

## **4 Casework Processing**

### **4.1 Analysis Methods**

The DNA section provides STR analysis. STR analysis using the Identifiler or Identifiler Plus 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.

The amount of human DNA must be quantified prior to nuclear DNA amplification. However, when a reference sample is re-extracted for extraction/exclusion confirmation purposes, it is not necessary to also re-quantify the sample. Quantification data from the initial extraction may be used for amplification, as the resulting profile is not used for interpretation.

Semen-containing samples shall be processed using a differential extraction method.

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 (see section 14).

### **4.2 Case Acceptance and Evaluation**

Before a case is accepted for analysis into the DNA section, 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 customer 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 section 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. When possible, unused evidence should be returned to the HPD Property Room or to the submitting agency when it is not HPD, once analysis is complete.

### 4.3 Evidence Evaluation

**Evidence** is defined as any original item submitted to the laboratory for analysis, related to a specific incident, and/or any cutting or swabbing taken from that item.

**Work product** is defined as any derivative item obtained as a result of the analysis of evidence including but not limited to:

- Microscopy slides
- DNA extracts
- PCR amplification products

Before the case is worked, and in an effort to support an efficient laboratory, an evaluation should be made to determine the quality and quantity of the evidence that is going to be analyzed initially. 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, including both evidence and work product, must be considered so that none are compromised through sample loss or deleterious change.

During the initial analysis of the case, DNA extracts may be stored refrigerated. After a report has been issued, DNA extracts should be relocated to a freezer for long-term storage. Repeated freezing and thawing of extracts should be minimized. DNA extract tubes should be clearly labeled with the case number and item number and sealed with parafilm™ prior to long-term storage. DNA extracts may be stored individually with remaining evidence from that case, or in “batches” that contain several items from multiple cases that went through the analysis process simultaneously. When stored as “batches”, the storage container must be clearly labeled with a unique batch # and sealed with tamper-evident evidence tape. Examination documentation must indicate to which “batch” a sample belongs so that a DNA extract may be easily located at a later time. Documentation of which box(es) the extract(s) will be stored in long-term should be on the extraction worksheet(s).

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 and permission to consume will be requested. Samples will not be consumed without first having documented permission, preferably in writing. Furthermore, wherever possible, efforts should be made to limit the consumption of DNA extracts.

### **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 records.

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 tests 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. Examination documentation should reflect the name and/or initials of the individual who performed the work.

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 or in LIMS:

- 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 (i.e., storage after the completion of analysis).

- 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.

#### 4.5 Naming 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. Alternatively, a LIMS generated item identifier may also be used. LIMS item identifiers include the incident #, the item #, and the portion #.

Samples must be marked in such a way to distinguish them throughout processing. Extract tubes containing the final eluate must include the incident # and item # unique to that sample. Labeling can be hand-written or printed (e.g., barcode label).

In the event a re-extraction of a sample occurs, efforts should be made to distinguish it from the original extraction. If being extracted by the same individual, 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. However, whether the unique identifier is LIMS-generated or not, the item # will include the next higher number for the portion number, so the item number will not remain the same. Item # 1, portion # 1 would be 1.1 during the first extraction; it would be 1.2 for the second extraction.

Extraction confirmations will be marked with the same original item name and "EC" to distinguish it from the original extraction, if being re-extracted by the same individual.

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. The sample description in LIMS can be modified to indicate a re-amplification by pressing F9 on the well to add comments, such as "RA".

#### 4.6 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 that the analyst performs two extractions or amplifications on the same day, the two events will be distinguished (e.g., RBQ010101RDG1, RBQ010101RDG2, POS010101RDG1, POS010101RDG2, etc.).

Alternatively, a LIMS-generated identifier that uses the date and the unique plate ID # assigned to a particular worksheet may be used for all controls.

#### **4.7 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?

#### 4.8 Casework Outsourcing

All outsourcing activities shall comply with the Quality Assurance Manual policies (see section titled Outsourcing of Work) as well as the following:

1. Vendor laboratories will demonstrate compliance with the most current version of the Quality Assurance Standards for Forensic DNA Testing Laboratories and accreditation requirements of federal law.
2. The technical leader will approve technical specifications of outsourcing agreements with a vendor laboratory before contracts are awarded.
3. For any work that may be uploaded into or searched in CODIS, vendor laboratories will not begin analysis of casework before the HPD DNA section technical leader has accepted ownership of DNA data.
4. HPD Crime Laboratory will perform technical review of DNA data from a vendor laboratory prior to upload or search in CODIS. Technical review will be performed by an analyst or technical reviewer employed by HPD Crime Laboratory who is qualified or previously qualified in the technology, platform, and typing amplification test kit used to generate the data and participates in the laboratory's proficiency testing program. This technical review shall include, at a minimum:
  - a. Review of all DNA types to verify that they are supported by the raw and/or analyzed data (electropherograms or images).
  - b. A review of all associated controls, internal lane size standards, and allelic ladders to verify that the expected results were obtained.
  - c. A review of the final report to verify that the results/conclusions are supported by the data. The report shall address each tested item (or its probative fractions) submitted to the vendor laboratory.
  - d. Verification of the DNA types, eligibility, and the correct specimen category for entry into CODIS.
5. On-site visits shall be performed as follows:
  - a. An initial on-site visit will be conducted prior to the vendor laboratory's beginning of casework analysis.
  - b. The HPD DNA section technical leader shall perform the on-site visit. Alternatively, the technical leader may delegate this task to a current employee who is a qualified or previously qualified DNA analyst in the technology, platform, and typing amplification test kit used to generate the DNA data.
  - c. If the outsource agreement extends beyond one year, annual on-site visits will be conducted every calendar year, at least 6 months and not more than 18 months apart. After the initial on-site visit, HPD Crime Laboratory may accept subsequent visits by another NDIS participating laboratory using the same technology, platform, and typing amplification test kit.

Should the outsource lab be an NDIS-participating laboratory who will routinely submit profiles for upload into CODIS as part of their analysis process, technical and administrative reviews are not required by HPD personnel upon receipt of the DNA report.

The technical leader shall review and approve of all DNA analysis outsource contracts with vendor laboratories before they are awarded. Approval of such a contract includes acceptance of ownership of the DNA data generated in analysis performed under that contract.

#### 4A Abbreviations

Abbreviations common to the Forensic Biology field or found in an American English dictionary may be used in case files without definition. Additional abbreviations defined in the HPD Crime Laboratory SOP – Biology Section or defined below are also permissible. Any other abbreviation used must be defined on the page on which it is used.

AF	alleged father
AMEL	amelogenin
AMP	amplification
ASCLD/LAB	American Society of Crime Laboratory Directors/Laboratory Accreditation Board
BLK	African American
bp	base pair
C	child
CAU	Caucasian
CO	COfiler®
CODIS	Combined DNA Index System
CPE	combined probability of exclusion
CPI	combined paternity index
DAB	DNA Advisory Board
DB	digest buffer
DTT	dithiothreitol
EC	Exclusion/Extraction confirmation
EDTA	ethylenediaminetetraacetic acid
IDF	Identifiler® Amplification Kit
IDP	Identifiler® Plus Amplification Kit
INJ	injection
IPC	internal PCR control
KBS	Known buccal swabs
KSS	Known saliva swabs
LDIS	Local DNA Index System
M	mother
MCON	Microcon®
NDIS	National DNA Index System
NR	no result
NRC II	Committee on DNA Forensic Science, National Research Council, <i>An Update: The Evaluation of Forensic DNA Evidence</i> . National Academy Press, Washington, D.C., 1996.
PCI	phenol:chloroform:isoamyl alcohol
PI	paternity index
POS	amplification positive control
PRO	Profiler Plus®
PK	proteinase K
QF	Quantifiler
qPCR	Quantitative PCR

QUANT	quantification
PCR	polymerase chain reaction
PK	Proteinase K
RB	reagent blank
RCF	relative centrifugal force
RE-AMP	re-amplification
RFU	relative fluorescence unit
SDIS	State DNA Index System
SDS	sodium dodecyl sulfate
SEB	stain extraction buffer
SEH	Southeast Hispanic
SPEC	specimen
STR	short tandem repeat
SWH	Southwest Hispanic
TE	Tris-HCL and EDTA buffer
TECH	technical
TNE	Tris/NaCl/EDTA solution
UNDET	undetermined
YF	Yfiler™ Amplification Kit

Loci:

CSF	CSF1PO
D2	D2S1338
D3	D3S1358
D5	D5S818
D7	D7S820
D8	D8S1179
D13	D13S317
D16	D16S539
D18	D18S51
D19	D19S433
D21	D21S11