CODIS and PCR-Based Short Tandem Repeat Loci: Law Enforcement Tools

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INTRODUCTION

The ability to type DNA from biological evidence is one of the most important developments in forensic science since the advent of fingerprint analysis. DNA technology affords the forensic scientist the ability to eliminate individuals who have been falsely associated with a biological sample and to reduce the number of potential contributors to a few (if not one) individuals. The technology today includes a myriad of genetic markers, a variety of valid DNA typing strategies, powerful computers, and specialized software. All of which make developing DNA profiles and searching DNA databanks relatively rapid and facile. Since cases can be analyzed more rapidly and DNA databanks can be generated more rapidly than a decade ago, DNA databanking can be established and used to search DNA profiles/records to help resolve a number of violent crimes.

More than 300,000 threatened, attempted, or completed sexual assaults occur annually in the United States (1). In 1989, the Technical Working Group on DNA Analysis Methods (TWGDAM) took cognizance of the large number of sexual assaults and tendency for sex offenders to repeat crimes (i.e., recidivism) and proposed the concept of combining forensic DNA technology with computer science capabilities to aid in resolving violent crimes. By taking advantage of the unfortunately high repeat offense rate by some individuals, combined with the power of forensic DNA technology and utilization of computerized DNA databanks, law enforcement individuals may be able to solve many cases that previously could not be addressed (i.e., cases without suspects). Another important fact contributing to the need for databases involves the entry of convicted offenders back into the community. Approximately 234,000 offenders convicted of sexual assault are under the control or care of corrections agencies, and 60% of these sex offenders are under some type of conditional supervision in the community (1). Finally, individuals convicted of sexual offenses will be back in society in a relatively short time. The average time served by a convicted rapist is about 5 years, and those incarcerated for other sexual assault sentences typically serve under 3 years (1).

This paper describes: 1) the structure and function of an integrated DNA databanking system based on the needs and infrastructure of United States law enforcement agencies and institutions, and 2) it briefly describes the basis and advantages of the use of the polymerase chain reaction (PCR) and the selection criteria for the core PCR-based DNA markers that have proven useful for human identity testing. The information may guide the planning or development of other national DNA Identification systems.

CODIS - COMBINED DNA INDEX SYSTEM

The realization of the full use of DNA typing technology has come to fruition by the development by the FBI of a national DNA databank called CODIS (Combined DNA Index System). The two main objectives for CODIS operations are: 1) assist investigators in the identification of suspects of violent crimes, and 2) increase the efficacy of forensic laboratories by providing software to conduct DNA casework and perform statistical calculations.

CODIS is a hierarchical database of DNA identification records. The DNA records in CODIS contain limited information to enable profile searching. In general, the identification record contains 1) a laboratory identifier, 2) a specimen identifier, 3) DNA characteristics, and 4) information to classify and review the integrity of the DNA record.

To be completely functional for comparing DNA profiles, the FBI Laboratory's CODIS must be a fully integrated network among local, state, and federal crime laboratories. CODIS enables federal, state and local law enforcement crime laboratories to exchange and compare DNA profiles, to link serial violent crimes, and to identify potential suspects by matching DNA profiles from crime scene evidence to convicted offenders' profiles.

DNA records from a number of sources can be obtained, stored, and compared in CODIS. The sources are:

1) Convicted Felons - persons convicted of crimes which, under state statutes, may be included in CODIS.

2) Victims - persons, living or dead, against whom a crime has been committed. The perpetrators may have carried away DNA from the victims as the result of a commission of a crime.

3) Missing persons and their close biological relatives - persons reported missing, and sought, and their biological relatives, such as parents, siblings and children.

The DNA records from these individuals will be stored in various indexes in CODIS. The indexes are:

1) Convicted Offender Index - contains DNA records from individuals convicted of certain crimes. This index is used to generate investigative leads in official criminal investigations, to create investigative leads in missing persons cases, and to determine if duplicate records exist in the index (i.e., alibi usage by repeat offenders).

2) Victims Index - This index can be used to search DNA profiles found on, but foreign to, a suspect and contains DNA records from victims, living or dead, from whom DNA may have been carried away by perpetrators.

3) Forensic Index - contains DNA records attributed to unknown individuals derived from lawfully collected specimens obtained during the course of a criminal investigation. This index generally contains DNA records from cases without a suspect.

4) Unidentified Persons Index - contains DNA from individuals whose identities are not known with certainty and who left the DNA at a crime scene or whose DNA was carried away from the crime scene.

5) Missing Persons Index - contains DNA records of individuals that have been reported missing.

6) Close Biological Relatives Index - contains DNA records from close biological relatives of missing persons. The Missing Persons Index (above) and Close Biological Relatives Index also are designed to assist in identifying an unidentified individual.

7) Population File - contains DNA types and allele frequency data from anonymous persons intended to represent major population groups found in the United States. These databases are used to estimate statistical frequencies of DNA profiles.

CODIS is implemented as a distributed database with three levels, i.e., local, state, and national. All three tiers contain the forensic and convicted offender indexes and the population database file. The Local DNA Index System (LDIS) is installed at crime laboratories operated by police departments, sheriff's offices, or state agencies. All forensic DNA records originate at the local level and are transmitted to the state and national levels. Each state participating in the CODIS program has a single State DNA Index System (SDIS) that enables exchange and comparison of DNA profiles within a state. SDIS also links the local and national levels and is typically operated by the agency responsible for maintaining a state's convicted offender DNA database program.

The National DNA Index System (NDIS) is a single central repository of DNA records submitted by participating states. NDIS is administered by the FBI. Currently, participating laboratories communicate via telephone lines using Secure Telephone Units (STU III) modems for data encryption.

The majority of data stored in CODIS is created and maintained by state and local crime laboratories (Federal convicted offender records will be maintained by the FBI). The current version of CODIS software supports the storage and searching of both restriction fragment length polymorphism (RFLP) and PCR-based DNA profiles. The software features include: 1) computer assisted analysis of RFLP profiles; 2) keyboard entry of PCR analysis results; 3) batch entry of RFLP and PCR-based results produced by contract service laboratories; 4) intra-laboratory searching of RFLP and PCR-based profiles; 5) intra-state searching of DNA profiles; 6) searching the database of DNA profiles at the national level; and 7) calculation of DNA profile frequencies.

The "DNA Identification Act of 1994" contained within the Omnibus Crime Control Act (The Violent Crime Control and Law Enforcement Act of 1994, Public Law 103-322, 108 stat. 1796) expressly authorizes the FBI to establish an index for law enforcement purposes. The Act also authorizes the FBI Director to establish quality assurance (QA) standards for laboratories performing forensic DNA testing.

In accordance with the DNA Identification Act, only information that fulfills the following criteria may be included in the national index:

1) Analyses performed by or on behalf of a criminal justice agency pursuant to the QA Standards issued by the FBI Director;

2) Analyses prepared by laboratories, and DNA analysts, that undergo external proficiency testing every 180 days.

Data maintained by federal, state, and local criminal justice agencies that allow disclosure of DNA samples and DNA analyses may be disclosed under the following circumstances:

1) To criminal justice agencies for identification purposes related to law enforcement;

2) In judicial proceedings, if otherwise admissible pursuant to applicable statutes or rules;

3) For criminal defense purposes, to a defendant who shall have access to samples and analyses performed in connection with the case in which such a defendant is charged; or

4) If personally identifiable information is removed, for a population statistics database, for identification research and protocol development purposes, or for quality control purposes.

Laboratories participating in NDIS and/or receiving Federal grant funding are required to certify their compliance with the above criteria.

System-wide standards have been established to ensure that only reliable and compatible profiles are contained in the NDIS files. These include QA standards for performing forensic DNA analyses (2). Initially, the DNA Identification Act recognized the TWGDAM "Guidelines for a Quality Assurance Program for DNA Analysis" as the standards for QA in forensic DNA typing laboratories. The TWGDAM standards are superseded by the "Quality Assurance Standards for Forensic DNA Testing Laboratories" issued by the Director of the FBI, which take effect October 1, 1998 (see appendix for QA standards). While any genetic marker can be maintained in LDIS, SDIS, and NDIS, a core set of loci for each DNA profile (particularly, those in the Convicted Offender Index) is required for DNA records uploaded to the national system.

DNA MARKERS

The tools of molecular biology now enable forensic scientists to characterize biological evidence at the DNA level. Currently, the methods available to the forensic scientist include a) RFLP typing of variable number of tandem repeat (VNTR) loci (3-5) and b) amplification of specified genetic loci by the polymerase chain reaction (PCR) (6) and subsequent typing of specified genetic markers (7-13). Any material that contains nucleated cells, including blood, semen, saliva, hair, bones, and teeth, potentially can be typed for DNA polymorphisms.

The typing of VNTR loci by RFLP analysis is the most discriminating, or individualizing, molecular biology technology for forensic identity testing. Although this approach is valid and reliable for forensic and paternity testing, it has certain limitations. These include: 1) a sufficient quantity of high molecular weight DNA (usually at least 50 ng) is required for RFLP analysis (14); 2) samples that have been substantially degraded can not be analyzed by RFLP typing; and 3) RFLP analysis is laborious as well as time-consuming, requiring two to eight weeks to obtain results on six VNTR markers.

An alternative strategy for forensic DNA typing is the use of PCR-based assays (6). Compared with the RFLP approach, the advantages a PCR-based technology affords include augmented sensitivity and specificity and decreased assay time and labor. Also, many degraded DNA samples can be amplified by PCR and subsequently typed because amplified alleles generally are much smaller in size compared with alleles detected by RFLP analysis. These features make PCR a particularly useful tool for analyzing biological material found at crime scenes.

The ability to employ the PCR has facilitated analyses of forensic biological samples. PCR is a sample preparation technique in which relatively large amounts of specific DNA sequences of DNA can be generated from relatively small (picogram or nanogram) quantities of DNA. A typical PCR is based on the annealing and extension of two primers that flank a specific target (template) DNA segment. Primers are single-stranded DNA oligonucleotides, usually 20 to 30 bases in length, that can be obtained commercially or synthesized in-house. The template DNA to be amplified by PCR is denatured by heating the sample to approximately 95^{o}C using a thermal cycler. By lowering the thermal cycler temperature following denaturation (typically 37-72^{o}C), each primer then anneals to a region of complementarity on one of the separated strands. The appropriate annealing temperature is determined empirically and is influenced by the length and sequence of the primers. The final phase in the PCR cycle, primer extension, is generally carried out at 70-72^{o}C, the temperature at which Thermus aquaticus (Taq) DNA polymerase, a commonly used thermostable DNA polymerase, most effectively synthesizes DNA, or extends the primers. These three steps (denaturation, primer annealing, and primer extension) constitute a single PCR cycle. The newly synthesized strand can serve as a template for subsequent PCR cycles. Upon repeated cycles of denaturation, primer annealing, and primer extension (usually 28 to 36 times), an exponential accumulation of specific DNA fragments is generated, and millions of copies of target sequence can be obtained.

PCR, in principle, is easy to accomplish. One needs only a DNA sample, primers, a mixture of four nucleic acid building blocks (dNTPs), buffer, and a thermostable DNA polymerase. All components are placed in a reaction tube that is inserted into a thermal cycler which enables a programmable change in temperature in the reaction tubes. Routinely, PCR can be carried out in this manner in one to two hours.

PCR-BASED GENETIC MARKERS - STRs

For purposes of applying PCR-based technology to human identity testing and hence the use of a national DNA databank, defined polymorphic genetic markers, robust analytical techniques, and population studies are required. Polymorphic loci whose alleles are the result of short tandem repeats (STRs) are the most informative PCR-based genetic markers for attempting to individualize biological material (11, 15). The STR loci are composed of tandemly repeated sequences (each of which is two to seven base pairs in length), are highly informative, and when amplified simultaneously in a multiplex PCR, can be extremely effective for individualizing a wide range of forensic samples. The decision to select STR loci as genetic markers for CODIS is obvious.

The FBI Laboratory sponsored a community-wide forensic science effort to establish the core STR loci for the national DNA index. On April 9, 1996, an organizational meeting, supported by the CODIS program of the FBI Laboratory, was held to establish a research project agenda to validate the forensic application of a number of STR loci for DNA typing of biological evidence.

The purpose of the meeting was to identify candidate STR loci that can be analyzed by fluorescent detection, to determine research assignments for the participating laboratories, and to report the results at specified intervals. The anticipated goals were (1) to test, evaluate, and/or optimize the PCR and typing conditions for commercially-available STR kits that contain the candidate STR loci; (2) for all laboratories to evaluate the protocols, once the desired typing conditions were established; (3) to establish relevant population databases; and (4) to perform environmental insult and casework evaluation studies. The STR loci that were evaluated were CSF1PO, F13AO1, F13B, FES/FPS, FGA, TH01, LPL, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S359, D18S51, and D21S11.

The laboratories participating in this effort are: FBI, Royal Canadian Mounted Police, Armed Forces Institute of Pathology, Alabama Department of Forensic Sciences, California Department of Justice, Detroit Police Department, Florida Department of Law Enforcement, Illinois State Police, Metro-Dade Police Department/ Miami Children's Research Institute, Michigan State Police, Minnesota Bureau of Criminal Apprehension, National Institute of Standards and Technology, New York City Office of the Chief Medical Examiner, North Carolina State Bureau of Investigation, Orange County Sheriff's-Coroner Laboratory, Oregon State Police, Palm Beach County Sheriff's Office, Suffolk County Crime Laboratory, University of North Texas Health Science Center, Virginia Division of Forensic Science, and Arizona Department of Public Safety.

At the STR Project meeting on November 13-14, 1997 the core loci for the national system were agreed upon by the participating laboratories. The 13 core loci are: CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S359, D18S51, and D21S11. To take full advantage of the power of STR typing and to ensure compatibility for the searching of DNA profiles, all participants agreed that typing all 13 STR loci would be attempted when analyzing casework.

However, there initially were different viewpoints among the participants of the STR project regarding the manner that the 13 loci would be analyzed for convicted offender samples. There were two general approaches proffered: 1) initially type all 13 STR loci for convicted offender samples prior to submission to NDIS, or 2) type a subset of the loci initially, send the subset profile to NDIS, and only type the remaining STR loci to resolve provisional "hits." Some factors considered in assessing these two approaches were cost, labor, potential sources of error, individualization potential, ability to resolve mixture samples, response time, investigative lead potential, public impression, avoidance of endorsing a particular manufacturer's STR typing kit, which loci to use in the initial subset, and database size and growth.

The advantages of typing convicted offender samples for all 13 STR loci are: the augmented discrimination potential even for mixtures or degraded samples, fewer provisional hits, better quality control, defined budgets, faster response time to the police, less documentation, enhanced nation-wide compatibility, minimal subsequent investment, no consequence of sample loss preventing a subsequent analysis, defined message to manufacturers, and sufficient data to obviate differences that may arise among primer sets from the different manufacturers.

The factors initially considered as disadvantageous to typing all 13 STR loci are: increased cost and labor, fewer laboratories to participate initially and fewer samples to be typed, the lack of availability of all 13 STR loci in commercial typing kits from a single manufacturer, and the increased time required to type all loci.

The pros and cons for typing convicted felons with a subset of the STR loci are the opposite of the advantages and disadvantages listed above for typing all the loci initially. However, additional factors need to be considered regarding the use of a subset of the loci, such as the availability of the loci in commercial kits. There are two

manufacturers of STR typing kits that are used predominately in the United States: Promega Corporation, Madison, WI, and Perkin Elmer, Foster City, CA. Between the manufacturer's kits, there are seven common loci - CSF1PO, TPOX, TH01, vWA, D5S818, D7S820, and D13S317. Obviously, selecting these seven STR loci as the initial core would be compatible with various laboratories' preferences and instrumentation and would provide short-term advantages to uploading DNA profiles to NDIS. However, selecting these seven loci for convenience would preclude usage of scientifically better STR loci and thereby generate more candidate matches after a profile search.

After discussion and evaluation, the STR project working group decided overwhelmingly that convicted felon samples would be typed for all 13 core STR loci before sending DNA records to NDIS. At the local and state levels, laboratories may elect to type any subset of the markers.

The coordination of CODIS and STR research, from both operational and scientific perspectives, was invaluable for enhancing the capabilities of forensic scientists to resolve violent crimes. This concerted effort has enabled identification of the core loci for CODIS, has promoted continuing scientific exchanges throughout the forensic community, and has provided a solid base for the validity of STR typing.

This is publication number 98-06 of the Laboratory Division of the Federal Bureau of Investigation. Names of commercial manufacturers are provided for identification only, and inclusion does not imply endorsement by the Federal Bureau of Investigation.

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APPENDIX I. The following are the current quality assurance standards, mandated by the DNA Identification Act, approved by the Director of the FBI, and required by CODIS, for performing DNA typing in a forensic laboratory.

Quality Assurance Standards for Forensic DNA Testing Laboratories

Preface

Throughout its deliberation concerning these quality standards, the DNA Advisory Board recognized the need for a mechanism to ensure compliance with the standards. An underlying premise for these discussions was that accreditation would be required to demonstrate compliance with the standards and therefore assure quality control and a quality program. Accordingly, the Board recommends that forensic laboratories performing DNA analysis seek such accreditation with all deliberate speed. Additionally, the Board strongly encourages the accrediting bodies to begin positioning themselves to accommodate the increasing demand for accreditation.

Proposed Mechanism to Recommend Changes to Standards

Once the Director of the FBI has issued standards for quality assurance for forensic DNA testing, the DNA Advisory Board may recommend revisions to such standards to the FBI Director, as necessary. In the event that the duration of the DNA Advisory Board is extended beyond March 10, 2000 by the FBI Director, the Board may continue to recommend revisions to such standards to the FBI Director. In the event that the DNA Advisory Board is not extended by the FBI Director after March 10, 2000, the Technical Working Group on DNA Analysis Methods [TWGDAM] may recommend revisions to such standards to the FBI Director, as necessary.

Effective Date

These standards shall take effect October 1, 1998.

Quality Assurance Standards for Forensic DNA Testing Laboratories

INTRODUCTION

This document consists of definitions and standards. The standards are quality assurance measures that place specific requirements on the laboratory. Equivalent measures not outlined in this document may also meet the standard if determined sufficient through an accreditation process.

REFERENCES:

American Society of Crime Laboratory Directors-Laboratory Accreditation Board (ASCLD-LAB), *ASCLD-LAB Accreditation Manual*, January 1994, and January 1997.

International Standards Organization (ISO)/International Electrotechnical Commission (IEC), *ISO/IEC Guide 25-1990*, (1990) American National Standards Institute, New York, NY.

Technical Working Group on DNA Analysis Methods, "Guidelines for a Quality Assurance Program for DNA Analysis," *Crime Laboratory Digest*, April 1995, Volume 22, Number 2, pp. 21-43.

42 Code of Federal Regulations, Chapter IV (10-1-95 Edition), Health Care Financing Administration, Health and Human Services.

1. SCOPE

The standards describe the quality assurance requirements that a laboratory, which is defined as a facility in which forensic DNA testing is performed, should follow to ensure the quality and integrity of the data and competency of the laboratory. These standards do not preclude the participation of a laboratory, by itself or in collaboration with others, in research and development, on procedures that have not yet been validated.

2. DEFINITIONS

As used in these standards, the following terms shall have the meanings specified:

(a) Administrative review is an evaluation of the report and supporting documentation for consistency with laboratory policies and for editorial correctness.
(b) Amplification blank control consists of only amplification reagents without the addition of sample DNA. This control is used to detect DNA contamination of the amplification reagents.
(c) Analytical procedure is an orderly step by step procedure designed to ensure operational uniformity and to minimize analytical drift.
(d) Audit is an inspection used to evaluate, confirm, or verify activity related to quality.
(e) Calibration is the set of operations which establish, under specified conditions, the relationship between values indicated by a measuring instrument or measuring system, or values represented by a material, and the corresponding known values of a measurement.
(f) Critical reagents are determined by empirical studies or routine practice to require testing on established samples before use on evidentiary samples in order to prevent unnecessary loss of sample.
(g) Commercial test kit is a pre-assembled kit that allows the user to conduct a specific forensic DNA test.
(h) Examiner/analyst is an individual who conducts and/or directs the analysis of forensic casework samples, interprets data and reaches conclusions.
(I) Forensic DNA testing is the identification and evaluation of biological evidence in criminal matters using DNA technologies.
(j) Known samples are biological material whose identity or type is established.

(k) Laboratory is a facility in which forensic DNA testing is performed.

(l) Laboratory support personnel are individual(s) who perform laboratory duties and do not analyze evidence samples.

(m) NIST is the National Institute of Standards and Technology.

(n) Polymerase Chain Reaction (PCR) is an enzymatic process by which a specific region of DNA is replicated during repetitive cycles which consist of (1) denaturation of the template; (2) annealing of primers to complementary sequences at an empirically determined temperature; and (3) extension of the bound primers by a DNA polymerase.

(o) Proficiency test sample is biological material whose DNA type has been previously characterized and which is used to monitor the quality performance of a laboratory or an individual.

(p) Proficiency testing is a quality assurance measure used to monitor performance and identify areas in which improvement may be needed. Proficiency tests may be classified as:

(1) Internal proficiency test is one prepared and administered by the laboratory.

(2) External proficiency test, which may be open or blind, is one which is obtained from a second agency.

(q) Qualifying test measures proficiency in both technical skills and knowledge.

(r) Quality assurance includes the systematic actions necessary to demonstrate that a product or service meets specified requirements for quality.

(s) Quality manual is a document stating the quality policy, quality system and quality practices of an organization.

(t) Quality system is the organizational structure, responsibilities, procedures, processes and resources for implementing quality management.

(u) Reagent blank control consists of all reagents used in the test process without any sample. This is to be used to detect DNA contamination of the analytical reagents.

(v) Reference material (certified or standard) is a material for which values are certified by a technically valid procedure and accompanied by or traceable to a certificate or other documentation which is issued by a certifying body.

(w) Restriction Fragment Length Polymorphism (RFLP) is generated by cleavage by a specific restriction enzyme and the variation is due to restriction site polymorphism and/or the number of different repeats contained within the fragments.

(x) Review is an evaluation of documentation to check for consistency, accuracy, and completeness.

(y) Second agency is an entity or organization external to and independent of the laboratory and which performs forensic DNA analysis.

(z) Secure area is a locked space (for example, cabinet, vault or room) with access restricted to authorized personnel.

(aa) Subcontractor is an individual or entity having a transactional relationship with a laboratory.

(bb) Technical manager or leader (or equivalent position or title as designated by the laboratory system) is the individual who is accountable for the technical operations of the laboratory.

(cc) Technical review is an evaluation of reports, notes, data, and other documents to ensure an appropriate and sufficient basis for the scientific conclusions. This review is conducted by a second qualified individual.

(dd) Technician is an individual who performs analytical techniques on evidence samples under the supervision of a qualified examiner/analyst and/or performs DNA analysis on samples for inclusion in a database. Technicians do not evaluate or reach conclusions on typing results or prepare final reports.

(ee) Traceability is the property of a result of a measurement whereby it can be related to appropriate standards, generally international or national standards, through an unbroken chain of comparisons.

(ff) Validation is a process by which a procedure is evaluated to determine its efficacy and reliability for forensic casework analysis and includes:

(1) Developmental validation is the acquisition of test data and determination of conditions and limitations of a new or novel DNA methodology for use on forensic samples.

(2) Internal validation is an accumulation of test data within the laboratory to demonstrate that established methods and procedures perform as expected in the laboratory.

3. QUALITY ASSURANCE PROGRAM

STANDARD 3.1 The laboratory shall establish and maintain a documented quality system that is appropriate to the testing activities.

3.1.1 The quality manual shall address at a minimum:

(a) Goals and objectives
(b) Organization and management
(c) Personnel Qualifications and Training
(d) Facilities
(e) Evidence control
(f) Validation
(g) Analytical procedures
(h) Calibration and maintenance
(I) Proficiency testing
(j) Corrective action
(k) Reports
(l) Review
(m) Safety
(n) Audits

4. ORGANIZATION AND MANAGEMENT

STANDARDS 4.1 The laboratory shall:

(a) have a managerial staff with the authority and resources needed to discharge their duties and meet the requirements of the standards in this document.
(b) have a technical manager or leader who is accountable for the technical operations.
(c) specify and document the responsibility, authority, and interrelation of all personnel who manage, perform or verify work affecting the validity of the DNA analysis.

5. PERSONNEL

STANDARD 5.1 Laboratory personnel shall have the education, training and experience commensurate with the examination and testimony provided. The laboratory shall:

5.1.1 have a written job description for personnel to include responsibilities, duties and skills.

5.1.2 have a documented training program for qualifying all technical laboratory personnel**.**

5.1.3 have a documented program to ensure technical qualifications are maintained through continuing education.

5.1.3.1 Continuing education - the technical manager or leader and examiner/analyst(s) must stay abreast of developments within the field of DNA typing by reading current scientific literature and by attending seminars, courses, professional meetings or documented training sessions/classes in relevant subject areas at least once a year.

5.1.4 maintain records on the relevant qualifications, training, skills and experience of the technical personnel.

5.2 The technical manager or leader shall have the following:

5.2.1 **Degree requirements:** The technical manager or leader of a laboratory shall have at a minimum a Master's degree in biology-, chemistry- or forensic science- related area and successfully completed a minimum of 12 semester or equivalent credit hours of a combination of undergraduate and graduate course work covering the subject areas of biochemistry, genetics and molecular biology (molecular genetics, recombinant DNA technology), or other subjects which provide a basic understanding of the foundation of forensic DNA analysis as well as statistics and/or population genetics as it applies to forensic DNA analysis.

5.2.1.1 The degree requirements of section 5.2.1 may be waived by the American Society of Crime Laboratory Directors (ASCLD) or other organization designated by the Director of the FBI in accordance with criteria approved by the Director of the FBI. This waiver shall be available for a period of two years from the effective date of these standards. The waiver shall be permanent and portable.

5.2.2 **Experience requirements:** A technical manager or leader of a laboratory must have a minimum of three years of forensic DNA laboratory experience.

5.2.3 **Duty requirements:**

5.2.3.1 **General:** manages the technical operations of the laboratory.

5.2.3.2 **Specific duties**

(a) Is responsible for evaluating all methods used by the laboratory and for proposing new or modified analytical procedures to be used by examiners.

(b) Is responsible for technical problem solving of analytical methods and for the oversight of training, quality assurance, safety and proficiency testing in the laboratory.

5.2.3.3 The technical manager or leader shall be accessible to the laboratory to provide onsite, telephone or electronic consultation as needed.

5.3 Examiner/analyst shall have:

5.3.1 at a minimum a BA./BS degree or its equivalent degree in biology-, chemistry- or forensic science- related area and must have successfully completed college course work (graduate or undergraduate level) covering the subject areas of biochemistry, genetics and molecular biology (molecular genetics, recombinant DNA technology) or other subjects which provide a basic understanding of the foundation of forensic DNA analysis, as well as course work and/or training in statistics and population genetics as it applies to forensic DNA analysis.

5.3.2 a minimum of six (6) months of forensic DNA laboratory experience, including the successful analysis of a range of samples typically encountered in forensic case work prior to independent case work analysis using DNA technology.

5.3.3 successfully completed a qualifying test before beginning independent casework responsibilities.

5.4 Technician shall have:

5.4.1 On the job training specific to their job function(s).

5.4.2 successfully completed a qualifying test before participating in forensic DNA typing responsibilities.

5.5 Laboratory support personnel shall have:

5.5.1 training, education and experience commensurate with their responsibilities as outlined in their job description.

6. FACILITIES

STANDARD 6.1 The laboratory shall have a facility that is designed to provide adequate security and minimize contamination. The laboratory shall ensure that:

6.1.1 Access to the laboratory is controlled and limited.

6.1.2 Prior to PCR amplification, evidence examinations, DNA extractions, and PCR setup are conducted at separate times or in separate spaces.

6.1.3 Amplified DNA product is generated, processed and maintained in a room(s) separate from the evidence examination, DNA extractions and PCR setup areas.

6.1.4 The laboratory follows written procedures for monitoring, cleaning and decontaminating facilities and equipment.

7. EVIDENCE CONTROL

STANDARD 7.1 The laboratory shall have and follow a documented evidence control system to ensure the integrity of physical evidence. This system shall ensure that:

7.1.1 Evidence is marked for identification.

7.1.2 Chain of custody for all evidence is maintained.

7.1.3 The laboratory follows documented procedures that minimize loss, contamination, and/or deleterious change of evidence.

7.1.4 The laboratory has secure areas for evidence storage.

STANDARD 7.2 Where possible, the laboratory shall retain or return a portion of the evidence sample or extract.

7.2.1 The laboratory shall have a procedure requiring that evidence sample/extract(s) are stored in a manner that minimizes degradation.

8. VALIDATION

STANDARD 8.1 The laboratory shall use validated methods and procedures for forensic casework analyses.

8.1.1 Developmental validation that is conducted shall be appropriately documented.

8.1.2 Novel forensic DNA methodologies shall undergo developmental validation to ensure the accuracy, precision and reproducibility of the procedure. The developmental validation shall include the following:

8.1.2.1 Documentation exists and is available which defines and characterizes the locus.

8.1.2.2 Species specificity, sensitivity, stability and mixture studies are conducted.

8.1.2.3 Population distribution data are documented and available.

8.1.2.3.1.1 The population distribution data would include the allele and genotype distributions for the locus or loci obtained from relevant populations. Where appropriate, databases should be tested for independence expectations.

8.1.3 Internal validation shall be performed and documented by the laboratory.

8.1.3.1 The procedure shall be tested using known and non-probative evidence samples. The laboratory shall monitor and document the reproducibility and precision of the procedure using human DNA control(s).

8.1.3.2 The laboratory shall establish and document match criteria based on empirical data.

8.1.3.3 Before the introduction of a procedure into forensic casework, the analyst or examination team shall successfully complete a qualifying test.

8.1.3.4 Material modifications made to analytical procedures shall be documented and subject to validation testing.

8.1.4 Where methods are not specified, the laboratory shall, wherever possible, select methods that have been published by reputable technical organizations or in relevant scientific texts or journals, or have been appropriately evaluated for a specific or unique application.

9. ANALYTICAL PROCEDURES

STANDARD 9.1 The laboratory shall have and follow written analytical procedures approved by the laboratory management/technical manager.

9.1.1 The laboratory shall have a standard operating protocol for each analytical technique used.

9.1.2 The procedures shall include reagents, sample preparation, extraction, equipment, and controls which are standard for DNA analysis and data interpretation.

9.1.3 The laboratory shall have a procedure for differential extraction of stains that potentially contain semen.

STANDARD 9.2 The laboratory shall use reagents that are suitable for the methods employed.

9.2.1 The laboratory shall have written procedures for documenting commercial supplies and for the formulation of reagents.

9.2.2 Reagents shall be labeled with the identity of the reagent, the date of preparation or expiration, and the identity of the individual preparing the reagent.

9.2.3 The laboratory shall identify critical reagents and evaluate them prior to use in casework. These critical reagents include but are not limited to:

(a) Restriction enzyme
(b) Commercial kits for performing genetic typing
(c) Agarose for analytical RFLP gels
(d) Membranes for Southern blotting
(e) K562 DNA or other human DNA controls
(f) Molecular weight markers used as RFLP sizing standards
(g) Primer sets
(h) Thermostable DNA polymerase

STANDARD 9.3 The laboratory shall have and follow a procedure for evaluating the quantity of the human DNA in the sample where possible.

9.3.1 For casework RFLP samples, the presence of high molecular weight DNA should be determined.

STANDARD 9.4 The laboratory shall monitor the analytical procedures using appropriate controls and standards.

9.4.1 The following controls shall be used in RFLP casework analysis:

9.4.1.1 Quantitation standards for estimating the amount of DNA recovered by extraction.

9.4.1.2 K562 as a human DNA control. (In monitoring sizing data, a statistical quality control method for K562 cell line shall be maintained.)

9.4.1.3 Molecular weight size markers to bracket known and evidence samples.

9.4.1.4 Procedure to monitor the completeness of restriction enzyme digestion.

9.4.2 The following controls shall be used for PCR casework analysis:

9.4.2.1 Quantitation standards which estimate the amount of human nuclear DNA recovered by extraction.

9.4.2.2 Positive and negative amplification controls.

9.4.2.3 Reagent blanks.

9.4.2.4 Allelic ladders and/or internal size makers for variable number tandem repeat sequence PCR based systems.

STANDARD 9.5 The laboratory shall check its DNA procedures annually or whenever substantial changes are made to the protocol(s) against an appropriate and available NIST standard reference material or standard traceable to a NIST standard.

STANDARD 9.6 The laboratory shall have and follow written general guidelines for the interpretation of data.

9.6.1 The laboratory shall verify that all control results are within established tolerance limits.

9.6.2 Where appropriate, visual matches shall be supported by a numerical match criterion.

9.6.3 For a given population(s) and/or hypothesis of relatedness, the statistical interpretation shall be made following the recommendations 4.1, 4.2 or 4.3 as deemed applicable of the National Research Council report entitled "The Evaluation of Forensic DNA Evidence" (1996) and/or court directed method. These calculations shall be derived from a documented population database appropriate for the calculation.

10. EQUIPMENT CALIBRATION AND MAINTENANCE

STANDARD 10.1 The laboratory shall use equipment suitable for the methods employed.

STANDARD 10.2 The laboratory shall have a documented program for calibration of instruments and equipment.

10.2.1 Where available and appropriate, standards traceable to national or international standards shall be used for the calibration.

10.2.1.1 Where traceability to national standards of measurement is not applicable, the laboratory shall provide satisfactory evidence of correlation of results.

10.2.2 The frequency of the calibration shall be documented for each instrument requiring calibration. Such documentation shall be retained in accordance with applicable Federal or state law.

STANDARD 10.3 The laboratory shall have and follow a documented program to ensure that instruments and equipment are properly maintained.

10.3.1 New instruments and equipment, or instruments and equipment that have undergone repair or maintenance, shall be calibrated before being used in casework analysis.

10.3.2 Written records or logs shall be maintained for maintenance service performed on instruments and equipment. Such documentation shall be retained in accordance with applicable Federal or state law.

11. REPORTS

STANDARD 11.1 The laboratory shall have and follow written procedures for taking and maintaining case notes to support the conclusions drawn in laboratory reports.

11.1.1 The laboratory shall maintain, in a case record, documentation generated by examiners related to case analyses.

11.1.2 Reports according to written guidelines shall include:

(a) Case identifier
(b) Description of evidence examined
(c) A description of the methodology
(d) Locus
(e) Results and/or conclusions
(f) An interpretative statement (either quantitative or qualitative)
(g) Date issued
(h) Disposition of evidence
(I) A signature and title, or equivalent identification, of the person(s) accepting responsibility for the content of the report.

11.1.3 The laboratory shall have written procedures for the release of case report information.

12. REVIEW

STANDARD 12.1 **The laboratory shall conduct administrative and technical reviews of all case files and reports to ensure conclusions and supporting data are reasonable and within the constraints of scientific knowledge.**

12.1.1 The laboratory shall have a mechanism in place to address unresolved discrepant conclusions between analysts and reviewer(s).

STANDARD 12.2 **The laboratory shall have and follow a program that documents the annual monitoring of the testimony of each examiner.**

13. PROFICIENCY TESTING

STANDARD 13.1 **Examiners and other personnel designated by the technical manager or leader who are actively engaged in DNA analysis shall undergo, at regular intervals of not to exceed 180 days, external proficiency testing in accordance with these standards. Such external proficiency testing shall be an open proficiency testing program.**

13.1.1 The laboratory shall maintain the following records for proficiency tests:

(a) The test set identifier.
(b) Identity of the examiner.
(c) Date of analysis and completion.
(d) Copies of all data and notes supporting the conclusions.
(e) The proficiency test results.
(f) Any discrepancies noted.
(g) Corrective actions taken.

Such documentation shall be retained in accordance with applicable Federal or state law.

13.1.2 The laboratory shall establish at a minimum the following criteria for evaluation of proficiency tests:

(a) All reported inclusions are correct or incorrect.
(b) All reported exclusions are correct or incorrect.
(c) All reported genotypes and/or phenotypes are correct or incorrect according to consensus genotypes/phenotypes or within established empirically determined ranges.
(d) All results reported as inconclusive or uninterpretable are consistent with written laboratory guidelines. The basis for inconclusive interpretations in proficiency tests must be documented.
(e) All discrepancies/errors and subsequent corrective actions must be documented.
(f) All final reports are graded as satisfactory or unsatisfactory. A satisfactory grade is attained when there are no analytical errors for the DNA profile typing data. Administrative errors shall be documented and corrective actions taken to minimize the error in the future.
(g) All proficiency test participants shall be informed of the final test results.

14. CORRECTIVE ACTION

STANDARD 14.1 The laboratory shall establish and follow procedures for corrective action whenever proficiency testing discrepancies and/or casework errors are detected.

14.1.1 The laboratory shall maintain documentation for the corrective action. Such documentation shall be retained in accordance with applicable Federal or state law.

15. AUDITS

STANDARD 15.1 The laboratory shall conduct audits annually in accordance with the standards outlined herein.

15.1.1 Audit procedures shall address at a minimum:

(a) Quality assurance program
(b) Organization and management
(c) Personnel
(d) Facilities
(e) Evidence control
(f) Validation
(g) Analytical procedures
(h) Calibration and maintenance
(I) Proficiency testing
(j) Corrective action
(k) Reports
(l) Review
(m) Safety
(n) Previous audits

15.1.2 The laboratory shall retain all documentation pertaining to audits in accordance with relevant legal and agency requirements.

STANDARD 15.2 Once every two years, a second agency shall participate in the annual audit.

16. SAFETY

STANDARD 16.1 The laboratory shall have and follow a documented environmental health and safety program.

17. SUBCONTRACTOR OF ANALYTICAL TESTING FOR WHICH VALIDATED PROCEDURES EXIST

STANDARD 17.1 A laboratory operating under the scope of these standards will require certification of compliance with these standards when a subcontractor performs forensic DNA analyses for the laboratory.

17.1.1 The laboratory will establish and use appropriate review procedures to verify the integrity of the data received from the subcontractor.