To ensure proper Autofocusing and operation of the instrument, always be sure that either of these filters (585 or 605) are in Channel 1 (Autofocus channel) whenever the instrument is turned on or for the first scan of the day.

2) CXR -- When using CXR, you must first scan with Channel 1 at 585nm and Channel 2 at 505nm THEN remove the 585 nm filter in Channel 1 and replace it with the 650 nm filter. Select <u>Channel 1 only</u> on the ReadImage window. <u>Keep all scanning and area parameters the same as for the previous scan with the 585 and 505 filters.</u>

- 11.10 Define a scan area by:
 - 11.10.1Selecting an area to PreRead by clicking on ALL AREA (entire scannable area) or by using the mouse to drag out a rectangular area in the image scan window.
 - 11.10.2Click PREREAD and the instrument will perform a brief scan of the defined area.
 - 11.10.3Based on the PreRead results, select area to be scanned by dragging inside the scan window with the mouse (Be sure your defined area includes all lanes as well as high and low bands).
 - 11.10.4When all parameters are set, click READ (A new screen will appear which



asks you to name the image) and which file you need to save to. 11.10.5Name the image and click on the SAVE.

- 11.11 At this point the counter for the FMBIO II -- will count down in minutes, until it reaches "0" at which time the scanning is complete.
- **Note Do not stop or pause the instrument until scanning is complete. Also do not change the scan area parameters between scans of the same gel. This will create different size image files and 3-color analysis will not be possible.
 - 11.12 At the completion of scanning, when 2 or 3 channels are selected, a suffix (.1 CH, .2CH, CXR) is automatically added to the end of the file name.
 - 11.13 To view scanned images, click on the designated file on the FMBIO II.



12 COLOR SEPARATION

- 12.1 Move file from server to a designated folder (optional).
- 12.2 Open the file and select CH.1 or CH.2 image.
- 12.3 For Database Images: Click on "File" and select "New"."Select Multi Analyze Image Box" appears. Open of the channels and save new project with file name.
- 12.4 Adjust Background and Signal Strength (Note: Some analysts may elect to adjust the background AFTER Color Separation. This is acceptable)
 - 12.4.1 Select MULTI (top menu bar)
 - 12.4.2 Select DISPLAY MODE: MONO
 - 12.4.3 Click on Gray Level icon or choose Gray Level Correction in the Image menu. The Gray Level Correction dialog box appears. You can click on this dialog box and move it around over the scanned image. The left side of the dialog box acts like a transparent opening letting you frame regions of interest.
 - 12.4.4 Adjusting the background (if necessary) by one or both of these methods:
 - 12.4.4.1 Double click on the "%" beside of in the "Low (Background)" box to highlight the number (if not highlighted already). Manually adjust by typing in a number accordingly (NOTE: A general guideline is that the Background Signal be set at 70-85%. Optimal numbers may vary depending on signal of bands and background noise in the gel).
 - 12.4.4.2 Double click on the "%" beside of in the "Low (Background)" box to highlight the number (if not highlighted already). Draw a box around area of background and the machine will automatically adjust the setting.
 - 12.4.5 Adjusting the signal manually (if necessary) double clicking on the "%" number and typing in a number. Adjusting the high value field down, will increase the intensity of your image (NOTE: A general guideline is the signal be set at 10,000 for Channel 1 and 3000 for Channel 2. Optimal numbers may vary depending on signal of bands and background noise in the gel).



12.4.6 Test the area of interest by clicking on TRY. Repeat, until you are satisfied with your image. Then click SET to apply changes.

NOTE: Some analysts may elect not to adjust background until after color separation

- 12.5 Perform Initial Color Separation.
 - 12.5.1 Go to Menu bar.

12.5.2 Click on MULTI.

12.5.3 Click on COLOR SEPARATION.

- 12.6 The COLOR SEPARATION dialog box opens. File names of components of the multicolor image appear on 2 lines labeled Basis Image and Target Image.
- 12.7 Use the file name pop-up to (downward facing arrow next to the SET button) choose a Basis Image file.6.
- 12.8 Enlarge the image as necessary. On the Image, use the Marquee tool to draw a rectangle inside an isolated band that MATCHES the color assigned to the Basis Image file for CH1.
- 12.9 Click the SET button.
- 12.10 Use the file pop-up to choose the Basis Image file for CH.2.
- 12.11 Draw a rectangle inside the chosen band that MATCHES the color assigned to this Basis Image file.
- 12.12 Click the SET button.
- 12.13 Click OK.





- 12.14 Click OK when asked to "Create Separate Images".
- 12.15 Go up to the top menu bar.
 - 12.15.1Click on MULTI
 - 12.15.2Go down to DISPLAY MODE
 - 12.15.3Click on MONO (This brings up the black and white image of the file that is highlighted in the project window).
 - 12.15.4View each channel to insure no "bleed through" is occurring. If there is still "bleed through", repeat COLOR SEPARATION.
 - 12.15.5If your images are correct, go to 1-D GEL ANALYSIS.



13 STR-FMBIO Analysis (1-D Gel Analysis)

- 13.1 Once scanning is complete and image is saved to desktop or HD (hard drive), double click on your personal file (This opens up your file and allows you to choose what image you want to analyze).
- 13.2 Double click on file containing image of interest, i.e, popdbase 561-575. The main file opens containing sub-files generated from Channel 1 (CTTV loci) and Channel 2 (DDDD loci)
- 13.3 Double click on file of interest. FMBIO Analysis will momentarily appear on the screen. The image will appear next. Make sure that FMBIO 8.0 (or newest revision) is used by going up to the small computer screen icon located at top right corner of screen. Click and hold down mouse and scroll down to FMBIO 8.0 (or newest revision). A check mark appear when program is on.
- **13.4** From the

top of the and hold Scroll down release the The 1-D gel the menu bar

tools appear the image

	1D-Gel
Single Lane	— 🚦 🛛 🖂 – Manual ban
Multiple Lane —	- 1
Show Range	- Show Lane
Show Bands —	Show Info
Auto Band	Auto Band - Open Table
Set Mark ———	- Set - Recalculate

menu bar at the screen, click on down TOOLS. to 1-D Gel and mouse button. menu appears in and the corresponding on the left side of window:

13.5 MIGRATION AREA BAR A migration area bar also appears at the top and bottom of your image when 1-D Gel is selected. Adjust to just above the top band and just below the bottom band by holding arrow on left square of bar and moving



it up or down.

- 13.6 MULTILANE SELECTOR TOOL. The Multilane Selector Tool will define the area of the scan that will be analyzed.
 - 13.6.1 To select the multilane selector tool, click on the icon from the 1-D Gel menu bar.
 - 13.6.2 Click and drag the multilane selector tool from the center of lane #1 to the center of the last lane (above migration bar area). Release the mouse button.
 - 13.6.3 Using the mouse, pull down below bottom migration area bar.
 - 13.6.4 Click mouse (Multi Lane Window appears).
 - 13.6.5 Enter # of

lanes on gel.

- 13.6.6 Click on the computer with blue vertical lines). OK. (Lanes are now identified by
- 13.7 CENTERING OF LANES. Once the computer recognizes the correct number of lanes by placing blue vertical lines on each lane, each lane must be centered.
 - 13.7.1 Click on icon until you get a single blue center line for each lane (Clicking on this icon several times changes the display from 3-line to center line to no lines).
 - 13.7.2 Click on arrow icon found in the menu bar. Go up to top of blue center line on each lane. Click on the line until a small box appears. Using the mouse, place the arrow on the box, click and move the line until centered over the lane (This moves line on lane left or right). Go to bottom of lane and click on the box. With the FMBIO II, you must click at the bottom of the lane for the small box to appear. (This moves line on lane diagonally). Center all lanes by using the mouse as described above.



- 13.7.3 Once all lanes are centered , press on keyboard "apple" + A to get "flashing lines".
- 13.8 AUTOBAND. identify bands.

This function instructs the computer to

- 13.8.1 Click on
- 13.8.2 Computer will identify bands in all lanes that were centered. If you look at bottom of gel scan you will see displayed (Autoband: lane #) as it goes through autobanding.
- 13.8.3 Once autobanding is complete, red and yellow horizontal bands will appear on bands (vertical blue lines will still be visible).
- 13.8.4 Remove vertical blue lines by clicking on until no blue lines are visible.
- 13.8.5 Click on icon until
 only red bands are

 visible (Clicking on this icon will allow you to go from yellow & red
 only, to no visible

 bands, to red bands
 only, to no visible
- 13.9 EDITING BANDS. At this point you can add or delete bands that the computer misidentified.
- 13.10 To add bands, click on peak finder and dogbone. Place dogbone on top of band of interest and click. A red band will appear denoting addition (recognition) of band.
- 13.11 To delete bands you can use:
 - 13.11.1The dogbone . Place on top of band and click. Band will turn from red to blue. Hit the delete button. The computer no longer recognizes this band.
 - 13.11.2The marquee . The marquee allows you to draw a box around the band or bands you want to delete. Once you have the box around the desired

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band(s), they will also turn blue. Hit the delete button. The computer no longer recognizes the band(s).

- 13.12 SET MARK . Once all band editing is complete you are ready to tell the computer what standards to use to calculate unknown band sizes.
 - 13.12.1Click on . TT-J to the Bruph of markets and chick Marker Setting 13.12.2The Lane [29] Lane1 [2] Lane2 [2] Lane3 [2] Lane4 [2] Lane5 [2] Lane6 [2] Lane7 [28] Lane8 Marker following screen will Options ... appear: Cancel 0K - 1 **a**

- 13.12.3Make sure Natural Logarithms is checked. Click OK.
- 13.12.4The Marker Setting window will appear: The Marker Setting dialog box displays the defined lanes with parentheses indicating the number of defined bands in each lane.



13.12.5For Powerplex, you should have the following number of ladder bands :

[35] ppCTTV ladder [52] pp2.1 TMR
[37] ppCTTVa ladder [86] pp2.1 Fluroscein
[36] DDDD ladder
[14] CXR ladder

- **13.12.6**Scroll up and down the Marker Setting window to check that the correct number of ladder bands has been identified.
- 13.12.7<u>If all ladders do not contain the correct # of bands</u>. Cancel out of this screen and go back to Editing Bands.
- 13.12.8If all ladders are correct press shift and click on each ladder lane in the Marker Setting window. They will turn black once highlighted as seen below.
- 13.12.9Click on down arrow in the Marker Setting window menu. Go to Group and click on desired ladder, i.e., ppCTTV.+ppCTTV will now appear on the lane next to the highlighted ladders.
- 13.12.10 Click on OK (window will close).
- 13.13
 RECALCULATE. Now that bands
 H

 and the computer knows what ladder to
 Set
 H

 sample band sizes can be calculated.
 H
 H
 - have been marked use, the unknown
 - 13.13.1Go up to 1-D Gel on the top menu bar.
 - 13.13.2Go down to Setting.
 - 13.13.3Go down to Table Info
 - 13.13.4Click on what items you want calculated. <u>Always click on bp. If</u> <u>determining % stutter click on OD</u>. Deselect the other options.



13.13.5Click on Recalculate icon.

- 13.14 TABLE INFO. The above calculated data is placed in a table format.
 - 13.14.1Click on Table icon.
 - 13.14.2A table will appear with calculated data. You must SAVE the data before exiting the program.
 - 13.14.3Go up to the top menu bar. Click on FILE.
 - 13.14.4Go down to SAVE ANALYSIS.
 - 13.14.5Go over to desktop or HD. Click on it (This brings up files found either on the desktop or HD).
 - 13.14.6Scroll down until you find your personal file. Click on it (Now you file is opened and the table data will be saved in it).
 - 13.14.7Click on SAVE. Display at bottom of this screen will already read, i.e.: RD251-266.1CH.DAT --- for data file.
 - 13.14.8Repeat for Channel 2 image.
 - 13.14.9Close out of program by clicking on little box in the upper left hand corner of your image. If program asks if you want to save changes to this image. Click SAVE.

YOU ARE NOW READY TO DO STAR CALL ON THE ABOVE DATA. IN ORDER TO ACCESS STAR CALL YOU MUST CLOSE OUT OF THIS PROGRAM AND GO INTO MICROSOFT EXCEL.





14 STaR CALL

- 14.1 Turn on computer by holding down far right button with arrow pointing left on it.
- 14.2 Open Microsoft Excel by clicking on the Microsoft Excel icon on the desktop or go up to top left corner of screen, click on the "Apple". Scroll down to Microsoft Excel alias or use the Microsoft Excel icon on the desktop.
- 14.3 New screen appears with a menu bar across the top and a "codisdbf.xls" dialog box will appear briefly. (DO NOT CLOSE OUT of this dialog box. It must be opened for STaR CALL to operate. It is the lookup table containing known bp sizes of the standards). A table titled "Workbook 1" will then appear.
- 14.4 From toolbar select "Star Call" > "Options". At this point choose the "descending" button. Later it will be changed back to "ascending".
- 14.5 From toolbar click on StaR Call, then Import STR.
- 14.6 A grey box will then appear which will allow the analyst to choose a file or files to open (xx.dat).

STaR Call – In	nport STR
Path/Filename	Lookup Table
DAT File 1	Browse 🗢
DAT File 2	Browse
DAT File 3	Browse 🔶
DAT File 4	Browse 🔶
DAT File 5	Browse 🔶
DAT File 6	Browse 🔶
Convert DAT(s) as	
🔾 STR 💿 STR with OD values 🔾 STF	R with IOD values
OK Reset	Cancel Help

Choose "Browse" to locate the desired file (xx.dat). Up to 6 files can be selected. Note: Both PP 1.1 and PP 2.1 files can be imported simultaneously, as long as the samples are the same and the gel lanes are identical.

14.7 Choose Look up Table by using the drop down arrows.{Eg. PP 1.1 choose either CTTV, CTTV + AM, Gamma STR DDDD (New D 7). PP 2.1 choose either (FL)



or (TMR).

14.8 Click on:

14.8.1 STR – to calculate bp and genotype14.8.2 STR with 1OD values -- to calculate bp, genotype, and % stutter.

- 14.9 New dialog box opens "STaR CALL. Evaluate Select Allelic Ladders".
- 14.10 Check to ensure your allelic ladders are highlighted and the number of alleles are correct.
- 14.11 Click OK
- 14.12 STaR CALL will now perform Genotype Analysis.
- 14.13 Once STaR CALL has completed its analysis an initial "Summary" screen will appear.
- 14.14 In the bottom left corner several grey tabs will be visible which represent different tables within the complete workbook such as an individual Excel table for each (xx.dat) file, a Look Up Table for each (xx.dat) file, a merged Excel table which combines all (xx.dat) files and a merged landscape table.
- 14.15 At this point the analyst should review the "Merged Landscape" table to make sure STaR CALL has functioned properly.
- 14.16 Evaluate the merged Excel Table to confirm all alleles have been properly identified and the correct # of repeats have been assigned. If alleles have been identified as "Out of Range" the analyst can correct the table by comparing the base pairs of the sample to the base pairs of the allelic ladder. Or the analyst can use the FMBIO Image to manually identify the number of repeats.
- 14.17 While using the toolbar "STaR CALL" select "OPTIONS". Box will appear allowing the analyst to change the presentation of data in the "Merged Landscape Table". It is suggested that data be viewed as Genotype in Ascending order.

Note: It may be necessary to change the table from ascending to descending during analysis and then back to ascending aiding the analyst to better visualize the data.



- 14.18 Upon competing corrections return to toolbar "STaR CALL" then to "Create Merged Landscape". This will apply the corrections to the "Merged Landscape Table".
- 14.19 Return to toolbar "Star Call" then to "CODIS STR Export". A "Star Call Header Information" box will appear. It is <u>important</u> that this box contain all of the correct selected information. If not the file will be unsuitable for importing into CODIS.

STaR Call – CODIS STR Header Information		
CMF Header Information		
CMF Header Version 1.0	Source Laboratory ORI 🛛 Imaa ORI 💽 🖅 –	
CMF Message Type IMPORT	Destination ORI NCBCI0094 💽 +/-	
CMF Message ID 1	Creation Date/Time 02-Jun-1999 22:0	
Imaging System	Hitachi 💿 🖅	
Imaging System Utilized FMBIO		
Packet Information Reading Information		
CMF Type DNA Analysis	Reading by BEARLY • +7-	
CMF Version 1.0	Reading Date 02-Jun-1995	
Technolog PCR	Reading Time 22:00:00	
	No.of Reading(s) 1	
Next >>) Cancel Help		

The following should be selected.

Source Laboratory ORI = Image ORI Destination ORI = NCBCI0094 Creation Date / Time = N/A Imaging System= Hitachi Imaging System Utilized = FMBIO

Reading Information

Reading By = Analyst's CODIS Login name.

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Reading Date = Day analysis complete (Eg. 02-Jun-2000). Note: CODIS will not except any dates that are prior to analysts CODIS Start Date. Reading Time = Optional Number of Readings = 1

Once complete press "Next".

14.20 "StaR Call-CODIS STR Specimen Information" box will appear.

STaR Call – CODIS STR Specimen Information		
Specimen Information		
Lane/Marker Name Lane6		
Specimen Number CAP-04		
Use lane names as specimen numbers		
Include/Exclude selected lane from export		
Exclude Lane Included/Excluded Lane List		
Sample ID 0		
Specimen Category Proficiency		
Tissue Type Blood 🗢 >>		
Tissue Form Stain 🗢 >>		
Population Group Unknown		
All >>		
(« Previous) (Next >>) (Export) (Close) (Help)		

- 14.21 Analyst will identify each lane except ones that contain allelic ladders. Type in specimen number in white field at the top of the box. Next select the proper categories from each of the following, Specimen Category, Tissue Type, Tissue Form, and Population Group. This will need to be completed for each specimen and controls. Note: If all or multiple specimens share information the double arrows to the far right or the "All" button followed by double arrows can be pressed after the specimen information has been entered to apply information to all specimens.
- 14.22 The Include/Exclude box should only be marked if a sample is to be excluded from the Merged Table and/or the CMF file (Common Message Format file used



to import data into CODIS). Press "Close". Note: This should not be done until all data has been second read.

- 14.23 The table should be made to fit the page by using the "Format" function on the toolbar.
- 14.24 Next go to "File" > "Page Setup" > "Page" select "landscape". Next go to "Header/footer" tab and select the "Custom Header" button. Three boxes will appear. In the left box type the date (eg. 01/24/2000), Center box type Workbook name (eg. DB99-100), Right box type Analyst Name. Press "OK". Press "Custom Footer" button. Center box type "NCSBI", Right box type "Verified ______". Press "OK".
- 14.25 Return to toolbar "File" > "Save as". Name the file such as (xxxx.wkbk).
- 14.26 Print the "Merged Landscape Table".
- 14.27 Once table has been printed and <u>SAVED</u>, delete all of the allelic information from the table and print a second blank table to use for second reads. DO NOT SAVE THE CHANGES.
- 14.28 Once the second read is complete the analyst will return to computer to create the CMF file. To create CMF file open the desired previously generated Workbook (xxxx.wkbk) file.
- 14.29 From the bottom left corner select the "merged" tab.
- 14.30 Next from the tool bar select "Star Call" then "CODIS STR Export".
- 14.31 Review "Header Information" press "Next".
- 14.32 Review "Specimen Information". Be sure all specimens that should be excluded are excluded. All controls should also be excluded.
- 14.33 Press "Export".
- 14.34 Select where the file should be stored.
- 14.35 Name the file (eg. 01DB00c.dat) 01=set #, DB =DataBase, 00=year, C=codis/cmf



14.36 Press save.

14.37 he file is ready to import to CODIS.

NOTE: Analysts are not required to make allele calls from STaR Call for case work analysis, but may manually read the gel scans if they prefer.



15 Re-Use of Polyacrylamide Gels

- 15.1 After its initial use, the acrylamide gel may be stored and/or reversed prior to its next use.
- 15.2 To store the gel, it may be wrapped in wet paper towels and wrapped in Saran wrap to keep it moist. If the gel should dry out, it will not be re-used. For extended life, the wrapped or bagged gels can be placed in the refrigerator. As an alternative (for immediate re-use), the gel could be placed back into the electrophoresis tank and covered with TBE buffer.
- 15.3 Before re-use, the polarity will need to be reversed and the gel electrophoresed at 60W for at least two hours to rid the gel of previously loaded DNA. At the end of the reversal time period, return the polarity back to normal run conditions.
- 15.4 At this point, the gel is ready to be loaded with fresh samples.
- 15.5 Analysts are to re-use gels only if they are of good quality, (ie. no broken wells or distortions, etc.). A gel may be re-used until it exhibits problems.



16 Directions for Drying Down Samples Using the Speed-Vac

- 16.1 One hour prior to use, turn on the refrigerated condensation trap and the heater on the speed vac concentrator unit. Check the fluid level in the concentrator unit to see that the fluid levels are appropriate. Add more methanol, if needed, by pulling the glass trap out of the unit so that more fluid can be added. Place samples in the rotor unit, shut the lid, and turn the rotor on.
- 16.2 Turn on the vacuum pump and turn the stopcock parallel so that a vacuum is introduced into the rotor unit.
- 16.3 Let the samples spin until dry (samples with 200 μ l may take as long as one hour to dry).
- 16.4 Re-introduce air into the rotor unit by turning the stopcock so that "BLEED" is facing the tube from the rotor unit.
- 16.5 Cap the tubes and place them in a labeled envelope for return with the case's remaining evidence.

Revision History		
Effective Date	Revision Number	Reason
Unknown	00	Original Document(s)
January 4, 2002	01	Addition of Speed-Vac Procedure
November 26, 2002	02	Procedure Updates; Collation of Procedures and use of WP Outline; change in page numbering