Houston Police Department

Crime Laboratory

Standard Operating Procedures

DNA SECTION

Table of Contents

1 Overview

The DNA Standard Operating Procedure manual (SOP) specifies procedures for routine DNA analyses of biological evidence for human identification; it is approved for use in the DNA section of the laboratory in conjunction with the Biology SOP.

Each approved revision of this manual will be version-controlled and archived for retrieval by date of authority. Only the approved revision in effect at the time of analysis governs the analysis. If necessary, changes in SOPs will be documented via memoranda issued between manual revisions, until such time as the SOP manual is revised. Any deviation from accepted protocol requires approval as outlined in the Methods section of the quality manual, and must be documented in the case file.

This SOP is only one part of the policies and procedures that govern all work performed by the DNA section. These other relevant documents include, but are not limited to, the:

- Houston Police Department General Orders
- quality manual
- safety manual
- Biology section SOP
- DNA training manual

1.1 Technical Leader

The *Quality Assurance Standards for Forensic DNA Testing Laboratories and Convicted Offender DNA Databasing Laboratories* (QAS) mandates that each DNA Laboratory employ a Technical Leader. The Technical Leader oversees the technical management of the laboratory, including

1. Evaluates all methods used by the laboratory.
2. Stays current in new technologies and methods, and proposes new or modified procedures.
3. Oversees validation of new instrumentation and methodology, and keeps the SOP manual up-to-date.
4. Provides technical problem solving of analytical procedures, and makes technical decisions on casework.
5. Oversees staff training, competency, continuing education. This includes monitoring of proficiency testing and courtroom testimony.
6. Oversees quality assurance and safety.
7. Performs technical reviews (if at one time proficient in the analysis being reviewed) and administrative reviews
8. Keeps the Crime Laboratory Director and Quality Manager updated on technical issues that may affect laboratory analysis or productivity.

The technical leader has the authority to stop work in the DNA section in the event of problems that affect compliance with standards and/or the quality of the DNA results.

1.2 Criminalists

Forensic DNA casework is performed by criminalists (including criminalist specialists), whose responsibilities are outlined in the quality manual. The authority to perform casework is given upon completion of the laboratory's training program (or an equivalent training program elsewhere) and completion of appropriate competency testing, as well as ongoing demonstrated compliance with the laboratory's quality program (i.e. continuing education and external proficiency testing). These individuals may also be referred to in this SOP as examiners or analysts.

2 Quality Assurance

The quality manual prescribes general requirements for the quality assurance program. These requirements are based on ASCLD/LAB standards. In addition to ASCLD/LAB standards, the DNA section adheres to the FBI DNA Quality Assurance Standards for DNA Testing Laboratories (based upon the DAB standards). In order to participate in the National DNA Index System (NDIS); the DNA section complies with these DAB standards, the FBI's *NDIS Standards for Acceptance of DNA Data*, and the FBI's prescribed NDIS procedures.

2.1 Quality Control

Examiners keep quality control records as required in the quality manual and DNA SOP. The following controls will be used in DNA analysis:

- Substrate controls, taken from an unstained area adjacent to evidence stains, may be included in body fluid identification tests as appropriate. The examiner will determine if the substrate control will aid in the interpretation of the results.
- A hair shaft negative control will be analyzed for each hair root analyzed.
- A reagent blank will be prepared for each set of DNA extractions. It will contain all reagents used in that extraction process, but no DNA. The reagent blank will be processed through the entire analysis in the same manner as samples. It will be handled in such a way that it may detect contamination in the most dilute evidence sample. For example, the reagent blank volume will be at least as low as the lowest volume of any sample in that batch. Additionally, if 10 µl is the greatest amount of template amplified for any evidence sample in the batch, 10 µl of reagent blank should be used as template during the amplification.
- A positive control consisting of human DNA of known type and an amplification blank (containing all reagents but no DNA) will be introduced at the amplification setup. The positive and negative controls will be processed through PCR setup and analysis in the same manner as samples. The amplification blank will be the last sample processed in the set and should be handled in such a way that it may detect contamination occurring during PCR setup.

Critical reagents

Critical reagents are those that require testing prior to use on evidentiary samples in order to prevent unnecessary loss of sample and must include commercial DNA typing kits. These are itemized in Critical Reagents Section, and are quality control tested using the forms in Forms Section. The human DNA controls, primer sets, and DNA polymerase contained within these kits must be quality control tested as part of the kit testing.

The critical reagent quality control log will contain reagent quality control worksheets as well as any necessary corrective action records pertaining to critical reagent testing. The

quality control worksheet will show reagent name(s), lot number(s), expiration date(s), quality control test instructions and evaluation criteria, and quality control test results.

Validation

This laboratory will not generally develop a novel methodology; but if the laboratory does, the methodology will undergo full developmental validation in compliance with all relevant standards prior to use in casework. Methodology that has undergone developmental validation elsewhere will be internally validated prior to use in casework. Substantial changes in an existing protocol will also be subjected to an appropriate internal validation evaluation.

No new or modified method, i.e., any method not already described in the SOP, is to be used without the documented approval of the Technical Leader, Quality Manager, and Crime Laboratory Director.

Education

Educational requirements for criminalists are detailed in the quality manual. The education requirements for Technical Leader include a master's degree or higher in a biology-, chemistry-, or forensic science-related area and successful completion of 12 semester or equivalent credit hours from a combination of undergraduate and graduate coursework covering the following subject areas: biochemistry, genetics, molecular biology, and statistics or population genetics. The coursework shall include at least one graduate-level course registering 3 or more semester or equivalent credit hours.

Analysis Training

Examiners will be provided fundamental DNA analysis training according to the training manual. This will include all protocols in the SOP that are used in the lab. DNA training may be undertaken as separate units of training and will each conclude when:

- All training samples are correctly analyzed;
- A competency test (qualifying test) is correctly analyzed;
- The trainee successfully completes the DNA Section Training Manual;
- The training notebook, other training records documenting completion of training requirements, and trainee credentials are reviewed and approved by the Technical Leader, Quality Manager, and Crime Laboratory Director
- These individuals all approve the examiner for independent casework.

In addition, each examiner's profile must be on file for the DNA typing systems in use in the laboratory. The laboratory maintains a database of DNA profiles of personnel for the sole purposes of identification of the source of contamination and evaluation of in-house competency tests.

Continuing education training

Continuing education training will be conducted for enhancement of an examiner's skills. The supervisor will recommend to management and coordinate training activities for personnel. As part of their continuing education, each examiner approved to perform DNA analyses will:

- Attend at least 8 cumulative hours of continuing education training annually for the enhancement of individual DNA analysis skills.
- Read current scientific literature as a part of their continuing education. Each examiner will keep an updated log documenting any scientific literature read. Examiners will attempt to read at least 1 scientific article per month.

The Technical Leader and the Quality Manager may identify areas for which remediation training is necessary based on the results of proficiency or competency test results, laboratory audits, or peer review activities.

Training notebook

The trainer and/or supervisor is responsible for creating, maintaining, and distributing training samples as well as records of their sources and known types. The trainee will examine the training samples according to the SOP, maintaining analysis documentation as for casework. The training samples will include a minimum of ten bloodstains, ten buccal swabs, five mixed stains of semen and epithelial cells for differential extraction, and five hairs.

The Trainer will review the training notebook prior to its presentation for review by the Quality Manager. The training notebook will include at a minimum:

- The training log showing the start of training and the activities and date on each day of training;
- Summary page of results of all training samples;
- Analysis documentation for all training samples; and
- Written tests taken during training.
- Other training records, such as practice sample analysis documentation, may be kept in the competency notebook.

Competency Test

Trainees must correctly complete a lab practical competency test prior to recommendation for approval to perform casework. This competency test should be in the form of a case-type scenario, and the trainer but not the trainees should know the test results. An example of an acceptable competency test is a current or old proficiency test from a commercial provider (although in-house tests may be made).

An experienced examiner is required to test 5 previously analyzed samples in order to demonstrate competency. Documentation will be retained in the laboratory.

Written examinations

Trainees will be tested during training according to the DNA Training Manual. Trainees must successfully complete all written examinations covering the entire training unit of DNA prior to approval to perform independent analyses. Exams will be designed to document the trainee's knowledge of the procedures as well as the trainee's understanding of the theoretical basis for those procedures. In addition, the trainee may be asked to demonstrate knowledge and understanding of quality assurance practices relevant to his or her work. Exams will be written and administered by the trainer or supervisor. Discussion and/or distribution of any exam is limited to those individuals actively participating through role or chain-of-command in the trainee's examination. The exam will not be discussed with, distributed or displayed to, or otherwise divulged to any other person.

Unusual samples

An approved examiner may use a valid procedure for analysis of a body fluid or tissue not encountered during training providing the analyst has previously demonstrated competence in that procedure. Examiners must undergo training and competency testing for extraction of bone and teeth prior to analyzing these samples.

Review of casework

All case files and reports will be administratively and technically reviewed prior to release from the laboratory. Reviews should follow the reviewer checklist, and a copy of the completed reviewer checklist should be included in each case file.

Technical reviews should be conducted by a second analyst qualified (or previously qualified) in the DNA platform currently in use in the laboratory. All reviews of DNA case folders shall include a second read of the raw data generated for the case, including creation of a new GeneMapper project on the computer using the original raw data from the case. All electropherogram plots in the case file should match the electropherogram plots generated during the reviewer's second read. The reviewer should indicate agreement with the second read by signing and initialing the GeneMapper project table for each run in the case folder.

The analyst who worked the case may perform the administrative review; however, it is recommended that another analyst or supervisor perform the administrative review when possible.

If there is a discrepancy between the analyst and the technical and/or administrative reviewer, every attempt should be made by both parties to reach an agreement about the points being argued. If no agreement can be reached, the Technical Leader shall be consulted and make the final determination.

All outsource cases will be technically reviewed prior to uploading cases into CODIS, according to the same standards as in-house cases.

Proficiency testing

Proficiency testing and review will follow the requirements of the quality manual. In addition, the Quality Manager will maintain a copy of the analysis documentation for each proficiency test. Proficiency tests will be analyzed and interpreted according to standard operating procedures including technical review. Administrative review may or may not be appropriate to a given test format and is to be performed at the discretion of the laboratory.

Audits

The Quality Manager will plan, arrange, and direct audits according to ASCLD/LAB and QAS requirements. This audit will be completed once each calendar year, with the intervals between FBI Quality Assurance Audits being no less than 6 months and no more than 18 months. The auditor(s) will use both the ASCLD/LAB and FBI Quality Assurance Audit Documents as a checklist for compliance.

Personnel records

The Quality Manager will maintain a transcript, approval memos, complete proficiency records, continuing education records, and testimony monitoring records for each examiner. The laboratory must maintain the competency notebook, original or copies of training records, and proficiency test files for each examiner. Original training records must be replaced with complete copies prior to separation of an examiner from HPD Crime Lab. The laboratory shall maintain a transcript, approval memos, and testimony monitoring reports for each examiner.

Chemical and reagent labels

Purchased chemicals and reagents will be marked on the container with the date received and/or date opened. An expiration date will be placed on the outer container. In general, the manufacturer's labeling will be followed to determine expiration dates of purchased chemicals and reagents. If no manufacturer information exists for a purchased reagent, it will be considered expired 5 years from the received date.

The prepared reagent label will include the reagent specification, lot number (consisting of preparation date and preparer's initials), and date of expiration if required. In general, most solutions prepared in the DNA laboratory shall expire 1 year from the date of preparation. Additional information may be documented in a reagent log.

Equipment

Equipment operation manuals will be readily available to each examiner approved to use the equipment. Calibration, maintenance, and repair activities will be recorded in an equipment calibration and maintenance log, or in a logbook dedicated to that specific piece of equipment. The equipment calibration and maintenance log will include at a

minimum the date, activity, laboratory personnel performing or overseeing the activity, non-HPD technician(s) performing or overseeing the activity, a record of quality control checks performed to verify operation prior to returning a piece of equipment to casework use.

Contamination log

Any and all contamination events will be summarized in a contamination log that will document the date detected, first date evident in analysis records, case numbers of affected cases, and location of documents detailing contamination source and corrective action. A copy of this documentation should be provided to the Quality Manager and the Laboratory Director. The section defines two types of contamination event. Both types are required to be documented in the contamination log and are as follows:

- Type 1: A contamination event that affects the DNA extract and requires a re-extraction of DNA from the sample.
- Type 2: A contamination event that does not effect the DNA extract (i.e. one involving contamination in the amplified product OR contamination in the formamide set-up) and does not require a re-extraction of DNA from the sample.

3 Facilities

Crime Laboratory security and facility requirements are described in the quality manual. The Forensic Biology-DNA Section floor plan can be found in the "Supporting Documents" section of the Crime Laboratory Forensic Biology intranet site. In addition to procedures and policies in the quality manual, the following policies apply to the DNA section.

3.1 Work Areas

The DNA laboratory will have designated space for the following activities. These activities must occur only in these designated areas

- **DNA extraction** - DNA extraction, purification, and concentration; microscopy may also be performed in this area.
- **PCR setup** - setup of real-time PCR quantification and PCR amplification reactions. A laminar flow hood or PCR setup hood dedicated to amplification setup is recommended.
- **Amplified DNA product** - generation, analysis, and storage of amplified DNA product.

The extraction of known samples will be performed at a separate time or location from the extraction of evidentiary samples to prevent known to unknown sample contamination. Decontamination of work areas should be performed between set-up of the evidentiary samples and set-up of the known samples.

The DNA extraction area and PCR setup area will be separate from each other. This is accomplished by maintaining separate physical spaces for each task or by conducting these tasks at separate times. If conducted in the same space at separate times, the space will be decontaminated between tasks.

The amplified DNA product area will be physically separate from all other areas. Doorway(s) to the amplified product area will have a door that is to remain closed at all times. Once amplified, no samples will leave the amplified DNA product area unless securely packaged. Equipment, reagents, and supplies in the amplified product area are dedicated and will not be removed unless properly decontaminated through treatment with UV or thorough wiping with a decontaminant. Each removable item will be readily identified as dedicated for use in the amplified DNA product area only, e.g., with a spot sticker.

3.2 Contamination

Samples can become contaminated with DNA from the environment, from other samples during sample preparation, or from amplified DNA product from a previous amplification. Reagent blanks, negative amplification blanks, and possible substrate controls are used to detect contamination.

Contamination will be suspected and investigated when a mixture is obtained in samples expected to be of one source, or when a reagent blank or negative control yields peaks above the minimum analysis threshold. If possible peaks below the minimum threshold are observed in reagent blank or negative control, the Technical Leader will determine if the event should be further investigated. In addition, contamination may be suspected and investigated under other circumstances at the discretion of the examiner, Technical Leader, or supervisor.

Prevention and decontamination

The following policies are designed to prevent contamination of DNA samples:

1. To minimize the potential for contamination from staff and/or visitors, unnecessary traffic into each of the work areas should be avoided.
2. Use 10% bleach or DNAway as a decontaminant. Other commercially available decontaminants may be used if they are shown to completely inactivate DNA for the purposes of amplification. UV treatment and/or autoclaving is also acceptable for decontamination.
3. In general, clean glassware after each use wearing gloves, and using an appropriate soap, e.g., Liquinox or Alconox, and water. Rinse with deionized or distilled water and allow to air-dry inverted. DNA reagent bottles require sterilization after cleaning; autoclave or rinse with sterile water prior to use. For glassware in the amplified product area, rinse thoroughly with water after each use, with a final rinse of distilled or deionized water, and invert to air-dry.
4. Wear disposable gloves during all testing. Change gloves frequently and whenever gloves may have become contaminated. Discard gloves when leaving a work area. Centrifuge all liquid to the bottom of closed microcentrifuge tubes before opening. A de-capper or a clean kimwipe may be used for opening microcentrifuge tubes. Use sterile, disposable pipet tips and microcentrifuge tubes. Use aerosol-resistant pipet tips while working with any sample that may be subsequently amplified. Change pipet tips between samples.
5. In the DNA extraction area, clean work surfaces thoroughly with decontaminant at least at the beginning and the end of each DNA extraction session. Limit talking during sample handling.
6. In the PCR setup area, add DNA template last to the PCR setup tubes to minimize inadvertent transfer between setup tubes and resultant cross contamination. Limit talking during sample handling. It is recommended that the lab irradiate work surfaces and equipment in the PCR setup area with ultraviolet (UV-C, λ = 245 nm) germicidal lamps, for 15-20 minutes. Surfaces not irradiated will be treated with decontaminant. Timers for UV lights are recommended; if operated manually, wear UV protective glasses when turning UV lights on and off.

7. In the amplified DNA product area, wear a dedicated, disposable lab coat when handling amplified samples. Do not wear the lab coat outside the amplified DNA product area. These lab coats will be disposed of when necessary. Clean work surfaces thoroughly with decontaminant after use. The lab may irradiate work surfaces and equipment in the DNA product area with ultraviolet (UV-C) germicidal lamps.
8. Each Biology section examiner's DNA profile will be determined for all systems currently in use. The DNA profile for other staff and visitors may also be required, in order to ensure the detection of contamination. All DNA profiles should be stored by the laboratory.

Detecting and Responding to Possible Contamination Events

Any suspected contamination incident must be immediately brought to the attention of the Technical Leader. The Technical Leader will define and direct the investigation and corrective action for the event. All actions will be documented via the incident reports/correction actions policy detailed in the quality manual.

Investigation and corrective action should be guided by the nature of the specific event, and may include the following:

1. Compare the unknown profile to the staff/visitors database.
2. Compare the unknown profile to profiles from samples worked with the contaminated sample.
3. Work backwards to determine where the contamination occurred:
    a. Re-inject the sample from the injection tray.
    b. Re-prepare the amplified product (addition of formamide and internal lane size standard) and re-inject.
    c. Re-amplify and analyze the DNA extract.
    d. Re-extract and analyze the sample (if this may be done without consuming the sample).
4. Extract, amplify, and/or inject known samples (to test suspected reagents and/or equipment).
5. Discard suspected buffers and prepared reagents, and clean reagent bottles.
6. Clean and decontaminate work areas, glassware, pipets, etc.

3.3 Safety

There are biological and chemical hazards in the laboratory. Each lab employee is responsible for familiarity with the Lab Safety Manual. Any incident or condition that occurs in or under the control of the laboratory that threatens the immediate or future health of any individual must be immediately brought to the attention of the section

supervisor and laboratory safety officer. Laboratory management will define corrective action.

4 Casework Processing

4.1 Analysis Methods

The DNA laboratory provides STR analysis. The STR analysis using the Identifiler amplification multiplex produces the DNA profile at the FBI's 13 core loci (D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, TH01, TPOX, and CSF1PO), amelogenin (a sex marker), and the D2S1338 and D19S433 loci.

Unknown or suspect profiles developed from evidence are routinely databased in CODIS for searching against other evidentiary profiles and convicted offender profiles at the state and national levels. Suspect reference profiles are also databased and searched at the state level.

Comparisons that yield a probative match between known and questioned items are evaluated to estimate statistical significance. Highly significant matches are reported as identification of the source of the evidentiary profile.

4.2 Case Acceptance and Evaluation

Before a case is accepted for analysis into the laboratory, the case will be evaluated. The examiner should be thoroughly aware of the requested examinations, the reason(s) for the requested analyses, the potential probative value of the evidence, and the quality and quantity of the evidence. Because each case is different, only guidelines can be prescribed; the case evaluation may include consultation with the investigator/prosecutor as necessary to determine what evidentiary items should be analyzed. Document conversations related to case evaluation fully, and ask the officer to change analysis requests, as appropriate. An offense report may be helpful in assessing the evidentiary material.

If the necessary equipment or expertise is not available to comply with a valid, pertinent request, the submitting officer should be so advised. If another non-HPD laboratory is known to be capable of performing the requested analysis, consider coordinating portions of the analysis or referring the investigator/prosecutor directly to the other laboratory.

Both suspect and non-suspect cases will be accepted. The laboratory supervisor may evaluate unusual submissions for acceptance on an individual basis.

Biological evidence should be submitted by the law enforcement agency to the HPD Property Room or to the Crime Lab Central Evidence Receiving Section.

4.3 Evidence Evaluation

Before the case is worked, an evaluation should be made to determine the quality and quantity of the evidence that is going to be analyzed. In order to expedite casework, it is recommended a maximum of 5 evidence stains undergo DNA analysis. Emphasis should be placed on items of significant evidentiary value. Additional items/stains may be analyzed at a later date depending on case development and initial DNA analysis results. Decisions have to be made concerning the analytical approach that must be taken to obtain the most useful information. It is often helpful to consult with another qualified examiner, the Technical Leader, and/or the supervisor. Cases must be evaluated to:

- Eliminate the loss of potentially valuable information.
- Maximize the meaningful information obtained from the evidence.
- Determine if the requested examinations can be performed with the submitted evidence and with the available resources.

Some of the considerations in evaluating the evidence and deciding which items should be analyzed for DNA include:

- The age of the evidence, especially when the evidence is biological material.
- The storage conditions of the samples prior to submission.
- Whether wet samples were dried before submission.
- Whether the evidence is moldy and/or putrefied.
- Possible dilution of the samples.
- Whether weapons or other objects require fingerprinting or have been fingerprinted.
- Whether all pertinent evidence has been submitted.
- The availability of suspect, complainant, and/or elimination reference sample.
- The analyses that should be run if sample is limited.
- Possibility of sample remaining after analysis.
- Possibility of cross-contamination.

4.4 Evidence Handling

Storage of Evidence

Biological evidence must be properly stored to preserve biochemicals assayed in body fluid identifications and DNA typing for current and future analyses. Storage conditions for all types of evidence present must be considered so that none are compromised.
During the initial analysis of the case, DNA extracts may be stored refrigerated. After a supplement has been reviewed and entered for the case, DNA extracts should be stored frozen. Repeated freezing and thawing of extracts should be minimized. DNA extract tubes should be clearly labeled with the case number, item number, and unique identifier and sealed with parafilm™ prior to long-term storage. Extract tubes should be placed in a sealed plastic bag if they are retained in the same paper envelope as the retained stains.

Reagent blanks will be stored separately from DNA extracts. All reagent blanks should be clearly labeled with name and unique identifier. Each analyst will have a storage box to be kept in the walk-in freezer. These boxes will be labeled with analyst initials and numbered sequentially from the number 1. All reagent blanks will be stored in these boxes.
It is not necessary to maintain or store amplified product, amplification controls, or dilutions of DNA extracts.

Consumption of Evidence

The evidence quality and quantity will be preserved as much as possible without sacrificing the quality of the analyses. Whenever possible, at least half of the evidence sample will be preserved for possible re-analysis. When this is not possible, appropriate personnel (submitting officer, prosecuting attorney, and/or defense attorney) will be notified prior to the consumption of evidence.

Documentation

Refer to the quality manual for chain-of-custody policies and procedures, and documentation of chain-of-custody, as well as documentation required in all Crime Laboratory case files.
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Documentation must be in such a form that another qualified examiner or supervisor, in the absence of the primary examiner, would be able to evaluate what was done and interpret the data. The reviewer of the case should be able to determine from the notes that sufficient testing, relevant testing, and correct methods of testing were used. To this end, all documentation of procedures, standards and controls used, observations made, results of test performed, charts, graphs, photographs, sketches, electropherograms, etc. that are used to support the examiner's conclusions must be preserved as a record. If original items cannot be retained or decrease in intensity over time, copies of the original item sufficient to retain the information during long-term storage should be retained. Abbreviations must be defined in the Crime Laboratory SOPs, a common American English dictionary, or on the page on which it is used.

Appropriately completed SOP worksheets should be used during the analyses. In addition to the documentation requirements of the quality manual, the following must be documented in the case file:

- Notes that help in the identification of the item of evidence. A written description may suffice for some items, whereas others may need a drawing, sketch, or photograph.
- Documentation of long-term storage of DNA extracts and reagent blanks.
- Certain quality control documentation such as a copy of the standard curve used for Quantifiler results, a copy of the allelic ladder used for Genemapper analysis, a copy of amplification results of any reagent blanks associated with the case, and copies of results of positive and negative amplification controls.

- Naming of DNA Extracts. Each DNA extract will be assigned a unique identifier at the beginning of analysis. This identifier is intended to assist the analyst in tracking the extract through the analysis process. The unique identifier will be indicated on the DNA Extract Log and any subsequent DNA analysis worksheets. Each analyst will number his or her extracts sequentially from 1 using this format: #initialslasttwodigitsofyear. Each calendar year, every analyst will start his or her unique identifier-naming scheme at 1. An example of a unique identifier series that was extracted in 2005 follows: 1VN05, 2VN05, 3VN05, etc. Reagent blanks will be given a unique identifier in sequence with DNA extracts. The unique identifier along with the laboratory number will be included on all extract tube labels.

4.5 Naming Controls

Controls will be named using this format: identifierdateinitials. Identifiers for controls are as follows:

- RBQ (for questioned stains)
- RBK (for known stains)
- RBS (for sperm cell fractions)
- RBE (for epithelial cell fractions)
- RBR (for hair roots), RBSH (for hair shafts)
- POS (for PCR positive controls)
- NEG (for PCR negative controls)

For example, the naming of controls for a differential extraction that was extracted on May 31, 2006 and amplified on June 1, 2006 is as follows: RBS053106VN, RBE053106VN, POS060106VN, and NEG060106VN.

In the event a re-extraction of a sample occurs, the sample will retain its original unique identifier and the designation “RE” will follow. Thus, a re-extraction of sample 8CDA06 would be identified as 8CDA06RE. Additional re-extractions of the same sample would be identified using sequential numbers starting from 2. Thus an additional re-extraction of sample 8CDA06RE would be identified as 8CDA06RE2.

In the event a re-amplification of a sample occurs, the sample will retain its original unique identifier and the designation “RA” will follow. Thus, a re-amplification of sample 8CDA06 would be identified as 8CDA06RA. Additional re-amplifications of the same sample would be identified using sequential numbers starting from 2. Thus an additional re-amplification of sample 8CDA06RA would be identified as 8CDA06RA2.

In the event that the analyst performs two extractions or amplifications on the same day, the two events will be distinguished with a 1, 2, etc.

4.6 Analytical Approaches

Once the case has been evaluated, the examiner decides on an analytical approach. The examiner should choose a scheme of analysis using recognized, accepted, and internally validated scientific procedures designed to develop the information in a logical sequence. In general, the analysis will enable the examiner to make conclusions regarding the source of the evidence.

Once an approach is chosen, the examiner should evaluate the results at each step in light of previous results. A repeat analysis may be indicated when the first analysis has produced inconclusive results. Internal inconsistencies should be investigated. The opinion of a second qualified examiner or the technical leader can be helpful when results are unclear.

Hair comparisons can be made using DNA characteristics or microscopic characteristics. With any attempt to DNA type a hair root, a result is not assured and, for a hair root in the telogen phase, not expected. DNA STR hair root analysis consumes the sample but may not yield results. Therefore, the evidentiary value of the hair must be carefully evaluated and the potential loss of information weighed before proceeding with DNA analysis. Typically, an evidentiary hair will be analyzed only after a microscopic examination of the hair by a qualified trace analyst and after consultation with the investigator/prosecutor to determine:

- What is the significance of the particular hair, e.g., collected by pubic combing vs. car vacuum?
- Is it permissible to destroy part of the evidence?
- Are there additional details of the case that may explain the hair?
- What is the condition of the hair, e.g., fragment, telogen root, etc.? What is the likelihood of a DNA typing result?
- Is it desirable to postpone DNA typing at this time?
- Would mitochondrial DNA analysis by another laboratory be possible?

4A Abbreviations

The majority of abbreviations used in the DNA section are defined in the **HPD Crime Laboratory Standard Operating Procedures – Biology Section** and may be used in DNA procedures and documentation. In addition, the following abbreviations are permissible:

BP	base pair
DTT	dithiothreitol
QF	Quantifiler
PCR	polymerase chain reaction
PK	Proteinase K
RB	reagent blank
RFU	relative fluorescence unit

5 Critical Reagents

In order to provide and maintain the quality of the work provided in the DNA section, it is necessary to identify certain reagents as critical. Critical reagents are those that require testing prior to use on evidentiary samples in order to prevent unnecessary loss of sample.

Reagents and supplies that have passed their expiration dates may not be used on casework or database samples. Outdated reagents may be used for training purposes only, but must be designated as such.

5.1 Reagents

The following reagents are considered critical reagents: Proteinase K, DTT, Quantifiler Human Kit, and AmpFlSTR Identifiler kit.

Proteinase K (PK)
PK digests proteins and is used to lyse cells in the organic extraction of DNA from body fluids. Each new lot of PK must be subjected to the appropriate in house quality control test by using it in the differential lysis of a known semen/epithelial cell mixture. Cell lysis is confirmed microscopically by the presence of very few to no epithelial cells per field after digestion.

Dithiothreitol
Dithiothreitol reduces disulfides to their corresponding thiols. This reagent is used in conjunction with Proteinase K to lyse spermatozoa heads to release the DNA. Each new lot of DTT must be subjected to the appropriate in house quality control test by using it to lyse a known spermatozoa sample. Sperm head lysis is confirmed microscopically by the presence of very few to no sperm heads per field after digestion.

Quantifiler Human Kit
The Applied Biosystems Quantifiler Human kit is used to quantify human DNA through the use of real-time PCR. These kits contain reaction mix, control DNA, and primer mix. Each new lot of Quantifiler Human kits must be subjected to an internal quality control test as follows:

1. The control DNA must be diluted to make a standard curve according to the procedure outlined in Section 8. This DNA must then be run in duplicate on a plate. IPC values must be obtained from all points on the curve to ensure the reagents are working correctly.
2. A negative control consisting of master mix only must be run on the same plate. This negative control must have an IPC value and less than 5 x 10-3 ng/ul of detectable DNA present.

Identifiler Kit

Applied Biosystems Identifiler kits are used in the amplification and typing procedure for 15 loci plus sex typing (Amelogenin). These kits contain reaction mix, AmpliTaq Gold polymerase, primer set, positive control DNA, and the allelic ladder.
Each new lot of Identifiler kits must be subjected to an internal quality control test as outlined below:

1. The positive control DNA must be run and the correct profile (as reported by the manufacturer) must be obtained.
2. An amplification blank must be run and shown to have no detectable alleles
3. All reagents in the kit must be used.
4. The allelic ladder must be run to determine that all of the appropriate alleles are detected.

5.2 Logbook

A logbook will be maintained for the quality control of all of the critical reagents. This logbook will contain the test date, signature of the analyst performing the quality control, expiration date for lot, lot number, and any supporting documentation necessary to demonstrate the reagent met all of the standards listed above.

If any new lot of critical reagent does not meet the above stated guidelines, it may not be utilized in casework. All inconsistencies will be documented and reported to the technical leader. The analyst must work backwards to determine where the problem occurred. Problems that cannot be resolved must be reported to the manufacturer.

6 Equipment Quality Control and Maintenance

6.1 Purpose

In order to provide and maintain the quality of the work provided in the DNA section, it is necessary to ensure laboratory equipment is in good working order. Routine quality control and maintenance accomplishes this. The calibration intervals listed below are generally considered to be the minimum appropriate in each case, providing that the equipment is of good quality and of proven stability and the laboratory has both the equipment capability and expertise to perform adequate internal checks. More frequent checks are not discouraged. If there is any question concerning the reliability of an instrument or piece of equipment, a maintenance check should be performed immediately.

Full records must be maintained and be readily available for inspection. Documentation must include the numerical result, date of calibration, analyst's signature, and any other relevant observations. The section supervisor and QA Manager are responsible for ensuring all systems are checked annually.

The following equipment must be maintained and subjected to quality control measures: water baths, pH meters, microcentrifuges, refrigerators/freezers, thermometers, genetic analyzers, pipettes, balances, thermal cyclers (including real-time PCR instruments), hoods, autoclaves, and water filtration system. Maintenance and quality controls for these instruments is detailed in the HPD Crime Laboratory SOP – Biology, except for thermal cyclers, genetic analyzers, and pipettes. Details for these instruments are provided below.

6.2 Equipment

Genetic Analyzers

The Applied Biosystems genetic analyzer AB 3100 is a capillary electrophoresis instrument used to separate DNA fragments based upon size and fluorescent tags. The main parts of the instrument include the CCD camera, laser, pump block, heat block, autosampler, and syringe(s). All of these parts must be working properly to ensure accurate and usable results are obtained. The laboratory has a Planned Maintenance agreement with Applied Biosystems for the maintenance of these instruments. This plan allows for 1 planned-maintenance visit per year by an Applied Biosystems Field Service Engineer.

To ensure the Genetic Analyzers are working properly after repairs, an allelic ladder must be run and analyzed under normal conditions to ensure all peaks are being called appropriately as indicated by the manufacturer. The GeneMapper ID™ data from this run must be printed and placed in the appropriate logbook. If the ladder does not contain all of the appropriate alleles after several injections, the technical leader will be notified

and a service call scheduled with ABI. No casework samples should be run on the machine until the machine has been serviced.

A new spectral calibration must be made once every 6 months or as needed for each instrument in the laboratory. Follow the manufacturer's guidelines for making the matrix and or spectral and verifying its accuracy. These guidelines can be found in the operations guides located in the post-amp room. Additionally, the User Bulletin for the AB 3100 dated January 2003 should be consulted; this bulletin is maintained with the operations guides. The AB 3100 may be run using the current array if 14 or more of the capillaries pass the spectral. If an array is used where some of the capillaries failed the spectral, a note should be attached to the 3100 indicating which capillary in the array is bad and samples should not be injected on that capillary.

The instrument pump block and syringe should be cleaned as needed. Please consult the operations guides for each instrument for specific instructions on how to clean the instrument. Additionally, the Wizards for the 3100 Collection Software can be consulted for useful information on how to maintain the 3100.

Implementation validation for any new genetic analyzers must include:

1. Precision study comparing 10 positive control injections from the same run and preparing a summary report to include justification of the +/- 0.5 bp window.

2. Sensitivity (dilution) study ranging from 0.156ng to 2 ng of template DNA and prepare a summary report.

3. Accuracy study using a CTS (or other commercial) proficiency test with published results OR using a NIST-SRM.

Pipets

Accuracy and precision in dispensing of volumes are important during many routine operations. The DNA laboratory is supplied with pipets that cover the volume range from 0.1 - 1000 µl. Calibration will be performed at least annually on these instruments. When a pipette is determined to be performing improperly, it will be returned to the manufacturer, or another qualified repair technician, so that the problem may be identified and corrected.

Quality control data should include the type of pipette, volume range, model, and serial number.

The operating manual should be consulted for operation instructions prior to utilizing a pipette.

In case of accidental entry of liquids into the pipette mechanism, remove the shaft from the assembly and clean with distilled water. Dry with lint free tissue (i.e., Kimwipe). Inspect the seal assembly and piston for liquid. Inspect the entire instrument for staining or corrosion. If evident, do not use the pipette, and submit for service. If no further problems are identified, reassemble.

Thermal Cyclers

Thermal Cyclers automate the polymerase chain reaction (PCR) for amplifying DNA. The Thermal Cycler contains a programmable heating and cooling block that performs repeated temperature cycling profiles on samples contained within the block.

The sample block and exterior surfaces should be cleaned at least once every 6 months. Temperature calibration, temperature uniformity, and diagnostic tests must be performed once every 6 months. Follow manufacturer's instructions for performing these tests. The operations manual for the cyclers can be found in the post-amp room. The thermocyclers have established parameters for determining pass or fail; the machine will report a pass or fail result at the end of every test. Any variations outside of established parameters will necessitate recalibration or repair of the instrument by the manufacturer or a qualified service technician. If the Thermal Cycler is damaged or not functioning, either the manufacturer or a qualified service technician may repair the instrument.

Implementation validation for new thermal cyclers must include:

1. Temperature calibration, temperature uniformity, and diagnostic tests.

2. Amplification and analysis of the Identifiler kit positive control for concordance.

Real-time PCR Thermal Cyclers

The ABI 7000 is a specialized thermocycler unit used to detect amplified product in real-time. This unit contains a programmable heating and cooling block, several filters, and a halogen lamp. The unit is used in conjunction with quantitation kits to estimate the amount of DNA in a given sample.

The ABI 7000 should be function tested and the block cleaned according to manufacturer instructions every month. Also, the computer should be defragmented, the wells on the instrument cleaned, and a background calibration performed. Additionally, an ROI calibration should be performed for the instrument every 6 months.

To ensure that the halogen bulb is working properly in the unit, it should be checked monthly. This can be accomplished by one of two procedures:

1. Place the green calibration plate in the instrument. Click "Instrument", "Calibrate", "ROI Inspector". Next, select Filter A and the highest exposure

setting in the pull-down menu. Finally, click "Generate Snapshot." All green wells indicates that the ROI (region of interest) is not shifted; red wells indicate a shift.

2. After a normal plate run, select well C3 and open the "Component View" tab. Verify that the ROX curve begins around 1000 and that the curve is smooth. If the ROX curve is approaching 400 or less, the bulb may need to be changed. The curve will also be wavy instead of smooth.

The analyst will change the bulb if it is determined to have burned out or weakened. Remember to always wear gloves and avoid direct contact when handling the bulb.
A background calibration should be performed on the 7000 each month. To perform a background calibration, take the following steps:

1. Select File, New from the main menu
2. From the pull-down menus in the pop-up box, choose "assay: background", "container: 96-well clear", and "template: blank document". Next click "ok".
3. Choose "File, Save As" and name your document.
4. Start the background assay by clicking the "Start" button in the Instrument tab. The assay takes 10 minutes to complete.
5. When the analysis is complete, view the results in the Results tab. All values displayed in the wells should be less than 1200 rfu. If a value is greater than 1200 rfu, clean that well and repeat steps 1 through 5.
6. After viewing the results, click the play button. The 7000 will automatically extract the background and save it as part of the system calibration.
7. Click Save and close the software.

The instrument laptop computer should be defragmented every month. To defragment the hard drive, take the following steps:

1. Double click on the desktop shortcut to Disk Defragmenter
2. Select the unmounted volume and click the "Defragment" button
3. Once this is complete, select the (C:) volume and click the "Defragment" button
4. If an error message is seen on either of the volumes, alert the technical leader immediately.

An ROI calibration should also be performed on the instrument twice a year and every time the bulb is changed. To calibrate the instrument, take the following steps:

1. Place the green calibration plate on the block
2. Open the ROI inspector and select filters A through D one at a time.
3. Once a filter has been selected, click "Generate Calibration"
4. Inspect the window view: the wells should be mostly white with green circles around them. The wells should NOT be red.
5. Once the calibration window appears as described in item 4, click "Save Calibration"

Validation of new real-time PCR thermal cyclers should include at a minimum:

1. A precision study determining the quantity of the same DNA sample at least two times on the same plate. Several plate runs may be used to add data points.
2. A reproducibility study using the same series of DNA samples run on at least three different plate runs

6.3 Quality Check of the DNA analysis system through NIST-SRM

A NIST-SRM or a NIST-traceable stain must be extracted, quantified, amplified, and analyzed one time per year to verify the laboratory's system of analysis as required in the QAS. This sample should be verified to have typed correctly by comparison with the known DNA profile. If the correct profile is not obtained from the NIST-SRM or NIST-traceable stain, the technical leader should be informed and the analyst should work backwards to determine where any deficiencies in the system exist. Analysis of casework samples in the laboratory should cease until NIST-SRM or NIST-traceable stain can be typed correctly.

7 DNA Extraction

This section details numerous procedures for the extraction of DNA from forensic evidence and reference samples, for the purpose of STR analysis. It is at the discretion of the analyst to determine which of these procedures is most useful for a particular sample.

These procedures indicate recommended amounts of sample to be processed. Evidence samples may be in limiting supply. The analyst should add as much evidentiary sample to the tube as possible, up to the quantities specified in the procedure, retaining sufficient sample for replicate analysis if possible. These amounts are recommendations, and the actual amount used for any sample is up to the discretion of the analyst.

Swabs and cuttings should be dissected into pieces of appropriate size. Use a clean cutting surface for each sample. Sections which are not to be analyzed immediately should be stored frozen.

When the sample is expected to be limiting, or when quantitation indicates that the amount of DNA is limiting, DNA extracts may be concentrated to as low as 15 µL, to maximize the concentration of DNA in subsequent amplifications.

Safety

Body fluids, tissues, and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Gloves must be worn during testing. Clothing may protect unbroken skin; broken skin should be covered.

Phenol, or solutions made from phenol (including the Phenol:Chloroform:Isoamyl alcohol used in the organic extraction procedures in this section), may be fatal if swallowed, inhaled, or absorbed through the skin in sufficient quantities. Phenol solutions should be used only in a chemical fume hood; avoid ingestion, inhalation, or skin contact.

Related Forms - DNA Extraction Worksheet

Standards, Control, and Calibration

One reagent blank must be processed for each extraction batch as a negative control. The extraction method used must be recorded on the DNA extraction worksheet.

7.1 This Section Not Currently In Use

7.2 DNA Extraction Using Digest Buffer – Organic Method

The procedure uses digest buffer along with other reagents to digest and extract DNA. Extracts are purified using phenol-chloroform-isoamyl alcohol, followed by Microcon concentration.

Equipment, Materials, and Reagents

- scissors, scalpel, tweezers
- digest buffer – See Reagents section
- Phenol:Chloroform:Isoamyl alcohol (v/v 25:24:1) – purchased reagent
- TE Buffer – see Reagents section
- 10 mg/mL Proteinase K – see Reagents section
- 1M DTT – see Reagents section
- Microcon concentrator
- tubes – 1.5 mL, microcentrifuge
- sterile toothpicks
- microcentrifuge with rotor for 2 mL tubes at room temperature
- water bath or dry bath ~56 ºC
- vortex

Procedure

1. Add one of the following to a 1.5 mL microcentrifuge tube:
    a. Blood samples- stains or liquid - 1 cm^2 bloodstain, 10 to 50 µl whole blood, or 2 to 10 µL buffy coat (approximately 105 white blood cells).
    b. Saliva samples- including but not limited to oral swabs, filter paper, stamps, envelope flaps, cigarette butts, oral contact swab: 1 cm^2 stain (including filter paper, stamps, and envelope flaps), 1 cm strip of paper covering end of cigarette butt, or one swab.
    c. Hair samples- Approximately 1 cm of root end of hair in one tube and a separate tube for a 1 cm portion of the adjacent shaft of each hair as a control. Hairs should be gently cleaned in sterile distilled water prior to extraction.
    d. Tissue samples- including but not limited to skin, muscle, and body organs. Approximately 3-5 mm^2. It is helpful to mince the tissue prior to adding to digest buffer.
    e. Bone and Teeth - One tooth or approximately 2 cm^3 of bone (preferably flat bone in adults-i.e. pelvis, sternum, ribs). The exterior surface of each sample should be cleaned thoroughly of all debris. Bones may be sanded using a dremel tool to remove debris. Teeth may be cleaned with sterile distilled water and bleach unless there are surface fractures in which case only sterile water is used. Bone and teeth samples are prepared by crushing them into a fine powder. This can be achieved by using a hydraulic press with a stainless steel chamber that is cleaned with bleach

between each sample, or using a single-use coffee grinder. Approximately 0.5g of sample will be placed into each 1.5 mL tube (this may take several tubes).

f. Miscellaneous- including but not limited to items containing shed skin cells, sweat or other body fluids that may contain sufficient quantities of recoverable DNA. Amounts to be used will be at the analyst's discretion.

2. Add 0.5 mL Digest Buffer.
3. Add 15 µl of 10 mg/mL Proteinase K solution (to a final concentration of 0.3 mg/mL). Mix gently. Incubate at ~56ºC for at least 1 hour. For evidence samples it is recommended that digestion continue for a minimum of 6 hours. Digestion may be performed overnight, but more than 24 hours is not recommended.
4. After digestion, substrate may be removed with a fresh sterile toothpick, sterile, disposable pipette tip, or tweezers. Discard substrate or spin the sample for 2 minutes at maximum speed to capture sample at bottom of tube. Alternatively, the substrate may be placed in a spin basket in a separate tube. Centrifuge the tube 3-5 minutes at maximum speed. The substrate in the spin basket will be discarded and the collected liquid transferred to the original extract tube.
5. Separate DNA from proteins. Caution: Wear gloves and eye protection.
    a. To 0.5 mL lysed and digested cells, add 0.5 mL buffered phenol-chloroform solution. Cap tube and vortex until a complete emulsion forms.
    b. Spin in a microcentrifuge for 3 to 5 minutes at maximum speed at room temperature to separate the two phases.
    c. Use a sterile pipette tip to transfer the upper aqueous phase to a fresh 1.5 mL microcentrifuge tube.
    d. Repeat Steps a-c an additional 2 to 3 times, if necessary, until the interface is clean and the aqueous (upper) phase is clear. For these additional extractions, the lower phenol-chloroform layer may be removed and discarded, thus eliminating the need for a fresh microcentrifuge tube after the first extraction.
6. Proceed to Microcon concentration. A pause in the procedure is acceptable at this point.

7.3 DNA Extraction Using Stain Extraction Buffer – Organic Method

The procedure uses stain extraction buffer along with other reagents to digest and extract DNA. Extracts are purified using phenol-chloroform-isoamyl alcohol, followed by Microcon concentration.

Equipment, Materials, and Reagents

- scissors, scalpel, tweezers

- microcentrifuge tubes, 1.5 ml, or spin basket extraction tubes
- microcentrifuge with rotor for 2 ml tubes, at room temperature
- vortex
- ~56°C water bath or dry bath
- sterile toothpicks
- Microcon® concentrator
- Phenol:Chloroform:Isoamyl Alcohol (v/v 25:24:1) - purchased reagent
- Stain Extraction Buffer - See Reagents section
- 10mg/ml Proteinase K - See Reagents section
- TE Buffer - See Reagents section
- 0.39M DTT - See Reagents section

Procedure

1. Add one of the following to a 1.5 mL microcentrifuge tube:
    a. Blood samples- stains or liquid - 1 cm^2 bloodstain, 10 to 50 µl whole blood, or 2 to10 µL buffy coat (approximately 105 white blood cells).
    b. Saliva samples- including but not limited to oral swabs, filter paper, stamps, envelope flaps, cigarette butts, oral contact swab: 1 cm^2 stain (including filter paper, stamps, and envelope flaps), 1 cm strip of paper covering end of cigarette butt, or one swab.
    c. Hair samples- Approximately 1 cm of root end of hair in one tube and a separate tube for a 1 cm portion of the adjacent shaft of each hair as a control. Hairs should be gently cleaned in sterile distilled water prior to extraction.
    d. Tissue samples- including but not limited to skin, muscle, and body organs. Approximately 3-5 mm^2. It is helpful to mince the tissue prior to adding to digest buffer.
    e. Bone and Teeth - One tooth or approximately 2 cm^3 of bone (preferably flat bone in adults-i.e. pelvis, sternum, ribs). The exterior surface of each sample should be cleaned thoroughly of all debris. Bones may be sanded using a dremel tool to remove debris. Teeth may be cleaned with sterile distilled water and bleach unless there are surface fractures in which case only sterile water is used. Bone and teeth samples are prepared by crushing them into a fine powder. This can be achieved by using a hydraulic press with a stainless steel chamber that is cleaned with bleach between each sample, or using a single-use coffee grinder. Approximately 0.5g of sample will be placed into each 1.5 mL tube (this may take several tubes).
    f. Miscellaneous- including but not limited to items containing shed skin cells, sweat or other body fluids that may contain sufficient quantities of recoverable DNA. Amounts to be used will be at the analyst's discretion.
2. Add 0.5 mL Digest Buffer.
3. Add 10 µl of 10 mg/ml Proteinase K solution. Mix gently. Incubate at ~56°C for at least 1 hour. For evidentiary samples, it is recommended that digestion continue

for a minimum of 6 hours. Digestion may be performed overnight, but more than 24 hours is not recommended.

4. After digestion, substrate may be removed with a fresh sterile toothpick, sterile, disposable pipette tip, or tweezers. Discard substrate or spin the sample for 2 minutes at maximum speed to capture sample at bottom of tube. Alternatively, the substrate may be placed in a spin basket in a separate tube. Centrifuge the tube 3-5 minutes at maximum speed. The substrate in the spin basket will be discarded and the collected liquid transferred to the original extract tube.
5. Separate DNA from proteins:
    a. To 0.5 ml lysed and digested cells, add 0.5 ml phenol-chloroform-isoamyl alcohol.
    b. Cap tube and vortex for 15 seconds until a complete emulsion forms.
    c. Spin in centrifuge for 5 minutes at maximum speed, to separate the two phases. Remove the top aqueous phase to a labeled 1.5 ml microcentrifuge tube (if additional extractions are needed) or an assembled Microcon tube.
6. If necessary, repeat steps a-c, using the aqueous layer (after it has been transferred to a labeled 1.5 ml microcentrifuge tube) an additional 2 - 3 times, until the interface is clean and the aqueous layer is clear. For these additional extractions, the lower phenol-chloroform layer may be removed and discarded, thus eliminating the need for a fresh microcentrifuge tube after the first extraction.
7. Proceed to Microcon concentration. A pause in the procedure is acceptable at this point.

7.4 DNA Extraction – QIAamp®

This procedure uses the QIAamp® DNA Mini Kit to digest and extract DNA. The QIAamp silica-gel membrane has an affinity for nucleic acids under certain buffer and temperature conditions. The membrane is supported in a microcentrifuge tube, which simplifies handling. A body fluid extract is centrifuged through the membrane. The adsorbed nucleic acids are washed and then eluted. Due to PCR inhibitors present in urine, this procedure is not recommended for urine samples.

Additional Safety Information

Buffers AL and AW1 are irritants and are incompatible with bleach. Buffer AW2 contains sodium azide, which is highly toxic and may react explosively with lead and copper drainpipes. Ethanol is an irritant and is flammable.

Equipment, Materials, and Reagents

- microcentrifuge with rotor for 2-ml tubes, at room temperature
- vortex
- water baths or dry baths at ~56°C and ~70°C
- QIAamp® DNA Mini Kit (QIAGEN) kit, containing the following:
    - QIAamp spin columns and 2-ml collection tubes

  - Buffer AL
  - Buffer ATL
  - Buffer AW1 concentrate
  - Buffer AW2 concentrate
  - Buffer AE
  - Proteinase K (20 mg/ml, 600 mAU/ml solution or 40 mAU/mg protein)
  - 1 M DTT – see Reagents section
- ethanol, denatured
- For hair extraction only, Stain Extraction Buffer – see Reagents section

General Instructions

- Do not wet the rim of the spin column when transferring liquid.
- Do not touch the membrane with the pipette tip.
- Swabs should be dissected into pieces of appropriate size. Use a clean cutting surface for each sample. Sections which are not to be analyzed immediately should be stored frozen.
- Equilibrate samples and solutions to room temperature before use. Dissolve any precipitate in Buffer ATL by incubating at ~56°C.
- Prepare Buffer AW1 and Buffer AW2 by adding denatured ethanol at the volume noted on the bottle when the kit is first used. These solutions are good for 1 year at room temperature.
- All centrifugation steps should be performed at maximum speed.

Procedure

1. From the evidentiary stain or swab, take:
   a. 0.1–0.5 cm^2 dried bloodstain
   b. 1 swab (buccal, suspected oral contact, etc.)
   c. 1 cm^2 suspected saliva stain (filter paper, stamp, envelope flap)
   d. 1 cm strip of paper from outside of cigarette butt
   e. 1 cm root end of hair
      i. Gently rinse hair in sterile diH_2O prior to removing root end.
      ii. 1 cm of adjacent hair shaft must also be extracted as a negative control.
2. Lyse cells and suspend.
   a. Place the sample into a labeled 1.5 ml microcentrifuge tube.
   b. For blood and saliva samples:
      i. Add 200 µl Buffer ATL (~56°C), 8 µl 1 M DTT, and 20 µl Proteinase K.
      ii. Incubate at about 56°C for at least an hour; 6 hours to overnight is recommended for evidence samples.
   c. For hair samples:

i. Add 300 µl stain extraction buffer and 7.5 µl Proteinase K.
ii. Incubate 18–24 hours at ~56°C.

3. Preheat AE Buffer for elution to ~70°C.
4. Adsorb nucleic acids to membrane:
    a. Briefly spin tubes. Remove and discard the swab or cutting while retaining as much liquid as possible in the tube.
    b. Shake Buffer AL and add 200 µl to liquid in sample tube. Incubate at ~70°C for 10 minutes. Briefly spin tubes.
    c. Add 210 µl denatured ethanol. Vortex vigorously. Briefly spin tubes.
    d. Transfer tube contents (including any precipitate) to a labeled QIAamp spin column in a collection tube.
    e. Centrifuge for 1 minute or until all solution has passed through the membrane.
5. Wash membrane:
    a. Transfer the column to a new collection tube. Add 250 µl Buffer AW1 to the column.
    b. Centrifuge for 1 minute or until all solution has passed through the membrane.
    c. Add 250 µl Buffer AW2 to the column and centrifuge for 3 minutes, or until all solution has passed through the membrane. No Buffer AW2 should remain in or on the spin column.
6. Elute nucleic acids.
    a. Transfer the column to a new, labeled collection tube.
    b. Add 50–100 µl Buffer AE (~70°C) to the column depending on the expected quantity of DNA. Incubate at ~70°C for 10 minutes.
    c. Centrifuge 1 minute.
    d. Transfer liquid from collection tube to a labeled storage tube.
7. Optional: Repeat elution step using the recovered DNA solution to increase yield.
8. Optional: Proceed to Microcon concentration. A pause in the procedure is acceptable at this point.
9. Store samples refrigerated or frozen until ready to perform PCR.

7.5 Differential Extraction of Semen Stains Using TNE

This method of DNA extraction is appropriate for use on stains or swabs mixed with or originating from seminal fluid containing spermatozoa. The differential lysis procedure separates DNA into a sperm fraction and a non-sperm (epithelial cell) fraction. Following the differential lysis of the cellular material, the DNA fractions are further purified using organic extraction (phenol-chloroform).

Equipment, Materials, and Reagents

- scissors, scalpel, tweezers
- tubes – 1.5 ml, microcentrifuge
- microcentrifuge with rotor for 2-ml tubes, at room temperature

- vortex
- water bath or dry bath (~56°C)
- TNE – see Reagents section
- 20% Sarcosyl – purchased reagent
- Proteinase K (10 mg/ml) – see Reagents section
- 0.39 M DTT – see Reagents section
- Phenol:Chloroform:Isoamyl alcohol (v/v 25:24:1) – purchased reagent
- TE Buffer – see Reagents section
- Ethanol, denatured – purchased reagent

Procedure

1. Place one of the folowing evidentiary semen stains into a 1.5 ml microcentrifuge tube:
    a) 0.25 - 1 swab
    b) 0.1 - 0.5 cm2 dried stain
2. Add:
    a) 400 µl Tris/EDTA/NaCl (TNE)
    b) 25 µl 20% sarcosyl
    c) 75 µl sterile diH_2O
    d) 5 µl Proteinase K

    Mix tube contents (a final volume of 505 µl) and incubate at ~56°C for 2 hours.
3. Place the swab or fabric into the spin basket and centrifuge for 5 minutes at ~12,500 rpm. Transfer the supernatant fluid to a fresh 1.5 ml microcentrifuge tube for epithelial DNA analysis beginning with step 7.
4. Optional: to obtain a cleaner sperm fraction, resuspend sperm pellet in TNE. Centrifuge for 5 minutes. Repeat two more times.
5. Resuspend sperm pellet in 160 µl TNE using a small-bore pipette tip such as a p100 or a p200. Optional: Aliquot 10 µl on microscope slide. Fix and stain slide using the Spermatazoa Examination protocol from the Biology Section SOP manual. Record the number of sperm per field on the DNA Extraction worksheet.
6. To the re-suspended sperm pellet add:
    a) 50 µl 20% sarcosyl
    b) 40 µl 0.39 M DTT
    c) 150 µl sterile water
    d) 10 µl Proteinase K

    Mix tube contents (a final volume of 400) and incubate at ~56°C for at least one hour. Note: for evidentiary material, it is recommended that digestion continue for a minimum of 6 hours. It is appropriate at this point to allow samples to incubate overnight (not more than 24 hours).
7. To the lysed and digested sperm or epithelial cells, add 500 µl of phenol chloroform. Cap tube and vortex until a complete emulsion forms. Spin in a microcentrifuge for 3–5 minutes at maximum speed at room temperature, until the

two phases separate. Use a sterile pipet tip to transfer the upper aqueous phase to a fresh, 1.5 ml microcentrifuge tube.

8. Repeat step 7 an additional 2–3 times if necessary, until the interface is clean and the aqueous phase is clear. For these additional extractions, the lower phenol-chloroform layer may be removed and discarded, eliminating the need for a fresh microcentrifuge tube after the first extraction. A pause in the procedure is acceptable at this point.
9. Proceed to Microcon concentration. A pause in the procedure is acceptable at this point.

7.6 Differential Extraction of Semen Stains with QIAmp Purification

This method of DNA extraction is appropriate for use on stains or swabs mixed with or originating from seminal fluid containing spermatozoa. The differential lysis procedure separates the stain components into a sperm fraction and a non-sperm (epithelial cell) fraction. Following the differential lysis of the cellular material, the DNA fractions are further purified using QIAamp® extraction.

Safety

Buffers AL and AW1 are irritants and are incompatible with bleach. Buffer AW2 contains sodium azide, which is highly toxic and may react explosively with lead and copper drainpipes. Ethanol is an irritant and is flammable.

Related Forms - DNA Extraction worksheet

Equipment, Materials, and Reagents

- scissors, scalpel, tweezers
- tubes – 1.5 ml, microcentrifuge
- microcentrifuge with rotor for 2-ml tubes, at room temperature
- vortex
- 2 water or dry baths (~56°C and ~70°C) – or 70°C oven
- sterile toothpicks
- Digest Buffer – See Reagents section
- TE Buffer – see Reagents section
- Proteinase K (10 mg/ml) – see Reagents section
- 1M DTT – see Reagents section
- Ethanol, denatured – purchased reagent

- QIAamp® DNA Mini Kit, QIAGEN catalog number 51304, 51306, or 51308 for 50, 250, and 1000 preparations respectively. Contains the following:
    - QIAamp spin columns and 2-ml collection tubes
    - Buffer AL
    - Buffer ATL
    - Buffer AW1 concentrate
    - Buffer AW2 concentrate
    - Buffer AE (10 mM Tris·HCl, 0.5 mM EDTA, pH 9.0)

General Instructions

- Do not wet the rim of the spin column when transferring liquid.
- Do not touch the membrane with the pipette tip.
- Swabs should be dissected into pieces of appropriate size. Use a clean cutting surface for each sample. Sections which are not to be analyzed immediately should be stored frozen.
- Equilibrate samples and solutions to room temperature before use. Dissolve any precipitate in Buffer ATL by incubating at ~56°C.
- Prepare Buffer AW1 and Buffer AW2 by adding denatured ethanol at the volume noted on the bottle when the kit is first used. These solutions are good for 1 year at room temperature.
- All centrifugation steps should be performed at maximum speed and room temperature unless otherwise noted.

Procedure

Differential Digest

1. Place one of the following evidentiary semen stains into a 1.5 ml microcentrifuge tube:
    - 0.25 - 1 swab
    - 0.1 - 0.5 cm^2 dried stain
2. Add 1 ml sterile TE buffer in a 1.5 ml microcentrifuge tube. Incubate at room temperature for at least 30 minutes. Ultrasonic agitation or vortexing for a short period of time may aid in recovery of cellular material.
3. Vortex 15-30 seconds. Remove the swab or fabric and toothpick. Store swab or fabric in a tube.
4. Centrifuge the sample for 1 minute.
5. Without disturbing the pellet, remove and discard all but 50 µl (or twice the volume of the pellet, whichever is greater) of the supernatant using a sterile 1 ml disposable pipet and discard. Resuspend the pellet in the remaining 50 µl by stirring it with a sterile pipet tip. Note: This pellet contains epithelial cells and sperm cells and is called the cell debris pellet.

6. To the approximately 50 µl resuspended cell debris pellet, add 0.5 ml Digest Buffer. Add 15 µl of 10 µl/ml Proteinase K solution. Mix gently. Incubate at ~56ºC for 1-2 hours to lyse epithelial cells, while leaving sperm cells intact.
   Spin the sample in a microcentrifuge for 5 minutes. Using a 1ml pipet, remove the supernatant to a fresh microcentrifuge tube and proceed to the QIAamp Spin Column Purification. These tubes may be placed in refrigerated storage overnight at this point. Note: Take care not to pipet too near the cell debris in the bottom of the tube to avoid pulling up sperm cells and insure a cleaner epithelial cell fraction.
7. Wash the sperm pellet as follows: Resuspend the pellet in 0.5 ml Digest Buffer by vortexing briefly. Spin the sample in a microcentrifuge for 5 minutes. Using a sterile 1 ml pipet tip, remove all but 50 µl of the supernatant and discard.
8. Optional: Repeat wash step 7 an additional 1 to 2 times. Additional wash steps are recommended when the ratio of sperm to epithelial cells is low.
9. Resuspend the pellet in 0.5 ml sterile deionized or distilled water by vortexing briefly. Spin the sample in a microcentrifuge for 5 minutes. Using a sterile 1 ml pipet tip, remove and discard all but 50 µl of the supernatant. Resuspend the pellet in the remaining 50 µl by stirring it with a sterile pipet tip. Optional: Aliquot 3-10 µl on microscope slide. Fix and stain slide using the Spermatazoa Examination protocol from the Biology Section SOP manual. Record the number of sperm per field on the DNA Extraction worksheet.
10. Add 0.5 ml Digest Buffer to the approximately 50 µl resuspended sperm cell pellet. Add 20 µl of 1M DTT and 15 µl of 10 mg/ml Proteinase K solution. Incubate at ~56oC for at least 1 hour. Note: For evidentiary material, it is recommended that digestion continue for a minimum of 6 hours. It is appropriate at this point to allow samples to incubate overnight.

QIAamp Spin Column Purification

1. Adsorb nucleic acids to membrane.
   a. Shake Buffer AL and add a volume of AL Buffer equal to the sample volume (approximately 500 µl) to sample tubes. Incubate at ~70°C for 10 minutes. Briefly spin tubes.
   b. Add an equal volume (~500µl) of denatured ethanol. Vortex vigorously. Briefly spin tubes.
   c. Transfer tube contents (including any precipitate) to a labeled QIAamp spin column in a collection tube. Centrifuge for 1 minute or until all solution has passed through the membrane.
   d. If the entire volume cannot be loaded in one application, repeat step c above until all the solution has been loaded. This may require that the collection tubes be emptied between spins.
2. Wash membrane.
   a. Transfer the column to a new collection tube. Add 250 µl Buffer AW1 to the column.
   b. Centrifuge for 1 minute or until all solution has passed through the membrane.

   c. Add 250 µl Buffer AW2 to the column and centrifuge for 3 minutes or until all solution has passed through the membrane. No Buffer AW2 should remain in or on the spin column.
3. Elute nucleic acids.
   a. Transfer the column to a new, labeled collection tube.
   b. Add 50–100 µl TE or Buffer AE which has been pre-warmed to ~70°C to the column depending on the expected quantity of DNA. Incubate at ~70°C for 10 minutes.
   c. Centrifuge for 1 minute.
4. Transfer liquid from collection tube to a labeled storage tube.
5. Store sample refrigerated or frozen.

7.7 Microcon® Concentration of DNA Solutions

Microcon® microconcentrators can be used for concentrating DNA solutions. This procedure is required following organic extractions, as it also serves to remove salts. It is optional for other extraction procedures.

Related Forms - Because it is optional in extractions other than organic extractions, its use in these situations should be documented on the DNA Extraction Worksheet.

Equipment, Materials, and Reagents

- Microcon® microconcentrators: consists of two components: filtration unit (sample reservoir) and filtrate/recovery tube
- Variable speed microcentrifuge with rotor for 2 ml tubes, at room temperature
- TE Buffer – see reagents section

General

- Do not touch filtration membrane with pipet tip or other object.
- Extended centrifugation can lead to dryness. If this should occur, add at least 10 µl TE Buffer to the sample reservoir, agitate gently for 30 seconds, then proceed with recovery.
- Excessive g-force by centrifugation at >10,000 rpm may result in leakage or damage to the membrane.

Procedure

1. Using a sterile pipet tip, add up to 500 µl of sample to sample reservoir. If sample volume is less than 500 µl, add TE to bring to a final volume of 500. Seal with attached cap.
2. Centrifuge 8 minutes at 10,000 rpm. Continue to centrifuge if an insufficient amount of filtrate has passed through filter.
3. If sample volume is greater than 500 µl, repeat steps 1 and 2 until all sample has been added to the Microcon.
4. Add 500 µl TE Buffer to sample reservoir to wash DNA and centrifuge as in step 2. Additional TE Buffer washes may be performed to assist in removal of pigments or other contaminants.
5. Invert sample reservoir into a fresh sample recovery tube.
6. Centrifuge inverted unit for 2-3 minutes at 3000 rpm to recover DNA sample. Recovery volume should be 10-40 µl.
7. Store the DNA extract refrigerated or frozen until ready to perform PCR.

7.8 This Section Not Currently In Use

7.9 Purification of Extracted DNA using QIAmp

Some DNA extraction procedures may yield DNA solutions containing inhibitors, identified during quantitation and/or STR analysis. This procedure uses the QIAamp® spin columns to remove PCR inhibitors from DNA extracts. Note that this procedure does not work well in removing inhibitors found in urine samples.

Related Forms - The use of this optional procedure should be documented on the DNA Extraction worksheet.

Safety and Equipment, Materials, and Reagents - See **Section 7.4 DNA Extraction – QIAmp**

Procedure

Add extracted DNA to 200 µl ATL buffer. Incubate at ~56ºC for at least one hour. Centrifuge briefly. Proceed to step 3 of **Section 7.4 DNA Extraction – QIAmp**.

Section 8 Quantitation

The Applied Biosystems Quantifiler Human kit contains all of the necessary reagents for the real-time PCR amplification, detection, and quantification of human DNA. This assay results in a determination of the amount of amplifiable human DNA in a sample. The internal PCR control (IPC) included in each amplification is also useful in detecting the presence of PCR inhibitors.

The reaction plate must be handled carefully to preserve its optical properties. Keep plate in plate base during setup, until you are ready to run the plate. Do not place directly on counter tops. Additionally, try not to touch the bottom of the well with pipette tips when adding reagents and samples to each well.

Related Forms – Quantifiler DNA Quantitation Worksheet

Equipment, Materials, and Reagents

- Quantifiler Human DNA Quantification Kit (AB part # 4343895) which includes
  - Primer Mix
  - PCR reaction mix
  - Human DNA standard
- TE – see Reagents section
- Vortex
- Centrifuge for microtube pulse spins
- AB7000 thermocycler, computer, and data collection and analysis software
- Computer with "QuantifilerHumanImport" Excel template
- 96-well Optical Reaction Plates (AB part # 4306737)
- Optical Adhesive Covers (AB part # 4311971)
- Centrifuge with plate adaptor
- MicroAmp Splash Free Support Base (AB part # 4312063)

Safety

Body fluids, tissues, and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Gloves must be worn during testing. Clothing may protect unbroken skin; broken skin should be covered.

Standards, Controls, and Calibration

Human DNA standards ranging from 0.023 to 50 ng/µl must be run in duplicate in each plate, to estimate the concentration of human DNA. At least one quantitation blank consisting of 23 µl of master mix and 2 µl TE will be run in one well of each plate as a negative quantitation control.

Import File and Worksheet Procedure

1. Open the "QuantifilerHumanImport" template (an Excel spreadsheet). Click the "enable macros" tab on the popup screen.
2. Select "sample sheet entry form" tab and type samples into the sample sheet. Enter each standard for the standard curve twice. Print the file for documentation in the case file(s).
3. Click the "Human Import" button. The screen will flash.
4. After the flash, select the "Import Human" tab and click the "Create Import button.
5. Click "Yes" on the popup screen to save the 7000Import.txt file.
6. Next click on the "7000Import" tab. When this tab is displayed, choose File, Save As. The file may be saved on a jump drive for transport to the 7000 computer.
7. Enter the correct location and file name and hit "save". Select "OK" on the first popup menu to save only the active sheet. Select "OK" on the second popup menu to keep the current format.
8. Close Excel without saving changes.

Reaction and Plate Setup Procedure

1. Allow Quantifiler kit components to thaw to room temperature. Mix each of the components by brief vortexing followed by a short spin.
2. Make standard curve consisting of

Standard	ng/µl
1	50
2	16.7
3	5.56
4	1.85
5	0.62
6	0.21
7	0.068
8	0.023

   To make this dilution series, add 10 µl of the stock Human DNA kit standard to 30 µl TE in a tube, mix well, and spin briefly for Standard 1. Place 20 µl of TE into 7 other tubes. Take 10 µl from the first tube and add to the next. Mix well, spin briefly, and take 10 µl from the second tube and add to the third. Continue until DNA has been added to all tubes. These standards must be made within 24 hours of use.
3. Make a master mix in a 1.5 mL tube using 11.5 µl of Primer Mix and 13.8 µl PCR reaction mix per reaction. Mix well and spin briefly.
4. Place a 96-well reaction plate into plate base.
5. Add 23 µl master mix to each well to be used.
6. Add 2 µl of DNA or control to the correct well and mix with the pipette tip.
7. After all components have been added, seal the plate with adhesive cover.
8. Bring plate in the PCR room. Spin plate in centrifuge to remove all bubbles.

9. Place plate in the 7000. Set compression pad, gray side down, on top of the plate. Close the door to the 7000.

Instrument Run Procedure

1. Turn on the 7000, then log onto the computer.
2. Open the "ABI Prism 7000 SDS Software" program.
3. In the "set up" tab, select the following:
    - Assay: absolute quantification (standard curve)
    - Container: 96-well clear
    - Template: "HUMAN.2"
    - Finish
4. Click "file", "import sample setup" and import the worksheet created in Excel from the jump drive.
5. Select "File" "save as" and save the plate setup in the QF folder using the analyst's initials and date (e.g. vn022805).
6. Select all blocks that DON'T contain samples. Use ctrl-click to highlight non-adjacent blocks.
7. With the blocks selected, click "View" "Well Inspector".
8. Uncheck all boxes in the "Use" column and click "Omit Well". Close the Well Inspector".
9. Verify that all unused wells/boxes have an X through them. Quantitation values will be generated for all boxes that do not have an X.
10. Click "Instrument". Verify that the "Thermal Profile" tab is selected and that it reads:

        Stage 1: 1 rep, 95, 10:00
        Stage 2: 40 reps, 95, 00:15 ramping to 60, 1:00

    Sample volume should be set to 25 µl and the 9600 Emulation should be chosen.
11. Save the file and click the "Start" button to begin the run.

Analysis and Interpretation Procedure

1. Once the run is finished, click on the "Results" tab and click the green "Play" button to analyze the data.
2. Click the "Standard Curve" button and verify the standard curve results. The slope of the curve must be between -2.9 and -3.3, and the r^2 value must be >0.98. With permission from the technical leader, a limited number of outlier points may be omitted from the curve to bring these values within acceptable range. If these values are not within these ranges, the procedure must be re-run.
3. Click on the "Report" tab. Highlight the row and/or samples to be displayed on the chart at the bottom of the screen.
4. Read the results at the top of the screen in the "Qty" column. These results are in ng/µl.
5. Check the internal PCR control (IPC) C_T values for all standards and samples. This value is typically between 27 and 28, and it should be similar for all samples and controls in the run. A higher value indicates the presence of inhibitors. The

absence of an IPC value and a quantitation value for a sample indicated either complete inhibition or a failure of the amplification reaction. Samples in which inhibition is suspected may be cleaned by either Qiagen or microcon and re-quantified.

6. Samples in which the concentration exceeds the highest standard must be diluted and re-quantified to obtain an accurate quantitation value. Samples in which the concentration is below the lowest standard should be amplified using the maximum volume.
7. Print the report by clicking “File” “Print”. Place a copy of the standard curve and data report containing the quantitation results in the case file.

Section 9 This Section Not Currently in Use

Section 10 DNA Amplification

This protocol uses the Applied Biosystems Identifiler PCR Amplification kit. This kit contains primers that are labeled with a light sensitive dye.

Related Forms - Amplification worksheet and amplification grid

Equipment, Materials, and Reagents

- calculator
- microcentrifuge
- microcentrifuge tubes, 1.5 ml
- microcentrifuge tube rack
- pipet tips
- pipettors, adjustable
- tube decapper
- vortex
- DNA Thermal Cycler 9700
- 0.2 mL reaction tube strips or 0.2 ml 96-well Optical Reaction plate
- Identifiler PCR Amplification Kit

Safety

Body fluids, tissues, and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Gloves must be worn during testing. Clothing may protect unbroken skin; broken skin should be covered.

Standards, Controls, and Calibration

- An amplification positive control, consisting of kit Control DNA 9947A must be included with each amplification set.
- An amplification negative control must be included with each amplification set. This negative control will consist of all amplification reagents with TE Buffer added in place of sample DNA.

Procedure

PCR Instrument	Times and Temperatures for Identifiler kits					
	Initial Incubation Step	28 cycles each			Final Extension	Final Step
		Melt	Anneal	Extend		

9700	95°C 11 min. hold	94°C 1 min.	59°C 1 min.	72°C 1 min.	60°C 60 min. hold	10-25°C hold (forever)

1. The thermal cycler should be programmed according to the chart above.
2. Label tube strip retainer tray or reaction plate with appropriate information.
3. Vortex PCR reaction mix, primer set, and AmpliTaq Gold™ and spin tubes briefly in a microcentrifuge to remove any liquid from the caps.
4. Prepare a Master Mix by adding the following volumes to a 1.5 ml tube:
    a. 10.5 µl Reaction Mix X # of samples
    b. 0.5 µl AmpliTaq Gold X # of samples
    c. 5.5 µl Primer Set X # of samples
5. Mix by vortexing for approximately 5 seconds
6. Spin briefly in microcentrifuge to remove liquid from cap.
7. Dispense 15 µl of Master Mix into each amplification tube.
8. Add approximately 1 ng of sample DNA to the appropriate amplification tubes, not to exceed 10 µl in total sample volume. Addition of more or less sample DNA is acceptable to obtain optimum results. For samples with high concentrations of DNA (i.e. >2 ng/µl), dilute these samples with TE buffer to attain an appropriate concentration.
9. Set up a positive control (5-10 µl of the Control DNA) and a negative control (10 µl of TE buffer) to tubes containing the Master Mix. The negative control should be the last tube set up.
10. Place the sample into the Thermal Cycler and start the appropriate program.
11. After amplification, remove the samples from the Thermal Cycler and store away from light. Store samples refrigerated short periods, or frozen for longer periods.

11 This Section Not Currently In Use

12 Genetic Analyzer Sample Preparation

Amplified samples can be analyzed by injection into a capillary on the Applied Biosystems Genetic Analyzer 3100. An internal lane size standard is loaded with each sample to allow for automatic sizing of the PCR products and to normalize differences in electrophoretic mobility between injections. GenemapperID software is then used for automatic analysis and genotyping of alleles in the collected data.

Safety

Body fluids, tissues, and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Gloves must be worn during testing. Clothing may protect unbroken skin; broken skin should be covered.

Exposure to formamide may have chronic health effects, and may be toxic to internal organs; avoid ingestion or inhalation.

Related Forms – None

Equipment, Materials, and Reagents

- Applied Biosystems 3100 Genetic Analyzer (instrument, computer and appropriate software)
- 3100 Genetic Analyzer capillary array (P/N 4315931)
- 3100 Genetic Analyzer 96-well reaction plate and plate septa (P/N n801-0560, 4315933)
- 3100 Genetic Analyzer Buffer Reservoirs
- 3100 Genetic Analyzer reservoir septa
- 250 µl and 5.0 ml glass syringe (P/N 4304470, 628-3731)
- GS500 LIZ (P/N 4322682)
- Matrix Standard Set DS-33 (P/N 4345833)
- deionized formamide- purchased reagent
- Performance Optimized Polymer (POP-4 polymer) (P/N 4316355)
- Genetic Analyzer 4 ml Buffer Vials (P/N 401955)
- vortex
- microcentrifuge tubes 1.5 ml
- microtube racks
- pipets
- pipet tips
- decapper

Standards, Controls, and Calibration

An appropriate allelic ladder, an amplification positive control and amplification negative control will be included with each Genetic Analyzer run. An internal size standard (GS500 LIZ) will be added to each sample.

Procedure

1. Set up the run plate:
    a. Prepare a master mix of deionized formamide and GS500 LIZ in a 1.5 ml microcentrifuge tube as follows and mix well:
        8.7 µl deionized formamide X # of samples, controls, and ladders
        0.3 µl GS500 LIZ X # of samples (include controls and ladders)
    The amount of GS500 LIZ may be adjusted as necessary to give optimal peak heights. Adjust the amount of formamide accordingly, such that formamide volume + GS500 LIZ volume = 9 µl per sample. Document the actual amout of GS500 LIZ per sample used.
    b. Label 96-well plate with appropriate information.
    c. Aliquot 9 µl of the deionized formamide/GS500 LIZ master mix into each well in the plate to be used.
    d. Add between 0.6 – 1.0 µl PCR product or 1.0 µl allelic ladder to each well. Cover the plate with a septum.
    e. Denature the DNA samples by placing the tubes on the 9700 thermalcycler for 3 minutes at 95ºC followed by a snap cool at 4ºC for 10 minutes.
2. Set up the Instrument. For more detailed information on instrument set-up, consult the 3100 operations manual located in the post-amp room. Additional information may also be located in the User Bulletin dated January 2003 and the Wizards that are part of the 3100 Collection Software.
    a. Complete the Plate Manager spreadsheet for the wells you have loaded, type a name for the plate using the analyst's initials and date (e.g rdg010109) and click OK. For each of the columns enter the appropriate data (sample name, dye, and module).
    b. Set the injection time for 5 – 20 seconds, and document the injection time used. A new Plate Manager spreadsheet must be made for each run even if the same plate is used for reinjections.
    c. Start the run.
3. Analyze collected data (refer to Section 13).
4. Document for each 3100 instrument:
    a. A copy of each new spectral file
    b. Capillary changes
    c. Performance Optimized Polymer 4(POP-4) lot numbers
5. Electronically archive all raw data. A copy of all archived data will be retained off-site.

13 Analysis and Interpretation of DNA Results

13.1 GeneMapper Analysis

Allele assignment occurs through a three-step process:

1. **Spectral separation:** The 4 dyes that Identifiler uses in the STR amplification are 6-FAM, VIC, NED, and PET. A fifth dye, LIZ, is used in the internal size standard. Although each dye emits its maximum fluorescence at a different wavelength, there is overlap in the emission spectra. Multi-component analysis is the process that separates the five different fluorescent dye colors into distinct spectral components. This analysis occurs automatically after each instrument run.

   Note: The precise spectral overlap is determined by separately analyzing DNA fragments labeled with each of the dyes (spectral standards). A spectral should be run on each 3100 at least every six months, following maintenance/repairs (other than routine cleaning), and anytime poor spectral separation is suspected.
2. **Peak BP sizing:** The internal size standard (GS500 LIZ, for the Identifiler multiplex) is used to calculate precise peak bp sizing. The internal size standard is used to normalize injection-to-injection variations. The Local Southern method is used to compare allele peaks with the LIZ peaks and calculate the bp size. See the GeneMapper User Manual for explanation of the Local Southern method.
3. **Allele designations**: Allele calls for each peak are made by comparing the bp size of the sample or control peak to the bp sizes of peaks in the allelic ladder. Thus for allele designations to be determined, an allelic ladder must be present and interpretable in each run, and each ladder must have the appropriate alleles present for each locus when analyzed.

Procedure

1. Login to GeneMapper using the appropriate username and password.
2. Add Samples to Project: Browse to appropriate data folders. Select folder, select all samples, click Add to List, and add to the right of window.
3. Verify that:
   a. the analysis range is set to exclude the primer peak
   b. the analysis smoothing option is “light”
   c. the size calling method is the Local Southern
   d. the minimum peak threshold is 100 RFU

   For more specific details about the analysis parameters, consult the Genetic Analyzer QC/maintenance binder.
4. Check that the Sample Type, Analysis Method, Panel, and Size Standard are correctly labeled.
   a. Sample types: allelic ladder or sample
   b. Analysis method: HPD 3100
   c. Panel:Identifiler_1
   d. Size standard: LIZ 01-09-06

5. Analysis settings are GeneMapper Default settings unless otherwise documented in this SOP. The Analysis Range may be changed as needed, assessed by viewing the Raw Data. Set the minimum threshold to 100 RFU for calling alleles. During analysis below, this threshold may be lowered to 50 RFU to investigate any potential alleles below the 100 RFU threshold. Note the presence of possible additional alleles with an asterisk at that locus in the allele chart, but do not use these in the interpretation of results.
6. Highlight a column heading and hit the green arrow on the toolbar. Name the project using the analyst's name and date.
7. At this point, the plots can be analyzed. Hit the Display Plots icon on the toolbar. A separate window will open containing all system plots. Click OFF the all colors except LIZ. The internal size standard for each lane appears in the window. Verify the following for each lane:
   - All of following peaks are present in LIZ: 75, 100, 139, 150, 160, 200, floater (245-250), 300, 340, 350, 400, and 450 bps.
   - Floater peaks are within +/- 0.5 bp of the allelic ladder floater peak. Choose one ladder. Delete all samples that do not match the ladder. A new project will have to be created for samples that are deleted, using a different ladder from the current plate record. Make sure each project contains only one ladder used for analysis.
8. Examine ladder and all controls and confirm:
   - Allelic ladder was correctly labeled (see amplification kit User's Manual).
   - All positive control peaks are present and labeled correctly. No extra peaks indicating contamination are present. Each amplification set and each analyzer run must have one acceptable positive control.
   - All negative controls (reagent blanks and amplification negative control) show no labeled peaks indicating contamination. Each reagent blank must have acceptable results for data from the corresponding extraction set to be used. The amplification negative control must have acceptable results for data from the corresponding amplification set to be used. Additionally, each analyzer run must have at least one acceptable negative control.
9. Examine all electropherograms for data quality and allele calls. Refer to the discussion below for guidance on evaluation data and editing artifacts and microvariant calls. Verify that "track changes" is turned on. Then if necessary, to change the allele type or call, click on the peak or allele call to be edited to select it. Choose either to Delete Allele or Rename Allele. After making selection, a dialog box will appear asking for sample comment. You must type in explanation of edit.
10. Upon completion of analysis, save the project. Print plots for all case samples, associated controls, and ladders to be included in the case file. Place in each case folder a printout of the complete GeneMapper project list for each run and the electropherograms (all 5 colors) from GeneMapper ID for the ladder, positive control, negative control, and all samples and reagent blanks for that case. If a run is not used, document why. Document each time a sample is manipulated including longer or shorter injection times and addition of more sample to the formamide/LIZ set-up.

11. Enter allele information for each sample and reagent blank into an allele chart.
12. A second analyst must perform the GeneMapper analysis independently and agree with the allele calls reported by the first analyst on the allele chart.

13.2 Artifacts and unusual results

True alleles are defined as any peak that meets established threshold values, which is clearly visible above baseline noise, is of a size that falls within a defined category as determined by the GeneMapper program, and is not an artifact. Peaks other than true alleles may be detected on the electropherogram and labeled by GeneMapper. The source of these artifacts should be determined where possible. If the technical leader or a supervisor agrees that such peaks can be definitely identified as an artifact and that the peak does not interfere with interpretation of the data, then data can be used for interpretation. If an artifact or suspected artifact may interfere with interpretation, the locus must be called inconclusive or the sample must be re-analyzed (re-inject or re-amplify) to resolve the issue. Commonly observed artifacts include:

1. **Spikes:** Spikes are sharp, narrow peaks generally present in all colors and occur at the same location. This is often caused by electrical anomalies.
2. **Stutter:** A stutter peak is a reproducible minor product peak four bases (1 repeat) shorter than the corresponding main peak allele peak. Rarely, stutter may be observed at two or three repeats shorter that the true allele, or one repeat greater than the true allele. Peaks in the -1 repeat position that fall below the maximum published % stutter values (see Identifiler User's Manual) may be assumed to be stutter, and GeneMapper does not label these peaks. If an apparent stutter peak exceeds the published maximum stutter percent, the sample must be carefully evaluated to determine if the peak may be a true peak or stutter.
3. **Minus A**: PCR amplification results in the addition of a single "A" nucleotide at the 3' end of double stranded PCR products, resulting in a product that is one bp longer than the actual target DNA sequence. PCR reactions have been optimized to favor this "A" addition, but incomplete "A" addition may occur when excessive amount of target DNA is present, or in other conditions that are less than optimal for the PCR reaction. Incomplete "A" addition, or "minus A" appears as a peak one bp shorter, and typically at a smaller peak height, than the true allele.
4. **Pull-up/Matrix Failure:** Pull-up is the result of the instrument's inability to completely separate the spectral components. Pull-up is identified as a smaller peak of the same location as a true allele but in another color. It is the result of either excessive DNA or a faulty spectral.

Microvariants

Microvariants are true alleles that vary by fewer than 4 bp from the typical repeating unit. The designation of alleles containing an incomplete repeat unit, falling within the range spanned by the ladder alleles, should include the number of complete repeats, a decimal

point, then the number of base pairs in the incomplete repeat (e.g. 9.3 for a THO1 allele with 9 full repeats plus three more bps). The determination of the number of additional bp present in a microvariant is made by comparing the bp size of the off ladder peak with the bp size of the flanking alleles in the allelic ladder.

If an allele falls greater than four bp away from the largest or smallest allele at a locus, it will be designated as greater than or less than the appropriate ladder allele (i.e. > 11 for THO1).

Any allele designated as off-ladder by GeneMapper and not determined to be an artifact is verified by reinjection. If the same microvariant or out-of ladder allele is called in matching known and questioned samples, the re-injection is not required. These off-ladder alleles will be used in match statements and statistical calculations using the minimum allele frequency for that locus.

13.3 Re-analysis and Additional Analysis

Any step in the process with unacceptable controls (as defined in this SOP) must be rerun. Refer to section 3.2 of this SOP for instructions on investigation and reanalyzing samples where contamination is detected or is suspected. Data from analysis with unacceptable controls may not be used for interpretation.

If low levels of DNA provide insufficient data upon initial analysis, the analyst may choose any of the following (as long as relevant maximums specified in the SOP's are not exceeded):

- Re-amplify the sample with more template DNA (or less template DNA, if inhibition is suspected).
- Re-inject for a longer amount of time.
- Add a greater amount of amplified product to the formamide/LIZ mixture and re-inject.

For all loci except amelogenin, the maximum analytical threshold is 8192 RFU. If this is exceeded at any locus except amelogenin, the sample must be re-analyzed to obtain suitable data, or the locus must be called inconclusive. The analyst may choose any of the following (as long as the minimum specified in the relevant SOPs are met):

- Re-inject the sample for a shorter time.
- Dilute the amplified product in TE buffer, add 1 µl to the formamide/LIZ mixture, and re-inject.
- Re-amplify the sample with less template DNA.

Results with artifacts that interfere with interpretation may be re-analyzed as needed to resolve the issue:

- Artifacts resulting from poor electrophoresis may simply be re-injected.
- Artifacts resulting from excessive DNA may be reinjected for a shorter time, diluted and re-injected, or re-amplified with less DNA. Artifacts that are a result

of poor amplification, such as excessive –A, are typically resolved best by re-amplification.
- Pull-up due to a poor spectral can only be resolved by running a new spectral or performing other instrument maintenance.

13.3 Interpretation Guidelines

Results and conclusions from DNA analysis must be scientifically supported by the analytical data with appropriate standards and controls. Interpretations are made as objectively as possible and consistently from analyst to analyst. Not every situation can nor should be covered by a specific rule, and situations may occur that require an analyst to deviate from stated guidelines. Deviations from stated guidelines must be documented in the case file and approved by the technical leader.

Types of Conclusions

Three types of conclusions are generally possible when both evidence (questioned) and reference (known) samples are tested and compared: (1) inclusion (the individual could have contributed to/been a source of the questioned profile), (2) exclusion (the individual could not have contributed to/been a source of the questioned profile), or (3) inconclusive.

Inclusion or exclusion is determined by qualitative and quantitative evaluation of the entire DNA profile produced at the various loci tested. Inconclusive results, or an uninterpretable profile, may result from, but are not limited to:
- Insufficient amounts of template DNA
- Degradation
- Inhibitors
- Mixtures of DNA from multiple donors

It should be noted that it may be acceptable for an inclusion or exclusion to be determined when one or more loci yield inconclusive results. An inclusion statement, and any resulting statistical calculations, will be based only on loci that yield conclusive results. An exclusion statement can be determined if even a single locus produces exclusionary results. However an exclusion will not be determined if technical issues such as the loss of an allele due to incomplete (preferential) amplification, stochastic effects, mutation, or other factors may have caused the non-match.

It also should be noted that it may be possible to obtain a conclusive result comparing one reference to a questioned sample, but an inconclusive result when comparing a second reference sample to the same questioned sample. This is most commonly observed when interpreting complex mixtures.

Types of Profiles

A number of different types of profiles (or a combination of these) may be obtained from evidence samples. The comparisons with references that can be made are determined by the type of profile.

No profile: No DNA results obtained at all. No comparison can be made to reference samples.

Full single source profile: A sample may be considered to be from a single person if the number of observed alleles at each locus is no more than two and the signal peak heights are balanced (the lower peak >60% of the higher peak) for heterozygous alleles. All loci must be evaluated when making this determination. If a sample has a third peak at just a single locus, with no other indication of a mixture, this may indicate an unusual mutation present in that individual; such a profile may be still considered single-source but must be interpreted with caution. If a sample has unbalanced peak heights with no other indication of a mixture, the sample may also be considered single-source but must be interpreted with caution.
Inclusions with full profiles can be made with respective statistics calculated and reported on probative samples. Exclusions with full profiles can be made if the individual being compared differs from the evidentiary profile at any one locus. If one or few non-matches are observed, the profiles should be evaluated carefully for evidence of dropout or artifacts that may have resulted in the non-match.

Partial profiles: Partial profiles exhibit allelic dropout in some, but not all, loci tested and can result from insufficient, degraded, or inhibited DNA. Typically, smaller loci amplify better under these conditions, and larger loci tend to drop out. Extremely low levels of template DNA may also lead to stochastic effects which may under represent one allele of a heterozygous locus. Partial profiles may be compared, but statistical interpretation will only be made using loci where peak heights are 100 RFU or higher. Exclusions with partial profiles may be made; again care must be taken to ensure that non-matches are not a result of drop-out.

Mixtures: Evidence samples may contain DNA from more than one individual. A mixture can consist of full and/or partial profiles from multiple individuals. One or more of the following may indicate the presence of a mixture:

- Greater than two alleles at a locus.
- A peak at the stutter position of significantly greater RFUs than is typically observed.
- Significantly unbalanced alleles at a heterozygous locus.

Two types of mixtures may be observed:

- **Mixture with Major/Minor Contributors:** Some mixed profiles may be clearly differentiated into major and minor components. A major component at a locus is determined if all minor peaks are 30% or less than the height of the shortest major peak at the same locus.

If a major component can be determined for at least 8 loci, a major profile may be reported. The major profile may be treated in the same manner as a single source profile for assessing inclusion or exclusion and for significance calculations.
If should be noted that not all alleles of a minor contributor to a mixture may be determined, since minor contributor alleles may be masked by the presence of the same allele in the major contributor. The minor component of a mixture is treated in the same manner as a mixture with indistinguishable contributors for assessing inclusion or exclusion and for significance calculations.

- **Mixtures with indistinguishable contributors (unresolved mixture):** A mixture should be considered indistinguishable when the major and minor contributors cannot be distinguished because of signal intensities. Individuals may still be included or excluded as possible contributors to the mixture. All interpretable loci should be used in evaluating whether a person is included or excluded as a possible contributor. When evaluating whether a person should be excluded as a possible contributor, if an allele is not present at a locus, care must be taken to consider whether the allele may be missing due to drop-out. This determination can be difficult to make, and consultation with a more experienced analyst or supervisor may be helpful.

14 Statistics

Once a match has been identified and an individual included as a possible source of evidentiary material, the significance of that match is estimated to allow investigators, the legal sector, and ultimately a jury of lay persons to place the appropriate emphasis on the conclusion. Although every locus analyzed is evaluated, some loci may provide no information with regard to a particular comparison. Because it is the significance of the match that is important, only the matching loci can be taken into account in estimating significance and only after the analyst has determined that the profiles match. The different methods of calculating significance, when each is to be applied, and the population data from which they are calculated are addressed in this protocol. If significance estimates are not presented in the report, the examiner should be prepared to provide that information during court testimony.

14.1 Significance Estimation for Forensic Profiles

For all estimates, statistical significance will be expressed as an inverse probability of inclusion and likelihood ratios will not be calculated.

Definitions

1. Source identification/attribution: When the estimate for each calculated population group exceeds 1 in 100 billion probability of a random match, the reporting statement may include identification of the source. This number reflects a 99% confidence limit, assuming a US population of less than 1 billion.
2. Single source significance: The single source significance calculation can be applied if the evidentiary profile compared fits the criteria for either a single source or a major component of a mixed source.
3. Single source: Evidence profiles can be identified as single source if there are no more than two alleles per locus and the alleles at each locus are balanced. For this determination, alleles are considered balanced if the smaller peak height is at least 60% of the larger peak height. Possible contribution of stutter is not subtracted prior to application of this 60% criterion.
4. Major component of a mixed source: Some mixed source profiles may be clearly differentiated into major and minor components. For STR analysis, if the major component fulfills certain criteria, it may be treated the same way that a single source profile is treated for estimating match significance. These criteria are: 1) Interpretable loci show a major component and 2) no minor peak is greater than 30% of the height of the shortest major peak in the same locus. As with the single source evaluation criteria, no prior adjustment for stutter is assumed in the 30% maximum. It is permissible to determine a major profile at most loci, even if at some loci the major component cannot be determined. In this case, only the loci for which the major component can be determined may be used in the single source significance determination.

Single source significance calculation

For evidence profiles that meet the criteria for either single source or major component of a mixture, each locus frequency calculation will use NRC II Recommendation 4.1 formulae:

For homozygotes, $f = p^2 + p(1-p)\theta$ where $\theta = 0.01$.

For heterozygotes, $f = 2pq$.

For the profile frequency, for all loci used to identify the match, F = (f1 x f2 x f3 x f4 x… x fk), where k is the number of loci.

Inverse probability (random match probability) = 1/F.

Mixed source significance calculation

Evidence profiles that do not fit the criteria for a single source are considered mixtures of two or more sources of biological material. A locus should not be used in the statistical interpretation if there is evidence of possible additional alleles below threshold, as determined by alleles called if the analysis minimum threshold is lowered to 50 RFU.

If a major component of a mixture does not meet the single source or major profile criteria above, use the mixed source significance calculation, using these formulae:

For the frequency of each locus, $f = (p1 + p2 + p3 + p4 + \ldots + pk)^2$ where p is the estimated frequency of the allele detected for each allele 1 through k, and k is the number of alleles detected at the locus.

For all loci used to identify the match, F = (f1 x f2 x f3 x f4 x… x fk), where k is the number of loci.

Minimum and null allele frequencies

Following NRC II recommendations, minimum allele frequencies are calculated using 5/2N where N is the number of individuals in the population database, and null allele frequencies are set to 0.

Off-ladder alleles

Off-ladder alleles that have been confirmed by reinjection (in the case of STR analysis) or appearance in more than one sample will be used to determine a match and estimate the significance of that match. The allele frequency will be the calculated minimum allele frequency for that locus and population group.

Software

The latest available and installable version of the FBI's Popstats software will be configured to use the above formulae and used to calculate significance estimates. The Forensic-Single Sample data input option will be used for single source significance calculations; the Forensic Mixture Case data input option and the Mixture Formula will be used for mixed source significance calculations.

14.2 Significance Estimation for Forensic Parentage and Relationship Cases

For parentage cases, statistical interpretation of results will follow the procedure outlined below, which follows guidelines established by the AABB Relationship Testing Standards. Greater details of these standards and recommendations can be found in their published Standards for Relationship Testing Laboratories and Guidance for Standards for Relationship Testing Laboratories. These references may also be used to calculate significance of other relationship testing cases. The Popstats software (which used these AABB-recommended formulae) is used to calculate parentage statistics.

This section refers to paternity calculations, as the vast majority of cases will involve questions of the paternity of a child. However the same statistical approach and formulas can be used in significance estimations in maternity cases.

Paternity analysis can be performed with a reference sample from the child and the alleged father (AF), with or without the biological mother. However the statistical results of a "not excluded" case will be more significant if the mother is included in the testing. Therefore a reference sample from the mother should be obtained when possible. A reference from the mother is required in the following two types of cases:
The child's sample is fetal tissue. For fetal tissue samples, the mother must be typed in order to confirm that the tissue sample is of fetal, not maternal, origin. Failure to test the mother could result in a false exclusion. Note: if the mother is unavailable and the tissue is determined to be from a male, it may be assumed that the tissue is of fetal origin and the results may be used in the paternity analysis.
The alleged father is a close biological relative of the biological mother. In this situation, the child is likely to share some alleles with the AF because of the biological relationship between the AF and biological mother. Therefore testing the mother is required in order to take into account this shared relationship.

Definitions

1. Likelihood Ratio: a baysian statistical calculation that estimates the likelihood of seeing the evidence in question under two competing hypotheses.
2. Paternity Index (PI): a specialized likelihood ratio estimating the likelihood of seeing the child's profile if (1) the AF is the biological father versus (2) the AF is unrelated (or another competing hypothesis)
3. Combined Paternity Index (CPI): The product of all individual Paternity Indexes
4. Probability of Paternity: The probability, expressed as a percentage, that the AF if the biological father of the child. This calculation is dependent on the CPI and prior probability assumption.
5. Mutation: A change in DNA resulting from a copying error during DNA replication. In STR analysis, this can result in a non-matching allele between a biological parent and child.
6. Obligate paternal allele: the child's allele(s) at a locus that must have been inherited from the biological father. If the child is heterozygous at a locus and it

cannot be determined which of the two alleles came from the biological father, then both alleles must be considered obligate.

Exclusion

An AF will be excluded as the biological father if in more than two loci, the AF does not share an obligate paternal allele with the child. In this case, no statistical calculations are required.

Not Excluded, or Included

If the AF shares an obligate paternal allele at all loci, he cannot be excluded as being the biological father of the child. In this case the PI at each locus will be calculated by Popstats using the formulas in the table below, where capital letters refer to the allele(s) present in each individual tested, and small letters refer to the frequency of the allele(s).

Formulas for Paternity Index Calculations

M	C	AF	PI
BD	AB	AC	1/2a
BC	AB	AC	1/2a
BC	AB	AB	1/2a
BC	AB	A	1/a
B	AB	AC	1/2a
B	AB	AB	1/2a
B	AB	A	1/a
AB	AB	AC	1/ [2(a+b)]
AB	AB	AB	1/ (a+b)
AB	AB	A	1/ (a+b)
AB	A	AC	1/2a
AB	A	AB	1/2a
AB	A	A	1/a
A	A	AB	1/2a
A	A	A	1/a
Unknown	AB	AC	1/4a
Unknown	AB	AB	(a+b)/4ab
Unknown	AB	A	1/2a
Unknown	A	AC	1/2a
Unknown	A	A	1/a

The CPI will be calculated by multiplying these individual PI's. The Probability of Paternity will be calculated, using the formula below, which assumes a prior probability of 50%:

Probability of paternity = (CPI/(CPI +1)) X 100

Mutations

Mutations in STR loci typically result in an allele one repeat shorter or longer than the parent allele. These mutations occur at different frequencies in different loci, and typically at a higher frequency in the larger loci. If a non-match is observed in only one or two loci, the possibility of mutational events must be evaluated.

If a non-match is observed at only one locus, the possibility of a mutation must be assumed, and taken into account in the CPI calculation. At the locus with the suspected mutation, a mutational PI is calculated for that locus, following AABB recommendations. This mutational PI is incorporated into the CPI calculation.

If two non-matches are observed after standard laboratory STR (Identifiler) testing, the results should be considered inconclusive. Supplemental testing, such as additional autosomal STR loci or Y STR analysis, may yield additional information in these cases.

14.3 Population Databases

The significance estimate calculations above use empirically determined allele frequencies for each of the represented population groups.

Analysts will routinely calculate and report significance estimates using Caucasian, Black, and Hispanic databases. STR allele frequency data is also available for Japanese, Chinese, and Vietnamese populations for use at the analyst's discretion.

STR allele frequency data for all thirteen NDIS core STR loci are configured for each Popstats installation in the form of data files. The analyst will routinely use the FBI Population database for allele frequency data and calculations. Although amelogenin is used for declaration of a match, it is not used in calculation of match significance.

Although D1S80 and DQα analyses have been discontinued in this lab, population data files for these loci will continue to be available for the statistical evaluation of previous test results. The D1S80 allele frequencies will be those presented in Budowle, et al., 1995 and the DQα allele frequencies will be those presented in Blake, 1994 For both D1S80 and DQα, null allele frequencies are equal to the minimum allele frequency for the population and locus.

Population frequency tables appear in the HPD Crime Laboratory Biology Section intranet site and may also be kept in a separate notebook accessible to all analysts.

15 Reports

The desired end of DNA analysis is to determine whether a particular person is or is not the source of an item of biological evidence. First, DNA profiles must be generated for the person and for the evidentiary item. Then the profiles must be compared to determine if they are different or matching or if it isn't possible to know if they match. Finally, if the profiles match, it is important to understand the significance of the match.

The notes and other documentation must support the reported conclusions of the examiner. The laboratory report must communicate both the analytical results and the conclusions of the examiner, conveying the essence of what he or she would say if asked for an expert opinion in court. The report informs the opposing counsel during discovery what physical evidence was examined and what its significance may be. Decisions may be made by police officers, attorneys and the courts based on the report alone without examiner clarification, so the report should be able to stand alone. At this time, the reporting system (OLO) does not accommodate inclusion of an allele table in the case report; however, alleles from profiles generated during casework analysis are located in the DNA Analysis Results Chart included in every case folder. Case information, analysis results, and reports will only be released to authorized individuals according to Laboratory Quality and Operations Manual requirements.

The report must contain the information required in the quality manual. The requirements in this manual cover several items also required by the QAS (case identifier, description of evidence, results and/or conclusions, a qualitative or quantitative interpretive statement, date issued, and signature and title of the responsible person). The following are also required in each DNA report:

1. A statement indicating that an allelic table is provided with the signed copy of the report.
2. Description of DNA methodology
3. Loci analyzed, if DNA analyzed
4. Disposition of evidence

When statistics are provided, they will be reported to two significant digits. If not all loci are used in the statistical calculation, the loci used should be noted in the report.

All samples received by the analyst will be listed in the report. For each sample, report the results obtained, or indicate that no analysis was performed. The general types of results are:

1. Whether or not results (or interpretable results) were obtained.
2. Whether the profile obtained is from a single source or mixture of DNA, and whether a full or partial profile was obtained.
3. Whether or not tested/known individuals can be excluded. (Note that sometimes the results are inconclusive as to whether an individual can be excluded.)

4. The statistical significance of a "not excluded" or "inclusionary" result. This will be reported, at a minimum, for Caucasians, Blacks, Southeast Hispanics, and Southwest Hispanics.
    a. This may include source attribution when this criterion is met.
    b. For parentage cases, both the CPI and Probability of Paternity will be reported.

The analyst will include in the report the DNA profile(s), in the form of a table. Note: this table cannot be imported into OLO. If a locus is inconclusive or not responsive to testing, the profile at that locus will not be reported and will not be included in calculations of statistical significance estimations. Statistical calculations for samples not known to be probative need not be performed or reported, however the analyst may be required to perform these calculations at a later time. Non-probative samples may include profiles from epithelial cell fractions on the victim's vaginal swabs or the victim's clothing that match the victim's type.

The wording of the conclusions in reports will depend on the specific nature of the results. Example wording for report conclusions as well as formats for the reports are available on the Crime Laboratory Biology section intranet site.

16 CODIS (Combined DNA Index System)

The 1994 Crime Act included provisions establishing the FBI's Combined DNA Index System, a national DNA database program. As of June 1998, all 50 States require the collection of DNA samples from some or all convicted offenders.

In 1995, the Texas 74th Legislature passed House Bill 40 which provided for the collection of blood or other samples from convicted sex offenders, and for a DNA analysis of those samples, with the DNA data stored in a computer database. In 1999, the Texas 76th Legislature passed House Bill 1188 which expanded the collection of blood or other samples to include persons convicted of murder, aggravated assault, burglary of a habitation, or an offense or conviction of which registration as a sex offender is required.

In June 1998, the FBI announced the establishment of a set of 13 core STR loci for use in the National DNA Index System (NDIS). The set of core loci required for participation in NDIS is as follows: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, TH01, TPOX, and CSF1PO. For the HPD Crime Lab, STR typing with the AmpFlSTR Identifiler PCR Amplification kit will satisfy this core loci requirement for CODIS entry.

It is a requirement to follow NDIS guidelines in order to obtain Federal financial support for the CODIS system. Refer to the NDIS Procedures Manual for additional information.

16.1 Case Evaluation

All cases containing biological evidence should be evaluated for possible entry into CODIS. Appropriate evidence stains should be fully characterized utilizing Identifiler for inclusion into the appropriate index for CODIS.

Indexes

- LDIS (Local DNA Index System) - does not require a minimum number of loci for searching. Our Houston Police Department Crime Laboratory Database is an LDIS.
- SDIS (State DNA Index System) - requires a minimum of 6 loci (not including Amelogenin) for searching.
- NDIS (National DNA Index System) - requires that all 13 core loci be attempted and will accept specimens with data for 10 loci (not including Amelogenin) for searching. Amelogenin is accepted at NDIS although not searched.

Specimen Identification

Specimen naming policies are up to the laboratory entering the profile. There is no statewide requirement for standardization of naming between laboratories as long as each laboratory can identify their own samples. The only requirement for CODIS is that each DNA profile has a unique specimen ID. The specimen ID for HPD cases will include the case number and item number.

Forensic Unknowns

Appropriate evidence profiles that are either single source or major profiles of mixtures will be entered in the "Forensic Unknown" Index. A forensic unknown is a DNA profile obtained from an evidentiary sample relating to a crime scene. All evidence profiles are considered unknown, even when they are consistent with a known reference sample. This category allows the inclusion of DNA records obtained from forensic samples recovered directly from the victim, sexual assault evidence kit, victim's clothing, or crime scene which are believed to be attributed to the putative perpetrator. At this time only one profile from the perpetrator which is the most complete should be entered into CODIS; this is done to prevent multiple hits on evidence within the same case during searching.

Suspect Knowns

Texas CODIS law allows for the inclusion in our state database of reference/known samples legally obtained in the investigation of a crime, under certain circumstances. The law further allows for the inclusion of voluntarily submitted samples. Suspect knowns are not accepted at NDIS.

A suspect profile will be entered in the "Suspect Known" index if:

1. the sample was obtained legally
2. it is collected for the investigation of a crime
3. it a biological sample from a suspect in a criminal investigation (this excludes complainants and elimination samples)
4. there is evidence in the case probative to the crime that is also analyzed, and
5. results are obtained at a minimum of 9 STR loci.

Eligible suspect knowns will be regularly searched against the forensic database at the local level and will be regularly uploaded to SDIS. Any suspect known profile will be expunged from the database if court ordered to do so. Suspect profiles are identified in the database by the case number and sample designation; the name of the individual is not included.

All suspect profiles remain the property and responsibility of the submitting local laboratory. The records in the CODIS database are confidential and not subject to open records disclosure. A record includes both the profile and the identity of the individual whose profile is in the database. Neither the profile nor the identity of the individual whose profile is in the database will be released except:

1. to a criminal justice agency for law enforcement identification purposes
2. for a judicial proceeding, if otherwise admissible under law
3. for criminal defense purposes to a defendant, if related to the case in which the defendant is charged.

Forensic Mixtures

An STR profile may be determined to be a mixture based on factors such as more than two alleles at more than one or two loci, imbalance in alleles within a locus, and case-specific information. When considering whether a particular STR mixture profile is suitable for entry in NDIS, the concern should be how many potential candidates it will match at moderate stringency every time the particular profile is searched at NDIS. A mixture STR profile suspected of being unsuitable for entry into LDIS/SDIS/NDIS should be tested by conducting a keyboard search of the profile prior to entry. A large number of moderate stringency matches is detrimental to both parties involved in the match. The following rules should be applied to determine suitability:

1. A mixture profile from three or more contributors shall not be entered into LDIS/SDIS/NDIS.
2. If the profile of interest can be reliably and completely extracted from a mixture profile, ONLY that portion of the mixture will be entered into LDIS/SDIS/NDIS. This extraction can be conducted by visual or quantitative interpretation of the alleles in each locus. If the profile is a "clean" profile of one individual, then the profile should be entered into the "Forensic unknown" index. .
3. For mixture profiles where a "clean" profile of one individual cannot be determined for at least 10 CODIS core loci, all alleles will be entered for those loci where the "extraction" cannot be reliably and completely performed. Any alleles that can unambiguously attributed to the complainant or an elimination reference cannot be entered. The profile will be entered into the CODIS "Forensic Mixture STR Index" and will contain single source alleles at some loci and multiple source alleles at other loci.
4. Obligate alleles may be designated where appropriate. These are alleles that are foreign to the known profile of the complainant at a locus where more than one allele is present and that the analyst wishes to designate as required for a match. Obligate alleles should only be used for mixture profiles where a "clean" profile of one individual cannot be determined. An obligate allele is designated as a "+" to the right of the allele. There can only be one obligate designated per locus and no obligates can be designated for a homozygous locus. In order to enter a profile in the Mixture Index STR, a minimum of 7 loci must contain single source alleles.

5. Mixture profiles to be entered into CODIS must meet the 4 x 4 rule. Therefore, profiles containing more than 2 alleles at more than 4 loci will not be entered into CODIS. Additionally, there can be no more than 4 alleles entered for a single locus.

16.2 Case File Documentation

Fill out the CODIS entry form for the case profiles to be entered into CODIS. Forensic unknown/mixture samples that are not obviously attributable to the complainant's sexual assault kit or clothing may require documentation that the item is associated with the crime scene before the profile may be entered into CODIS.

The technical and administrative reviewers will review the profiles to ensure that the correct specimen name, index, and alleles to be entered are valid and that the profile is CODIS eligible. Discrepancies will be referred to the CODIS Manager.

After review, the analyst will enter profiles into CODIS and a copy of the specimen report will be printed for the case file. The administrative reviewer will check the specimen report against the CODIS entry form to ensure that the profiles have been entered correctly.

If profiles have been entered incorrectly, the administrative reviewer and the analyst should notify the CODIS Manager immediately so that corrective actions can be taken.

The entry of a profile into the CODIS system should be documented in OLO. This can be accomplished by incorporating the CODIS information into the DNA analysis report.

16.3 Uploads

Uploads to SDIS should be performed weekly unless no new data has been entered. The upload must occur by Friday at noon for the data to be included in state upload into the national database for the following week, and for it to be included in that weeks statewide and nationwide search.

Hits

Autosearch, which performs searches within the LDIS (HPD) database, should be run after new profiles are entered into CODIS, or a minimum of weekly if new profiles are entered frequently. The state and national databases are searched weekly. If a hit, or match is made:

1. **Forensic Hits (Case To Case Hits):** The local CODIS Administrator or their designee will generate a letter when the laboratory is involved with a case to case hit. The letter will include specific case information along with contact information for all investigating officers involved in any of the cases.
2. **Offender Hits (Case to Offender Hits):** An affidavit will be generated by the State CODIS Manager after confirmation of the Offender STR data by the CODIS Offender laboratory in Austin. A new sample from the Offender shall be obtained and run in the HPD laboratory. Follow up of any match between an offender profile and a forensic profile is the responsibility of the local laboratory that

submitted the matching profile. The Local CODIS Administrator is responsible for notification of Management and the HPD Public Information Office. See Appendix 14A for Local CODIS Administrator duties and qualifications.

3. **Case to Suspect Known Hits:** Matches between suspect profiles and previously unlinked forensic profiles provide an investigative lead and may be sufficient probable cause for suspicion. Such a match is not to be used as the basis of a final comparison in any case. In order to make a comparison for a final case report, the suspect profile must be verified with a newly collected suspect sample. Follow-up of any match between a suspect profile and a forensic profile, whether identified at the local or state level, is the responsibility of the local laboratories that submitted the matching profiles and will be initiated by the laboratory that submitted the forensic profile. Therefore, a letter will be generated from the HPD laboratory when involved with a case to suspect known hit. The letter will include specific case information along with contact information for all investigating officers involved in any of the cases.

Backups

Backups should be performed after entry of new profiles into CODIS or a minimum of weekly if new profiles are entered frequently. Two backup copies should be generated with one copy stored off-site.

17 Reagents

The following reagents are used in these DNA SOPs. Reagents not listed in this section are purchased premade. Reagent preparation generally required the following equipment and supplies:

- Stir plate and bar
- Sterile beaker and/or bottle
- Sterile microtubes and/or tubes
- pH meter

All glassware, stir bars, and diH_2O used in reagent preparation should be sterilized prior to use in reagent preparation. Microtubes and tubes used for reagent storage may be purchased pre-sterilized or may be sterilized prior to use.

17.1 Digest buffer (Sperm Wash Solution) pH 7.5

Digest buffer (10mM Tris-HCl, 10mM EDTA, 50mM NaCl, 2% SDS) is used in organic DNA extraction procedures in combination with other reagents. The chemicals in digest buffer are identical to the sperm wash solution used in washing sperm cells during differential extraction procedures.

Specification - Digest buffer or Sperm wash

Safety

Tris-HCl and EDTA solutions are irritants. 20% SDS is toxic and an irritant. Gloves and protective clothing (lab coat) must be worn during preparation. Gloves should be worn during use. Clothing may protect unbroken skin during use; broken skin should be covered.

Reagents

1 M Tris-HCl, pH 7.5, ultra pure grade (purchased)	1 ml
0.5 M EDTA, pH 8.0, ultra pure grade (purchased)	2 ml
5 M NaCl, ultra pure grade (purchased)	1 ml
20% SDS (Sodium Dodecyl Sulfate), ultra pure grade(purchased)	10 ml
diH_2O	86 ml

Procedure

Mix together 1 ml Tris-HCl, 2 ml EDTA, 1 ml NaCl, 10 ml 20% SDS, and 86 ml diH2O. Aliquot as necessary into sterile tubes for long storage.

Storage, Expiration, and Disposal

Store tightly closed at room temperature. Minimum labeling includes specification above, initials, and date prepared. Digest buffer or sperm wash can be stored for one year. Discard in regular sink, flush with water.

17.2 Dithiothreitol, 0.39 M and 1 M

Dithiothreitol (DTT) is a reducing agent used in DNA extraction buffers to allow lysis of cells with thiol-rich membrane proteins, such as spermatozoa. Instructions for use and interpretation are in the test procedures for extraction.

Specification - DTT, 0.39 M or 1 M

Safety

Dithiothreitol is an irritant. It is incompatible with bases, oxidizing and reducing agents, and alkali metals and may decompose on exposure to moisture. Gloves, goggles, and protective clothing (lab coat) must be worn during preparation. Gloves must be worn during use. Clothing may protect unbroken skin during use; broken skin should be covered.

Reagents

dithiothreitol (DTT), molecular biology grade	601.2 mg or 1.54 g
diH_2O	10 ml

Procedure

Dissolve 601.2 mg DTT and bring to 10 ml with diH_2O for 0.39 M. Dissolve 1.54 g DTT and bring to 10 ml diH_2O for 1 M. Aliquot single-use volumes into sterile tubes. Do not autoclave.

Storage, Expiration, and Disposal

Store tightly closed. Minimum labeling includes specification above, initials, and date prepared. DTT can be stored at -20°C for two years.

17.3 Proteinase K

Proteinase K (PK) in combination with other reagents is used for cell lysis in DNA extraction procedures.

Specification - PK

Safety

PK and solutions of PK can be irritating to mucous membranes. Avoid inhalation and skin contact. Gloves and protective clothing (lab coat) should be worn during preparation. Gloves should be worn during use. Clothing may protect unbroken skin during use; broken skin should be covered.

Reagents

Proteinase K, molecular biology grade	100	mg
diH_2O	10	ml

Procedure

Dissolve 100 mg Proteinase K in 10 ml sterile diH_2O. Aliquot single-use volumes into tubes.

Storage, Expiration, and Disposal

Store in tightly closed microtubes and place tubes in a storage container in the freezer. Minimum labeling includes specification above on the tubes. Label storage box with specification, lot #, initials, and date prepared. Store aliquots frozen for two years. Thaw tubes as needed for appropriate number of extractions and discard unused portion. Discard in sink, flush with water.

17.4 Stain Extraction Buffer

Stain Extraction Buffer (10mM Tris, 100 mM NaCl, 39 mM DTT, 10 mM EDTA, 2% SDS) is used to lyse cells during the isolation of nucleic acids. This buffer contains DTT, a reducing agent used in DNA extraction buffers to allow lysis of cells with thiol-rich membrane proteins, such as spermatozoa.

Specification - Stain Extraction Buffer or SEB

Safety

Tris base is an irritant. DTT is an irritant, is incompatible with bases, oxidizing and reducing agents, and alkali metals, and may decompose on exposure to moisture. SDS is toxic, is fetotoxic, is an irritant and is incompatible with strong oxidizing agents. Hydrochloric acid is corrosive, is toxic by inhalation, causes burns, and reacts violently in water. It is incompatible with bases, amines, alkali metals, copper, and aluminum. Never add water to hydrochloric acid. Use concentrated hydrochloric acid only in a chemical fume hood. Gloves, goggles, and protective clothing (lab coat) must be worn during preparation. Gloves should be worn during use. Clothing may protect unbroken skin during use; broken skin should be covered.

Special Equipment and Supplies –

chemical fume hood
pH meter

Reagents

Tris base (Tris(hydroxymethyl)aminomethane)	1.21	g
sodium chloride (NaCl)	5.84	g
diH_2O	to 1 L	
EDTA solution, 0.5M	20	ml
20% w/v SDS	100	ml
hydrochloric acid, concentrated (HCl)	to pH	
DTT, molecular biology grade	6.02	g

Procedure

1. Dissolve 1.21 g Tris base, 5.84 g NaCl, and 6.02 g DTT in about 500 ml sterile diH_2O.
2. Add 20 ml 0.5 M EDTA solution and 100 ml 20% w/v SDS.
3. Adjust the pH to 8.0 (+/- 0.2) with HCl.
4. Bring to a final volume of 1 L with sterile diH_2O.

Aliquot in single-use volumes into tubes and stored frozen. Do not autoclave.

Testing, Storage, Expiration, and Disposal

Store tightly closed. Minimum labeling includes specification above, initials, and date prepared. Stain Extraction Buffer with DTT can be stored frozen for two years. Discard unused portion of thawed buffer in regular trash.

17.5 TE Buffer, 10/0.1 mM, pH 8.0

TE Buffer (10/0.1 mM, pH 8.0) contains a common buffering agent for nucleic acid analysis as well as EDTA to chelate DNases.

Specification - TE

Safety

Tris-HCl and EDTA solutions are irritants. Gloves, goggles, and protective clothing (lab coat) should be worn during preparation. Gloves should be worn during use. Clothing may protect unbroken skin during use; broken skin should be covered.

Reagents

Tris-HCl, 1 M, pH 8.0	10	ml
EDTA solution, 0.5M	200	µl
diH_2O	990	ml

Procedure

Add 10 ml Tris-HCl and 200 µl EDTA to 990 ml sterile dIH2O and mix thoroughly. Aliquot as necessary for long storage of working solution and to prevent possible contamination.

Storage, Expiration, and Disposal

Store tightly closed. Minimum labeling includes specification above, initials, and date prepared. TE Buffer can be stored at room temperature for one year. Discard in regular sink, flush with water.

17.6 TNE solution, pH 8.0

TNE (10 mM Tris, 100 mM NaCl, 1 mM EDTA, pH 8.0) solution is used in an organic differential DNA extraction procedure in combination with other reagents.

Specification - TNE

Safety

Tris base may be an irritant to skin, eyes, and mucous membranes. NaCl is an irritant to eyes, respiratory system and skin. EDTA is an irritant. Gloves and protective clothing (lab coat) must be worn during preparation. Gloves should be worn during use. Clothing may protect unbroken skin during use; broken skin should be covered.

Reagents

2M Tris (pH 8.0)	5 mL
5M NaCl	20 mL
0.5 M (pH 8.0) EDTA	2 mL
diH_2O	100 ml

Procedure

1. Add 5 ml 2M Tris (pH 8.0), 20 ml 5M NaCl, and 2 ml 0.5 M (pH 8.0) EDTA to 853 ml deionized water.
2. Titrate to pH 8.0.
3. Bring to final volume of 1 liter with deionized water.
4. Autoclave solution.

Storage, Expiration, and Disposal

Store tightly closed. Minimum labeling includes specification above, initials, and date prepared. TNE Solution can be stored at room temperature for one year. Discard in regular sink, flush with water.