This document sets forth background materials on the scientific research supporting examinations as conducted by the forensic laboratories at the Department of Justice. It also includes a discussion of significant policy matters. This document is provided to assist a public review and comment process of the related Proposed Uniform Language for Testimony and Reports (posted separately). It is not intended to, does not, and may not be relied upon to create any rights, substantive or procedural, enforceable by law by any party in any matter, civil or criminal, nor does it place any limitation on otherwise lawful investigative and litigative prerogatives of the Department.

SUPPORTING DOCUMENTATION FOR DEPARTMENT OF JUSTICE PROPOSED UNIFORM LANGUAGE FOR TESTIMONY AND REPORTS FOR THE Y CHROMOSOME AND MITOCHONDRIAL DNA TYPING DISCIPLINES

Background

Deoxyribonucleic Acid (DNA) is the genetic material found within our cells that provides a blueprint for our physical and metabolic characteristics. There are two types of DNA found within most of the cells in our body: nuclear DNA and mitochondrial DNA. Their designation refers to their location within the cell; nuclear DNA (nDNA) is housed within the nucleus, while mitochondrial DNA (mtDNA) is housed within energy-producing cellular structures called mitochondria.

While the majority of DNA is inherited from both parents (i.e., autosomal nuclear DNA), the exceptions involve the Y chromosome, which is inherited strictly from father to son, and mtDNA, which is passed from a mother to all of her children. The Y chromosome and mtDNA are both referred to as "lineage" markers, or "non-autosomal" markers. They are each inherited as a unit, which means that with the exception of mutational events, all individuals in the same lineage have the same type.[1] The uniparental inheritance pattern of the Y chromosome and mtDNA confers distinct advantages and limitations, some shared and some unique to each type of lineage marker, which must be considered in the context of forensic testing. This document focuses on the analysis and interpretation of lineage markers – the Y chromosome and mtDNA.

Principles of Y Chromosome and Mitochondrial DNA Typing

When conducting Y chromosome or mitochondrial DNA comparisons, a forensic examiner assesses whether the DNA types between an evidence sample and a reference or known sample are consistent with one another. By clearly expressing the scientific basis for the conclusion while remaining cognizant of the limitations, forensic examiners will testify within the bounds of science.

Uniparental inheritance

Although the mode of inheritance for the Y chromosome and mtDNA can both be described as uniparental, there are subtle differences in the paternal inheritance of the Y chromosome and the maternal inheritance of mtDNA that influence their application and interpretation. An

[1] Butler, JM. *Advanced Topics in Forensic DNA Typing: Methodology*. Elsevier Academic Press, San Diego, 2012 (hereinafter *Butler 2012*), Chapters 13 and 14.

illustration of the paternal inheritance pattern of the Y chromosome can be found in Figure 1a, while the maternal inheritance pattern of mtDNA can be found in Figure 1b. In both pedigrees, males are represented by squares and females by circles. Related individuals are connected by vertical lines, while horizontal lines connect individuals with children. The different colors/letters represent different DNA types (Y chromosome types for Figure 1a, mtDNA types for Figure 1b).

Both uniparental inheritance patterns dictate that a person's DNA type will be the same as other related family members. Note that only male individuals have a Y chromosome.

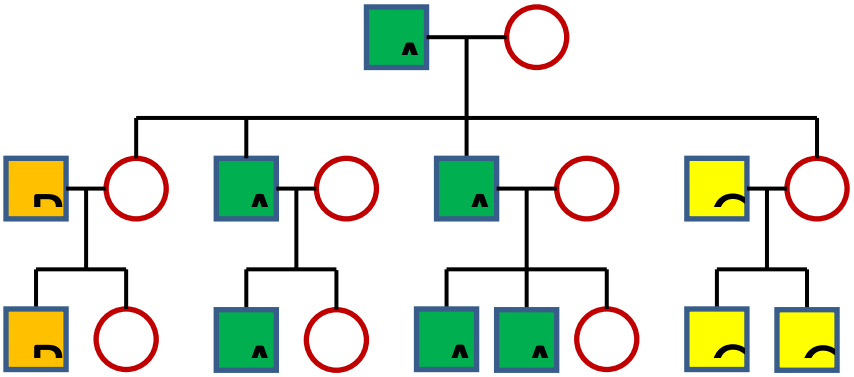

Figure 1a: Y Chromosome Inheritance.

In contrast, all individuals have mtDNA.

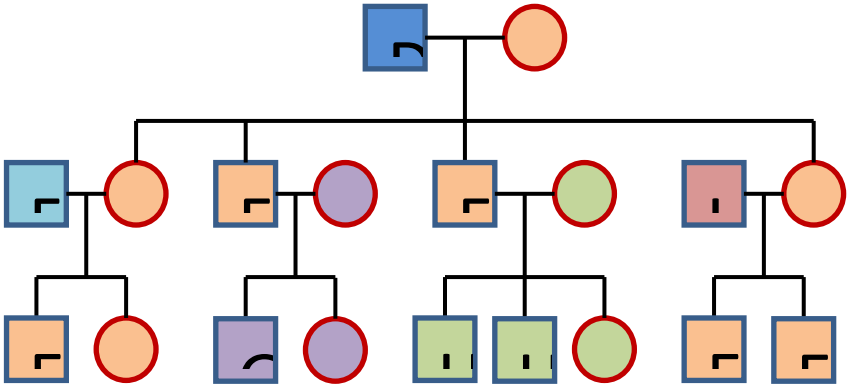

Figure 1b: mtDNA Inheritance.

A. Y Chromosome Testing

The Y chromosome is found in the nucleus of the cell as a part of the nuclear DNA. There are 23 pairs of chromosomes, and the Y chromosome is a part of the 23rd pair. The X and Y chromosomes are the sex chromosomes of humans. Only male individuals have a Y chromosome. Though the Y chromosome can be thought of as passing unchanged from father to son, there are regions on the tips of the Y chromosome that recombine with the homologous X

chromosome, which allows for some changes in that portion of the DNA. The remaining ~95% of the Y chromosome is known as the non-recombining portion of the Y chromosome (NRY). It is this region of the Y chromosome upon which forensic testing is based, and this region that passes unchanged from generation to generation.[2]

B. Mitochondrial DNA Testing

Mitochondrial DNA is located within mitochondria, the cellular structures responsible for energy production. Human cells can have hundreds to thousands of copies of mitochondrial DNA per cell, making it far more abundant than nDNA.[3] The structure and location of the mitochondrial DNA genome makes mtDNA less prone to degradation than nDNA.[4] The relative abundance and stability of mtDNA (compared to nDNA) make it well suited for particular types of forensic evidentiary samples, such as naturally shed hairs (i.e., hairs without a root), teeth, bones, and highly degraded tissues.

Theory of Haplotype Examinations

The utility of Y chromosome and mtDNA in forensic testing is based on the premise that the Y chromosome or mtDNA type of an individual is fully dependent on the parent from whom it was inherited: the father for the Y chromosome in males, and the mother for the mtDNA in both genders. Therefore, individuals who share a lineage will have concordant DNA types. This sharing of DNA types allows a Forensic Examiner to use appropriate relatives as references for haplotype testing in lieu of the individual in question.

A. General Examination Process

There are different methodologies and processes for conducting mitochondrial DNA and Y chromosome typing. The Department shares information regarding some appropriate processes below. The Department does not suggest that the processes outlined here are the only valid or appropriate processes.

The general laboratory processes for both Y-STR and mtDNA typing are the same. They begin with the identification of suitable samples. Following sample selection, a technique known as extraction is performed. Extraction is the process by which cells are broken open and the DNA is released and purified away from other cellular debris. Evidentiary samples then require a determination of how much DNA was recovered from the sample, which is accomplished by performing a quantification procedure. The quantity of DNA obtained from forensic samples varies, but in all cases the DNA must be exponentially increased, or amplified. Amplification of targeted portions of the Y chromosome or mtDNA is achieved via a process

[2] Butler 2012, *supra* note 3, p. 374.

[3] Robin E and Wong R, Mitochondrial DNA Molecules and virtual number of mitochondria per cell in mammalian cells, 136 (3) *J. Cell. Phys.* 507-513 (1988). Satoh M and Kuroiwa T, Organization of multiple nucleoids and DNA molecules in mitochondria of a human cell. 196 (1) *Exp. Cell. Res.* 137-140 (1991).

[4] Butler JM. *Forensic DNA typing: biology, technology, and genetics of STR markers,* Second edition, Elsevier Academic Press, San Diego (2005). Foran D, Relative degradation of nuclear and mitochondrial DNA: an experimental approach, 51 (4) *J. Forensic Sci.* 766-770 (2006).

known as the polymerase chain reaction, or PCR. Following amplification, the DNA is separated and detected utilizing a procedure called capillary electrophoresis. The discipline specific differences in Y chromosome and mtDNA processes will be addressed in following sections.

Controls are used throughout the process to monitor and detect contamination that may be inadvertently introduced during laboratory processing. A reagent blank, which contains all of the ingredients used in the extraction process, is created during extraction. Two additional controls are initiated at the amplification step, a negative control and a positive control.[5] The negative control contains all the ingredients of a sample amplification tube, without any added sample, and serves to monitor for the introduction of a contaminant at the amplification step. A positive control is a sample of known DNA type and serves to evaluate the success of the amplification. The controls are processed in parallel with the sample and are evaluated to ensure that the testing process is working as expected.

1. Y Chromosome Typing

The Forensic Examiner must determine when Y chromosome testing is appropriate for a case. Since the Y chromosome is found only in males, Y chromosome tests can produce interpretable Y-STR typing results when the male DNA may not have been detected using autosomal tests.[6] For example, sexual assault evidence such as bite mark swabs and fingernail scrapings from a female victim may benefit from Y chromosome testing because the female DNA present will not interfere with obtaining a Y-STR profile. Finally, Y chromosome testing is also useful in pedigree analysis or missing persons applications when male relatives are in question.

When nDNA is quantified during the analytical processing, the concentration of total human DNA and the concentration of male DNA are determined. By evaluating the ratio of the male DNA to the human DNA, the Forensic Examiner can determine whether Y chromosome testing may be useful.

a. Short Tandem Repeats

Forensic Y chromosome testing relies on short tandem repeat markers to develop DNA profiles. Several hundred short tandem repeat markers have been identified on the Y chromosome (Y-STR).[7] STRs are small repeating sequences between 2-6 base pairs that are sometimes referred to as microsatellites. The average mutation rate for Y-STRs is ~0.2% per generation,[8] which makes them capable of discriminating between haplotypes from different families with fairly high precision.[9]

---

[5] FBI Quality Assurance Standards for Forensic DNA Testing Laboratories (September 1, 2011) *available at* http://www.fbi.gov/about-us/lab/codis/qas-standards-for-forensic-dna-testing-laboratories-effective-9-1-2011 (hereinafter *QAS*).

[6] Butler 2012, *supra* note 3, p. 372.

[7] Keyser M, et. al. A comprehensive survey of human y-chromosomal microstatellites, 74(6) *Am. J. Hum. Genetics* 1183-1197 (Jun 2004) (hereinafter *Keyser 2004*).

[8] Keyser M, et. al. Characteristics and frequency of germline mutations as microsatellite loci from the human Y chromosome, as revealed by direct observation in father/son pairs. 66 *Am. J. Hum. Genetics* 66: 1580-1588 (2000)

The AmpF*l*STR® Yfiler™ PCR Amplification Kit (Life Technologies), tests 17 Y-STR locations, or loci, at one time. Figure 2 is an example of a Y-STR profile developed using the AmpF*l*STR® Yfiler™ PCR Amplification Kit. The alleles are detected in the form of peaks on an electropherogram. Note that there is a single allele, or variant, at each locus, because males have a single copy of the Y chromosome. The single-allele exception is the locus DYS385, which is generally present in two copies on the Y chromosome, and therefore may have more than one allele in an individual. Generally, other Y-STR loci are present at only one location on the Y chromosome, and therefore have only one allele.

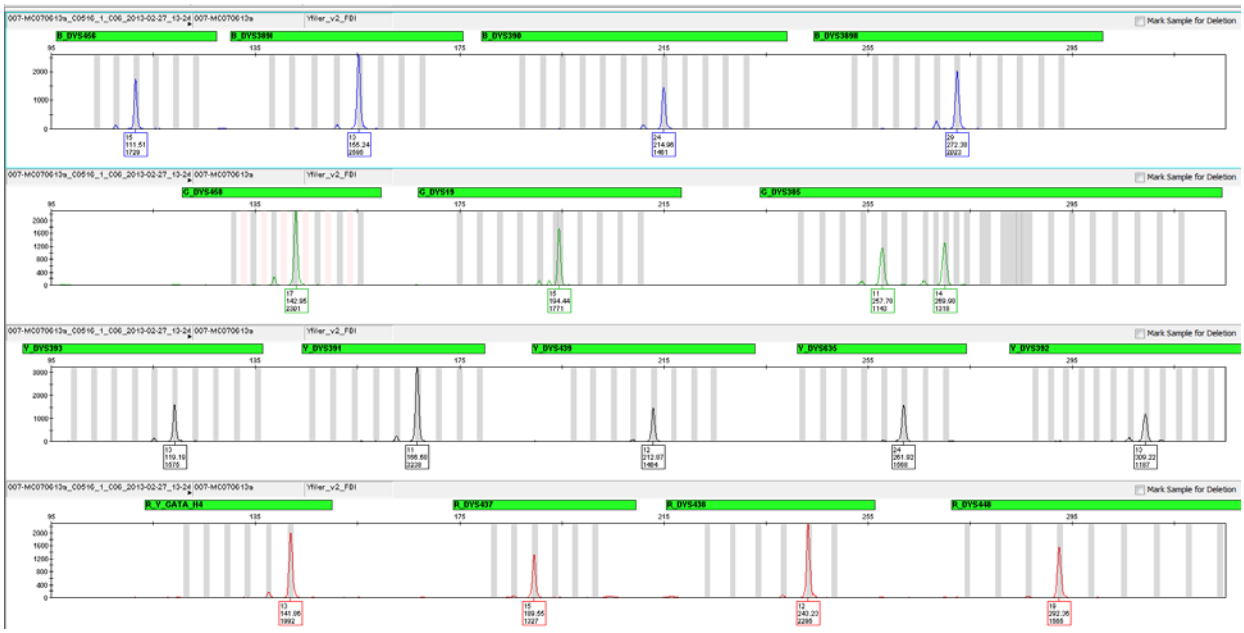

Figure 2: Sample Y-STR Electropherogram.

b. Y-STR Profile Interpretation

After PCR amplification and detection, the final step of Y-STR analysis is the interpretation of the electropherograms and comparison of the DNA profile results between the known and questioned DNA samples. While the previous steps of the process relied on commercially available and validated kits and equipment, the comparison of DNA profiles relies on the skill and training of Forensic Examiners as well as a detailed interpretation standard operating procedure.

To aid the forensic DNA community with interpretation, the Scientific Working Group on DNA Analysis Methods (SWGDAM) formed a subcommittee to provide guidance on the interpretation of Y STR typing results. The resulting guidance document entitled *Y-*

(hereinafter *Keyser 2000*). Dupuy BM. Y-chromosomal microsatellite mutation rates: differences in rate between and within loci. 23(2) *Human Mutation* 117-124 (Feb 2004) (hereinafter *Dupuy 2004*).

[9] Butler 2012, *supra* note 3, p. 376.

Chromosome Short Tandem Repeat (Y-STR) Interpretation Guidelines was published in January 2009 and updated in 2014.[10]

Evaluation of the Y-STR Profile

The first step in the interpretation of a Y-STR profile is to perform a preliminary evaluation of the DNA data and controls to assess that the outcome of all controls (reagent blank control, positive amplification control, and negative amplification control) is as expected and the data is of requisite quality for analysis. Following the preliminary evaluation, technical artifacts are assessed, thresholds and peak height ratios are applied, and the sample is evaluated to determine if it is a mixture or partial profile.

Technical Artifacts

The Forensic Examiner must first determine if a peak is an allelic peak or an artifact.[11] Artifacts that may be observed include stutter,[12] raised baseline,[13] minus-A,[14] spikes,[15] dissociated primer dye peaks,[16] and bleed-through/pull-up.[17] A Forensic Examiner uses specific

---

[10] Scientific Working Group on DNA Analysis Methods, *SWGDAM Interpretation Guidelines for Y-Chromosome STR Typing by Forensic DNA Laboratories* (2014), *available at* www.swgdam.org (hereinafter *SWGDAM Y-STR Guidelines*). SWGDAM is currently composed of approximately 50 scientists from federal, state, and local forensic laboratories in the United States and Canada. The group meets twice a year and makes recommendations to the Director of the Federal Bureau of Investigation regarding revisions to the Quality Assurance Standards for Forensic DNA Testing Laboratories (QAS, *supra* note 7).

[11] An artifact is a non-allelic product of the amplification process (stutter, minus-A), an anomaly of the detection process (pull-up/bleed-through, spikes) or a by-product of primer synthesis (dissociated primer dye peaks). *SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Typing Laboratories* (2010), available at www.swgdam.org (hereinafter *SWGDAM Interpretation Guidelines*), § 1, Definitions.

[12] Stutter is a minor peak typically observed one repeat unit smaller than a primary STR allele resulting from strand slippage during amplification. SWGDAM Interpretation Guidelines, *supra* note 13, § 1, Definitions.

[13] Raised baseline appears in an electropherogram as a non-specific elevation of the horizontal axis between one or more peaks. This elevation can result from excessive template DNA (i.e., >2 ng) or be instrument related (e.g., misaligned capillary). Nuclear DNA Analysis Procedures Manual, Procedures for the Interpretation of DNA Typing Results from the AmpF*l*STR® Identifiler® Plus and MiniFiler™ PCR Amplification Kits, Rev. 2 (05/08/15), § Glossary (hereinafter *nDNA SOP 229*).

[14] *Taq* DNA Polymerase is known to add an additional nucleotide (i.e., typically "A") to the 3' ends of double-stranded PCR products in a non-template-dependent manner. This phenomenon, called "adenylation," results in the generation of the "plus-A" fragment. This plus-A fragment is the allele (N) produced by the PCR amplification. Per allele, only a minority of amplified fragments do not have this additional nucleotide added (i.e., generally less than 15% for peaks that are not off-scale). These "minus-A" fragments are one bp shorter than the allelic fragment. Minus-A fragment generation is sequence-dependent and thus kit- and locus-specific. It is more likely to be observed when excessive template DNA amounts are used in the PCR and/or when an inhibitor of *Taq* DNA Polymerase is present in the sample. Butler, JM. *Advanced Topics in Forensic DNA Typing: Interpretation*. Elsevier Academic Press, San Diego, 2014 (hereinafter *Butler 2014*), p. 68.

[15] Spikes are non-specific, non-reproducible peaks that may result during electrophoresis from electrical fluctuations in the power source, from the interference of urea crystals, bubbles, or inherently fluorescent materials such as detergent in the capillary. nDNA SOP 229, *supra* note 15, § Glossary.

[16] Dye peaks are non-specific, reproducible peaks that result from dye that has become disassociated from the primers. nDNA SOP 229, *supra* note 15, § 7.4.1.4.5.

[17] Pull-up/bleed-through refers to the resultant artifact that is observed due to the inability of the genetic analyzer to properly resolve the different dye colors used to label the PCR product. Butler 2012, *supra* note 3, p. 383.

criteria established by the laboratory during its validation process to assess and interpret these artifacts.

Thresholds

Threshold values for peak height are established by each individual laboratory based on internal testing. The two thresholds recommended by SWGDAM and used by Department laboratories are the analytical threshold and the stochastic threshold.[18] The analytical threshold, also called the peak amplitude threshold (PAT), defines the minimum height at which a detected peak can be reliably distinguished from background noise.[19] The stochastic threshold is used by Department laboratories at the multicopy locus DYS385a/b to determine if a single allele is fully representative of the DNA type of the sample. Additionally, the stochastic threshold is used to establish the presence of a null allele.[20]

Peak Height Ratios

A peak height ratio of two alleles at a locus is the height in relative fluorescent units of the shorter allele divided by the height of the taller allele, generally expressed as a percentage. Peak height ratios (PHRs) in single source samples are only applicable to the multicopy locus DYS385a/b. Because individuals are expected to have two copies of the DYS385a/b locus, there is the expectation that the dosage of the two allelic peaks will be equivalent; therefore, peak height ratios can be used to associate the two alleles to a common source.[21]

Y-STR mixtures

Profiles are assessed to determine whether the DNA is from a single male or a mixture of multiple males.[22] A specimen is generally considered to have originated from more than one male individual if two or more alleles are present at two or more loci (other than DYS385a/b). This criterion is based on the knowledge that single-source samples may contain duplications, which can result in multiple loci with more than one allele present. Though rare, these duplications should be considered when determining the number of contributors to a Y-STR profile.[23]

Y-STR mixtures can be classified as distinguishable or indistinguishable. A distinguishable mixture is a sample that "contains a predominance of one individual's DNA…. In such instances, major and/or minor contributors may be determined."[24] Indistinguishable mixtures occur when the amounts of biological material from multiple donors are similar and cannot be distinguished

---

[18] SWGDAM YSTR Guidelines, *supra* note 12.

[19] Noise is background signal inherent to the instrumentation in the absence of DNA.

[20] A null allele is an allele that fails to amplify for various reasons.

[21] Nuclear DNA Analysis Procedures Manual, Procedures for the Interpretation of DNA Typing Results from the AmpF*l*STR® Yfiler™ PCR Amplification Kit, Rev. 4 (04/17/14), § 7.5.1 (hereinafter *nDNA SOP 215*).

[22] SWGDAM YSTR Guidelines, *supra* note 12 § 7.

[23] See SWGDAM YSTR Guidelines, *supra* note 12, § 3 for examples of reported single-source samples containing duplications.

[24] SWGDAM Interpretation Guidelines, *supra* note 13, § 3.5.

because of similarity in signal intensities.[25] In some instances, the profile of a male contributor known to be present in a mixture can be used to deduce the remaining profile. When Y-STR mixtures are deconvoluted, either because there is a major/minor contributor or because one of the profiles in the mixture is known or assumed, the probability calculations detailed for single source profiles can be used to assess the weight of a match.

Partial Y-STR Profiles

SWGDAM recommends that, "The laboratory should have guidelines for the interpretation of partial profiles (i.e., profiles with fewer loci than tested)."[26] Department laboratories require a greater distinction in the PHRs of major/minor alleles when a partial profile is identified compared to a full profile. Department laboratories define a "full profile" as any profile that contains at least one allele above the PAT at every locus, and a "partial profile" as any profile that failed to produce alleles above the PAT at one or more loci, with the exception of profiles that contain a confirmed null allele.[27]

c. Comparison of Y-STR Profiles

After the analysis and interpretation of the evidence samples and the known reference samples, a comparison of the Y-STR profiles is performed. The Forensic Examiner assesses whether the Y-STR typing results observed in the evidence sample and the known reference sample are the same or different. The general categories of conclusions that can be reached are inclusion or match, exclusion or non-match, inconclusive or uninterpretable, and no results.

When an inclusion/match occurs, the profile between the evidence sample and the known reference sample are the same. Therefore, the known individual is a possible contributor to the DNA obtained from an evidentiary item. All inclusionary conclusions must be accompanied by a statistical estimate to provide weight to the conclusion.

When an exclusion or non-match occurs, the profiles of the evidence and the reference are different. Therefore, the known individual could not have left the DNA obtained from the evidentiary item.

When the evidentiary profile is inconclusive or uninterpretable, the profile is at best only suitable for exclusionary purposes. Finally, the evidentiary sample may yield no results; therefore, it cannot be compared to reference profiles.

The conclusions that can be drawn between evidence samples and known reference samples are dependent upon whether direct (i.e., a comparison between a reference profile and an evidentiary sample) or lineage comparisons (i.e., comparison between a paternal relative's profile and an evidentiary sample) are performed. Direct and lineage comparison conclusions are the same when the Y-STR profiles obtained from the evidence and the known reference sample are identical. However, when the profiles from the evidence and known reference

[25] SWGDAM Interpretation Guidelines, *supra* note 13, § 3.5.

[26] SWGDAM Interpretation Guidelines, *supra* note 13, § 3.3.

[27] nDNA SOP 215, *supra* note 23, § 7.7.2.1.1 and 7.7.2.1.2.

samples are different, the conclusions for direct and lineage comparisons may differ. For a direct comparison, a difference at a single location is exclusionary. However, for lineage comparisons, the observation of two or more differences is generally necessary to exclude due to the potential for a mutation between generations.[28] If the profiles differ at a single locus in a lineage comparison, no conclusion as to the possible biological relationship between the individuals can be made. If two or more differences are present in a lineage comparison, the Forensic Examiner must consider whether a single mutation could have caused the result.[29]

2. Mitochondrial DNA Typing

Forensic mtDNA analysis targets the portion of the genome called the control region, which is highly variable between unrelated individuals. Within the control region, there are three regions in which the variability between individuals is concentrated, called hypervariable region I (HV1), hypervariable region II (HV2),[30] and hypervariable region III (HV3).[31]

The data derived from mtDNA analyses provide the analyst a graphical representation of the order, or sequence, of each DNA base in the targeted region of DNA. Figure 3 depicts a portion of an electropherogram. Forensic mitochondrial DNA analysis involves the comparison of the order of bases between a reference sample to the order of bases[32] from an evidentiary item.

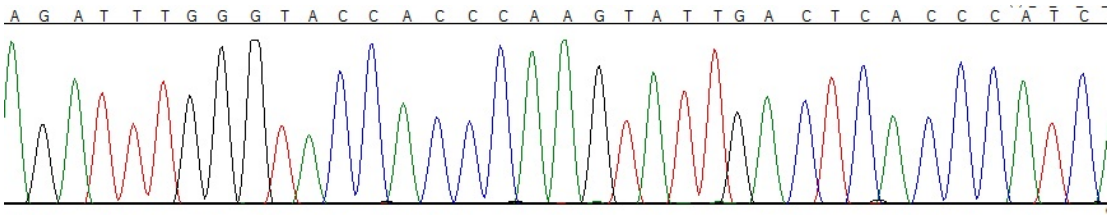

Figure 3: Sample of a portion of a mtDNA electropherogram.

[28] Keyser M, Sajantila A. Mutations at Y-STR loci: implications for paternity testing and forensic analysis. *Forensic Science International*. 2001; 118: 116-121.

Keyser 2000, *supra* note 10.

Keyser 2004, *supra* note 9.

Dupuy 2004, *supra* note 10.

[29] nDNA SOP 215, *supra* note 23, § 8.4.2.2.

[30] Greenburg B et al., Intraspecific nucleotide sequence variability surrounding the origin of replication in human mitochondrial DNA, 21 *Gene* 33-49 (1983).

[31] Lutz S et al., Location and frequency of polymorphic positions in the mtDNA control region of individuals from Germany, 111 (2) *Int. J. Legal Med.* 67-77 (1998). Bobillo M et al., Amerindian mitochondrial DNA haplogroups predominate in the population of Argentina: towards a first nationwide forensic mitochondrial DNA sequence database, 124 (4) *Int. J. Legal Med.* 263-268 (2010).

[32] Bases are the building blocks of DNA and are referred to by the first letter of their chemical name: A (Adenine), C (Cytosine), G (Guanine), and T (Thymine). The order of bases can differ between individuals and is the basis for mtDNA forensic comparisons.

a. Heteroplasmy in Mitochondrial DNA

Heteroplasmy is defined as the presence of more than one mitochondrial DNA type found within an individual. This phenomenon has been studied extensively by the Department and by many other laboratories, as evidenced by the wealth of published literature on this topic.[33] For the purposes of forensic testing, heteroplasmy is operationally defined as two or more types of mtDNA within an individual present at a detectable level. There are two types of heteroplasmy that are universally recognized in published literature: point heteroplasmy (also called sequence heteroplasmy) and length heteroplasmy. Point heteroplasmy is defined as the presence of more than one base at a single position, while length heteroplasmy is characterized as two or more mtDNA types within an individual that differ in length within a stretch of DNA that has the same repeated base. Traditional sequencing methods used at Department laboratories can detect a second mtDNA type when it reaches a threshold of 15% or more of the more abundant sample type.[34]

Point and length heteroplasmy are very well characterized in the published literature.[35] From these studies and others, we know that point heteroplasmy typically occurs at one sequence location within an individual (in approximately 6% of the population), and much less often at two sequence locations within an individual (in approximately 0.14% of the population), within the control region.[36] A tissue sample from a heteroplasmic individual may display both mtDNA types, while other samples from the same individual may only display one of the heteroplasmic types present within the individual. Additionally, samples from maternal relatives can share heteroplasmic variants, or can display fixation for one maternally inherited type over another.[37] Heteroplasmic variation within and between individuals is accounted for by the Department's interpretation standard operating procedures established for mtDNA sequence comparisons, which are based on published literature and internal validation studies.

b. MtDNA Testing Methods

MtDNA analysis employs the same general processing method as previously described in section II subsection A: sample selection, extraction, quantification and amplification. Following amplification, there are some techniques that are unique to mtDNA processing, including post-amplification quantification and cycle sequencing. These processes are described below.

---

[33] Melton T. Mitochondrial DNA heteroplasmy, 16 (1) *For. Sci. Rev*. 1-19 (2004).

[34] Irwin J et al., Investigation of heteroplasmy in the human mitochondrial DNA control region: a synthesis of observations from more than 5000 global population samples, 68 *J. Mol. Evol*. 516-527 (2009) (hereinafter *Irwin 2009*).

[35] Wilson MR et al., A family exhibiting heteroplasmy in the human mitochondrial control region reveals both somatic mosaicism and pronounced segregation of mitotypes. 100 *Hum. Genet.* 167-171 (1997) (hereinafter *Wilson 1997*). Stewart JEB et al., Length variation in HV2 of the human mitochondrial DNA control region. 46 (4) *J. Forensic Sci*. 862-870 (2001). Melton T et al., Forensic mitochondrial DNA analysis of 691 casework hairs, 50 *J. Forensic Sci*. 73-80 (2005) (hereinafter *Melton 2005*). Nelson K and Melton T, Forensic mitochondrial DNA analysis of 116 casework skeletal samples. 52(3) *J. Forensic Sci*. 557-561 (2007).

[36] Melton 2005, *supra* note 37. Irwin 2009, *supra* note 36.

[37] Wilson 1997, *supra* note 37.

i. Post Amplification Quantification

A post-amplification quantification is used to estimate the amount of DNA in the sample, and to assess negative and positive controls for contamination and predicted success, respectively. Based on extensive studies,[38] DNA detected in the negative control and/or reagent blank that is at a level of ≤10% of the sample concentration will not be detected within the associated sample upon sequencing. If the concentration of DNA in either the reagent blank or negative control is above 10% of the concentration of DNA in the associated sample, the amplified sample is not processed further, and analyses may be repeated from either the extraction or amplification stage, as appropriate. If the PCR failed to yield sufficient product, the sample may be re-amplified or re-extracted as appropriate.

ii. Sequencing

DNA sequencing is a method used to determine the specific order of the bases in the amplified region of DNA. At Department laboratories, sequencing is accomplished using the Sanger cycle sequencing method[39] coupled with injection onto a capillary electrophoresis instrument equipped with fluorescent detection.

c. Interpretation of mtDNA Profiles

The final step of mtDNA analysis is the evaluation of the sequence data and the comparison of the mtDNA profiles between known and questioned DNA samples. While the laboratory methods used for mtDNA analysis rely on commercially available and validated reagents and equipment and standard operating protocols, the evaluation and comparison of mtDNA sequences relies on the skill and experience of Forensic Examiners.

To assist the forensic mtDNA community, the Scientific Working Group on DNA Analysis Methods (SWGDAM) formed a mtDNA subcommittee to provide guidance on interpretation. The resulting guidance document, entitled *SWGDAM Interpretation Guidelines for Mitochondrial DNA Analysis by Forensic DNA Testing Laboratories*, was approved in July 2013.

i. Evaluation of the mtDNA Profile

A preliminary evaluation of the mtDNA sequence data and controls is performed to assess whether the results for all controls (reagent blank control, positive amplification control, and

[38] Wilson MR et al., Extraction, PCR amplification and sequencing of mitochondrial DNA from human hair shafts, 18 (4) *Biotechniques* 662-669 (1995) (hereinafter *Wilson 1995a*). Wilson MR et al., Validation of mitochondrial DNA sequencing for forensic casework analysis, 108 *Int. J. Legal Med.* (1995) (hereinafter *Wilson 1995b*).

Stewart JEB et al., Evaluation of a multicapillary electrophoresis instrument for mitochondrial DNA typing, 48 (3) *J. Forensic Sci.* 571-580 (2003) (hereinafter *Stewart 2003*).

[39] Sanger F et al., DNA sequencing with chain-terminating inhibitors, 74 (12) *Proc. Natl. Acad. Sci. USA* 5463-5467 (1977).

negative amplification control) are acceptable and the data is suitable for interpretation.[40] In addition, samples are evaluated for the presence of contamination, mixtures, or heteroplasmy.

ii. Contamination

While the high copy number and stability of mtDNA are advantageous for some sample types, they make forensic mtDNA testing particularly prone to contamination. Hence, mtDNA examinations must incorporate especially stringent quality assurance procedures designed to prevent and monitor for contamination.[41] Although these preventive measures are effective, they cannot prevent all instances of contamination. Based on extensive research and validation studies,[42] there are procedures in place to assess whether detected contamination could interfere with mtDNA typing.

The contamination procedures followed by Department laboratories comply with the FBI Director's Quality Assurance Standards as well as the SWGDAM guidelines which recommend that laboratories determine what level of contamination, if present, will not interfere with obtaining the correct mtDNA type of the sample.

iii. Naming of mtDNA sequences

To simplify sequence comparisons, as well as to provide a short-hand method for notation of DNA sequences, a sequence reference standard, called the revised Cambridge Reference Sequence (rCRS),[43] is used. The rCRS is a published sequence of the entire mtDNA genome from one individual that established, among other things, a numbering system for mtDNA base locations.[44] An mtDNA type is obtained by noting the position and nucleotide present in each position that differs between the sample and the rCRS (e.g., 73 G notes that a sequence is different from the rCRS at position 73, and has a guanine at that position). The nomenclature, or naming of an mtDNA type, is based upon published peer-reviewed literature and guidance contained within the SWGDAM mtDNA Interpretation Guidelines.

---

[40] Scientific Working Group on DNA Analysis Methods, *SWGDAM guidelines for mitochondrial DNA (mtDNA) nucleotide sequence interpretation* (2013) (hereinafter *SWGDAM mtDNA guidelines*).

[41] QAS *supra* note 7, Definitions. SWGDAM mtDNA guidelines, *supra* note 42 § 1.

[42] Wilson 1995a, *supra* note 40; Wilson 1995b, *supra* note 40; Stewart 2003, *supra* note 40.

[43] Anderson S et al., Sequence and organization of the human mitochondrial DNA genome, 290 *Nature*, 457-465 (1981). Andrews RM et al. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. 23 *Nature Genetics* 147 (1999).

[44] The Cambridge Reference Sequence (a.k.a. Anderson Sequence) was the first complete human mtDNA sequence published by Anderson et al. in 1981 in the journal *Nature* (*supra* note 45) and has been used as a reference sequence since that time. A revised Cambridge Reference Sequence (rCRS) was published by Andrews et al. in 1999 in the journal *Nature Genetics* (*supra* note 45), where a reanalysis of the original CRS sample was performed with more current technology. This reanalysis resulted in the acknowledgment of a few rare polymorphisms in CRS and the correction of minor errors that had been a result of the sequencing methodology at the time. None of the revisions to the rCRS affected the portion of the mtDNA genome that has been and currently is used in forensic mtDNA analysis.

iv. Confirmation

Once the sequence data and controls for a sample have been analyzed by a Forensic Examiner, a second Forensic Examiner performs an additional analysis of the data in a process termed confirmation. The confirmation results in a consensus profile derived from both Forensic Examiners, which is then used for sequence comparisons.

d. mtDNA Conclusions

After a Forensic Examiner has evaluated the mtDNA profiles obtained from questioned and known reference samples, a comparison of the differences from the rCRS for each sample is used to determine if they have the same mtDNA sequence. Typically, the mtDNA profile from each questioned item tested is compared with the mtDNA profile from each reference item. However, each case is different and may require an evaluation of which comparisons will answer the question asked. The following outlines criteria for the possible conclusions for typical comparisons between questioned and known items:

A cannot exclude interpretation occurs when samples have the same sequence or demonstrate sequence concordance (i.e., share a common base at each position in the sequence). For example, if one sample has evidence of a C and a T at a given position (i.e., point heteroplasmy), and the other has a C, they share the C in common at that position and are concordant. However, if one sample has evidence of a C and a T at a given position, and the other has a G, these sequences are not concordant.

An inconclusive interpretation occurs when samples differ at a single position only (and share a common length variant at positions 303-309), or do not share a common length variant at positions 303-309 (with all other positions concordant), or differ at a single position *and* have no common length variant at positions 303-309.

An exclusion interpretation occurs when samples differ at two or more positions.

Several scenarios may preclude sequence comparisons with a particular sample, including: insufficient mtDNA, mtDNA that is not of the requisite quality, or a mixture of mtDNA present within a sample. Additionally, mtDNA sequence analysis may also be requested for single samples for entry into the appropriate database, thereby negating the requirement for sequence comparisons and interpretation.

B. Limitations of Y Chromosome and mtDNA testing

The following are shared limitations of Y chromosome and mtDNA testing that the Forensic Examiner must consider when interpreting data derived from forensic specimens.

1. Uniparental Inheritance

The uniparental inheritance pattern of the Y chromosome and mtDNA confers both advantages and limitations for forensic testing. While it allows the Forensic Examiner to use relatives as references when individuals are not available for direct sampling, it also means that all males in the same paternal lineage will have the same Y-STR type, and all individuals in the

same maternal lineage will have the same mtDNA type. Therefore, relatives also cannot be excluded as a source of the evidentiary sample when there is a cannot exclude interpretation for Y-STR or mtDNA typing. Additionally, although mtDNA and Y-STR types most often differ between unrelated individuals, it is possible that unrelated individuals can share the same Y-STR or mtDNA type. Y-STR and mtDNA typing results simply cannot be attributed to a single individual to the exclusion of all others.[45]

2. Mixtures

A mixture is defined as the presence of more than one DNA type from more than one individual present within a sample. Mixtures can be identified as multiple peaks at several loci within a Y-STR profile, or as multiple positions of mixed mtDNA bases within an mtDNA sequence. According to the Scientific Working Group for DNA Analysis Methods, each individual laboratory must develop interpretation guidelines for mixtures based on validation studies.

SWGDAM is currently considering the best approach to give weight to an association between an individual and an indistinguishable Y-STR mixture. Therefore, "Indistinguishable mixtures may be used for exclusionary purposes only"[46] with further clarification stating that we are "continuing to evaluate statistical approaches for calculating the rarity of mixed Y-STR profiles."[47]

Unlike nuclear DNA analysis, mitochondrial DNA sequence data is not quantitative using traditional sequencing methods.[48] As a result, the analyst cannot determine the relative proportions of contributors to a mixed sample from the data, and therefore cannot determine which bases at each mixed-base position can be assigned to a specific contributor.

According to validation studies, mixtures of mtDNA cannot be deconvoluted, and therefore are not interpretable. Accordingly, no comparisons or interpretations are performed using data determined to be a mixture of mtDNA from two or more individuals. For this reason, mtDNA analysis is not appropriate for evidence containing possible mixed sources of DNA (e.g., semen stains from sexual assaults).

3. Ethnicity

Over evolutionary time, maternal and paternal genetic lineages will accumulate differences via normal mutational processes, while still retaining a signature of past mutational events that link them to closely related (maternal or paternal) lines. Evolutionary studies of contemporary mtDNA have mapped common mtDNA characteristics shared by individuals with a common

[45] SWGDAM Y-STR Guidelines, *supra* note 12, Background.

[46] nDNA SOP 215, *supra* note 23, § 7.8.2.

[47] nDNA SOP 215, *supra* note 23, § 8.4.1.2.

[48] Parker L. et al. AmpliTaq DNA polymerase, FS dye-terminator sequencing analysis of peak height patterns. 21 *BioTechniques* 694-699 (1996).

ancestry using molecular phylogenetic techniques, and the same can be said for Y-STRs.[49] Yet, neither lineage marker can be used to definitively predict ethnicity of an individual for several reasons. First, ethnicity has both a genetic[50] and a cultural component. Because individuals of mixed ancestry may identify with the ethnicity of one parent over another, that cultural identification may not reflect their (maternal or paternal) lineage. Secondly, an individual may have an unknown maternal or paternal genetic contribution from generations ago. These scenarios are reflected in studies of ethnicity, where some mtDNA types are more common in one ethnic group than another, but are often shared between ethnic groups to some extent.

4. Other Limitations

Other limitations of Y-STR and mtDNA testing are common to all types of DNA testing: the Forensic Examiner cannot determine when the detected DNA was deposited on the item, and, though highly sensitive, there is a detection limit associated with each technology. That is, without a sufficient quality or quantity of DNA present, Y-STR and mtDNA typing will be unsuccessful.

C. Statistical Evaluation of Y-STRs and mtDNA

In order to assist judges and juries in assessing the significance of an inclusion or match conclusion, statistical calculations are performed on the evidentiary DNA profile to indicate the weight of a match (i.e., cannot exclude) conclusion.[51] Estimates for a random match with Y-STR haplotypes and mtDNA sequences are done by the counting method, where the number of times the haplotype of interest is observed is divided by the total number of haplotypes in the database used. Because Y-STRs and mitochondrial DNA are each inherited as a unit (but independently from each other), the rarity of the profile as a whole unit must be considered. To account for database sampling bias, both Y-STRs and mtDNA apply a confidence interval calculation, which gives a statistical estimate for how often that type is expected to occur in the actual population.[52]

1. Frequency of a Haplotype within a Database

When concordance between Y-STR profiles or mtDNA sequences from an unknown sample and a known sample is obtained, a search of the appropriate population database is used to provide a frequency (*p*) of that type within all relevant database groups using the following formula:

$$p = x/N$$

[49] For example, see Stoneking M and Soodyall H, Human evolution and the mitochondrial genome, 6 *Curr. Op. Gen. Devel.*, 731-736 (1996). For example, see Forster P. et al. A short tandem repeat-based phylogeny for the human Y chromosome. 67(1) *Am. J. Hum. Genetics*, 182-196 (2000).

[50] Conner A. and Stoneking M. Assessing ethnicity from human mitochondrial DNA types determined by hybridization with sequence-specific oligonucleotides. 39(6) *J. Forensic Sci.*, 1360-1371 (1994).

[51] NRCII *supra* note 2, pp. 166-167.

[52] Butler 2014, *supra* note 16, Chapter 15.

Where *p* is the frequency in the population group, *x* is equal to the number of observances in a population group containing *N* number of profiles, or samples.

2. Confidence Intervals

The frequency of a Y-STR or mtDNA type is highly dependent on the sample size of the group: as the size of a database group increases, the frequency estimate within the actual population will become more refined (and theoretically, closer to the true frequency of the haplotype in the population).[53] However, unless every individual within the population is sampled, there is a degree of uncertainty about the true frequency of the type in the population. To account for this uncertainty, a statistical calculation called a confidence interval can be applied, which gives a range of possible values of how common that type is, based on the number of observances in the database and the number of individuals in the database. The upper bound of the confidence interval calculation is reported for each ethnic group within the database, which gives the maximum number of people expected (with 95% confidence) to have the same type (Y-STR or mtDNA) in the population. The application of a confidence interval corrects for both database size and sampling variation.

i. When the haplotype has been observed in the database

The Clopper and Pearson (C&P) method is an exact method for calculating the confidence limit based on the binomial distribution. The formula for the exact confidence interval is a cumulative binomial distribution for all values from 0 to *x* that match given a sample of size *N* and frequency *p*,[54] and is as follows:

$$\sum_{k=0}^{x} \binom{n}{k} p_0^k (1-p_0)^{n-k} = \alpha$$

Where $\alpha = 0.05$, the confidence coefficient for a 95% confidence limit; n = database size; x = number of observations of the haplotype in the database; p = haplotype frequency at which x or fewer observations are expected to occur 5% of the time; and $k = 0,1,2,3,\ldots x$ observations.[55] Solving for p yields the upper bound of the 95% confidence interval.

ii. When the haplotype has not been observed in the database

The formula for calculating the upper 95% confidence limit from zero proportion is a special case of C&P where $x = 0$:

$$1 - \alpha^{1/N}$$

[53] Butler 2012, *supra* note 3, p. 386.

[54] nDNA SOP 215, *supra* note 23, § 9.1.7: footnote 43. Also see Butler 2012, *supra* note 3, p. 388.

[55] USYSTR User Directions p. 1, and nDNA SOP 215, *supra* note 23, § 9.1.7

Where N = database size and $\alpha = 0.05$, the confidence coefficient for a 95% confidence interval.[56]

3. Y-STR Population Databases

Online Y-STR databases contain collections of anonymous individuals and can be used to estimate the frequency of specified Y-STR haplotypes. Additionally, online databases contain tools to calculate the upper bound confidence interval based on the observed data.

i. US Y-STR Database (US Y-STR)

The US Y-STR Database, which is the primary database for estimating haplotype frequency at Department laboratories,[57] is "a searchable listing of 11- to 29-locus Y-STR haplotypes … funded by the National Institute of Justice and managed by the National Center for Forensic Science (NCFS) in conjunction with the University of Central Florida."[58] It is available online at https://usystrdatabase.org. It was launched in 2007 and currently houses 9537 African American, 4284 Asian, 10903 Caucasian, 6377 Hispanic, and 4194 Native American profiles as of Release 4.1 in August 2014. Duplicate samples, identified by nDNA markers, "were removed to ensure that each sample in the consolidated database is from a unique individual."[59] Since March 2014, the US Y-STR Database calculates a match probability in addition to the 95% confidence interval. The match probability is recommended in the 2014 SWGDAM Interpretation Guidelines for Y-Chromosome STR Typing by Forensic DNA Laboratories and "…is the probability of observing (a) haplotype… given that it has already been seen once in another individual of the same subpopulation."[60]

ii. Y-STR Haplotype Reference Database (YHRD)

"The ... Y-STR Haplotype Reference Database (YHRD), was created by Lutz Roewer and colleagues at Humbolt University in Berlin, Germany, and has been available online since 2000."[61] It is available at http://www.yhrd.org. As of November 2014, Release 48 includes 136,184 haplotypes typed in 917 different populations.[62] YHRD can be searched based on population group or geographic location.[63]

---

[56] USYSTR User Directions p. 1, nDNA SOP 215, *supra* note 23, § 9.1.7.1, and Mitochondrial DNA Procedures Manual, Procedures for mtDNA Sequence Analysis, Comparison, Interpretation, Weight Assessment, and Report Writing, Rev. 2, § 6.3 (04/22/14).

[57] nDNA SOP 215, *supra* note 23, § 9.1.

[58] USYSTR info p. 1.

[59] USYSTR info, release 4.1 descriptive statistics.

[60] SWGDAM YSTR Guidelines, *supra* note 12, §10.3.

[61] Butler 2012, *supra* note 3, p. 383.

[62] YHRD "News & Updates" page (http://www.yhrd.org/posts), visited 01/26/15.

[63] Butler 2012, *supra* note 3, p. 383.

Department laboratories do not regularly use YHRD. However, it may be appropriate in situations where a specific population(s) other than those provided in the US Y-STR Database is needed.[64]

4. mtDNA Population Databases

The SWGDAM mtDNA population database is a compilation of mtDNA sequences used to provide an estimate of mtDNA sequence rarity in relevant US populations, as well as populations outside of the US. It contains mtDNA sequences covering the entire control region from specimens collected and submitted by the Armed Forces DNA Identification Laboratory (Dover, DE). The database is provided with the Combined DNA Index System (CODIS) software, is maintained by the FBI CODIS unit, and is available to all CODIS participating laboratories.

The current version of the database, CODIS + mito Popstats v.1.4, CODISmpPop _10629 v1, released in 2014, is comprised of 10,629 individuals categorized into 10 different population groups based on the self-identified ethnicity of the sample donor, including individuals classified as African American, Caucasian, Hispanic, US Asian, and US Native American, as well as groups from China (unspecified region), China – Hong Kong, Japan, Korea, and Vietnam.

Several publications support the separation of the population groups within the database,[65] as well as the representativeness of the groups within it.[66] Based on these publications, the major population groups within the database are representative of the corresponding population groups within the United States, and the population categories are appropriate based on current knowledge and the distribution of the sequences within each group.

Department laboratories employ the SWGDAM mtDNA population database in routine casework, but may also report estimates from the European DNA Profiling Group MtDNA Population database (EMPOP)[67] if additional population groups contained within EMPOP are relevant to the case. EMPOP includes the same sequences contained within the SWGDAM database, as well as sequences from approximately 40 additional population groups contained within Africa, Asia, Europe and Asia, for a combined total of 34,617 sequences.

---

[64] nDNA SOP 215, *supra* note 23, § 9.2.

[65] Budowle B. et al. Mitochondrial DNA regions HVI and HVII population data, 103, *Forensic Sci Int*, 23-25 (1999). Melton T et al. Diversity and heterogeneity in mitochondrial DNA of North American populations, 46 (1) *J Forensic Sci* 46-52 (2001). Zimmerman B. et al. Forensic and phylogeographic characterization of mtDNA lineages from northern Thailand (Chiang Mai). 123 *Int J Legal Med.* 495-501 (2009).

[66] Allard M. et al. Evaluation of variation in control region sequences for Hispanic individuals in the SWGDAM mtDNA data set 51 (3) *J. Forensic Sci*. 566-573 (2006). Allard M. et al. Characterization of human control region sequences of the African America SWGDAM forensic mtDNA data set. 148 *Forensic Sci Int.* 169-179 (2005). Allard M. et al. Characterization of the Caucasian haplogroups present in the SWGDAM forensic mtDNA dataset for 1771 human control region sequences. 47 (6) *J. Forensic Sci*. 1215-1223 (2002). Allard M. et al. Control region sequences for East Asian individuals in the Scientific Working Group on DNA Analysis methods forensic mtDNA data set. 6 *Legal Med*. 11-24 (2004). Irwin J. et al. Mitochondrial DNA control region variation in a population sample from Hong Kong, China. 3 *Forensic Sci Int.* e119-e125 (2009). Scheible M. et al. Mitochondrial control region variation in a Korean population sample. 128 (5) *Int J Legal Med.* 746-746 (2014).

[67] Parson W. and Arne D. EMPOP – A forensic mtDNA database. 1 *Forensic Science International: Genetics* 88-92 (2007).

5. Population Substructure and Theta (θ)

Populations have the tendency to mate within geographic or social confines rather than mating at random throughout the population as a whole. This nonrandom mating causes some amount of population substructure, which can be observed when databases are studied for various populations. F_{ST} values quantify the amount of substructure observed in a database, which is reflective of the substructure that exists in the population it represents.[68] A correction factor, theta (θ), may be used in the calculation of haplotype frequencies to account for observed F_{ST} values.

In 2014, the SWGDAM Y-STR Guidelines recommended incorporating a correction factor of θ. Studies with current population databases have shown that the magnitudes of multi-locus θ values are inversely proportional to the number of Y-STR loci analyzed: if more Y-STR loci analyzed, the multi-locus θ values are smaller.[69] SWGDAM recommended the calculation of the match probability, which is the probability of observing haplotype *A* given that it has already been seen once in another individual of the same subpopulation. The match probability for the haplotype is calculated using the theta (θ) values currently recommended by SWGDAM:

$$Pr\,(A \mid A) = \theta + (1 - \theta)\, p_A$$

where *A* is the haplotype of interest and $Pr\,(A \mid A)$ is the match probability. Sampling uncertainty is accommodated by using the 95% upper confidence limit for p_A. The current SWGDAM Y-STR Interpretation Guidelines (available at swgdam.org) list θ values that are incorporated into the above formula based on the number of loci typed and the amplification kit used.[70]

Currently, there are no recommendations in the mtDNA community for θ corrections. Based on this, Department laboratories do not currently correct for low level regional substructure in mtDNA analyses.

Policy Considerations

A. A Framework for Safeguarding Against Error in DNA Testing

The Department maintains a comprehensive quality system that employs quality assurance and quality control measures (e.g., extensive training, proficiency testing, detailed standard operating procedures, technical and administrative reviews) to ensure that the risk of an error is minimized and, if an error should occur, it is detected and the sample can be re-tested.[71] Additionally, if an error occurs, the Department laboratory formulates a corrective action plan to address the root cause of the error and documents action steps to correct the error and ensure that the same error is prevented in the future.[72]

---

[68] NRCII, *supra* note 2, at 90, 102-104.

[69] SWGDAM Y-STR guidelines, *supra* note 12 § 10.3.

[70] SWGDAM Y-STR guidelines, *supra* note 12 § 10.3 and nDNA SOP 215, *supra* note 23 § 9.1.8.

[71] NRC II, *supra* note 2, at 87.

[72] QAS, *supra* note 7, Standard 18.

There has been informed and comprehensive debate about how to best develop quality assurance standards in DNA testing. This debate has led to the creation of a number of bodies that define and establish quality assurance standards, and – ultimately – perform accreditation of laboratories performing DNA testing.

The 1994 DNA Identification Act requires all DNA laboratories that are federally funded, receive federal funds, or participate in the National DNA Index System (NDIS) to comply with quality assurance standards approved by the FBI Director. Pursuant to the Act's directives, the FBI Director approved and issued Quality Assurance Standards for Forensic DNA Testing Laboratories in 1998 and Quality Assurance Standards for DNA Databasing Laboratories in 1999. These standards were most recently updated in 2011. These FBI approved standards cover the areas of organization and management, personnel, facilities, evidence/sample control, validation, analytical procedures, equipment calibration and maintenance, reports, review, proficiency testing, corrective action, audits, safety and outsourcing[73] and are specific to the forensic DNA discipline. While mandatory only for crime laboratories that receive federal funding or participate in NDIS, these documents also serve as "benchmarks for assessing the quality practices and performances of DNA laboratories throughout the country."[74] Compliance is assessed through annual audits, which must be performed by DNA analysts who are external to the laboratory a minimum of once every other year.[75] To ensure a uniform interpretation of the standards by laboratories and auditors, the FBI Laboratory developed audit documents for both sets of standards which provide explanatory information about the standards.[76] SWGDAM meets twice a year and makes recommendations to the FBI Director regarding revisions to the QAS.

While there are a number of entities that provide laboratory accreditation, the American Society of Crime Laboratory Directors/Laboratory Accreditation Board (ASCLD/LAB), Forensic Quality Services (FQS) and American Association for Laboratory Accreditation (A2LA) are accrediting bodies for forensic science laboratories employing management and technical requirements following ISO/IEC 17025 standards. These standards pertain to document control, corrective action, audits, personnel, validation, equipment, assuring the quality of test results, reporting of results, reports, proficiency tests, technical records, education of personnel, competency testing, security of facilities and evidence, evidence handling, training, and technical and administrative review.[77] In contrast to the FBI Director approved QAS, which are specific to the discipline of forensic DNA, these ISO/IEC 17025 standards may be employed by laboratories conducting a variety of forensic analysis, for example, fingerprint analysis or firearms analysis. Through an agreement with the FBI and ASCLD/LAB (and FQS and A2LA accrediting bodies), for a laboratory to be accredited in forensic DNA testing to the ISO/IEC 17025, it must also comply with the requirements of the QAS.

---

[73] QAS, *supra* note 7.

[74] QAS Forensic Audit Document, *available at* http://www.fbi.gov/about-us/lab/biometric-analysis/codis/forensic-qas-audit-9-1-11 (hereinafter *QAS Audit Document*).

[75] QAS, *supra* note 7, Standard 18.

[76] QAS Audit Document, *supra* note 76, at 3.

[77] ISO/IEC 17025 - General Requirements for the Competence of Testing and Calibration Laboratories, International Organization for Standardization, Geneva, Switzerland (2005) and American Society of Crime Laboratory Directors/Laboratory Accreditation Board, ASCLD/LAB-*International* Supplemental Requirements for the Accreditation of Forensic Science Testing Laboratories, Garner, N.C. (2011).

B. National Research Council (NRC) / National Academy of Sciences (NAS) Reports

Forensic DNA analysis has been the subject of review and scrutiny on several occasions, culminating in reports on the status of forensic DNA analysis by the National Research Council (NRC) in 1992 and 1996. Although the fields of forensic Y-STR and mtDNA typing were not established during the NRC review process, both disciplines benefitted from the recommendations that came from those reviews during their formative phases of research, validation, and implementation.[78] Later, the status of forensic analysis of multiple disciplines, to include nuclear, Y-STR and mitochondrial DNA analysis, was considered by the National Academy of Sciences (NAS) in 2009. Though the NAS report focused on autosomal[79] nuclear DNA analyses; ultimately, the field of forensic DNA analysis as a whole was regarded as the gold standard to which other disciplines were compared.

[78] The FBI Laboratory implemented mtDNA analysis in 1996 and Y-STR analysis in 2011.

[79] Autosomal DNA refers to DNA found within the nucleus inherited from both parents.