This is to certify that the
thesis entitled

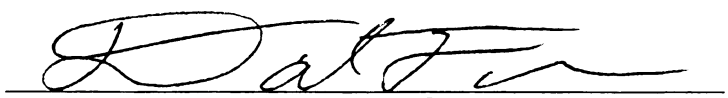
An Evaluation of Pubic Hair
Characterization Techniques for Forensic Casework

presented by

Lynne Karla Burley

has been accepted towards fulfillment
of the requirements for the

MS degree in Forensic Science



Major Professor's Signature

11-29-04

Date

AN EVALUATION OF PUBIC HAIR CHARACTERIZATION TECHNIQUES

By

Lynne Karla Burley

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Criminal Justice

2004

ABSTRACT

AN EVALUATION OF PUBIC HAIR CHARACTERIZATION TECHNIQUES

By

Lynne Karla Burley

A study was conducted to investigate the different methods used by forensic laboratories to characterize pubic hairs collected following a sexual assault. Techniques included visual examination, microscopic examination, nuclear DNA analysis of 15 short tandem repeat (STR) loci, and mitochondrial DNA (mtDNA) typing using a sequence-specific oligonucleotide (SSO) probe hybridization assay. Fifty participants donated reference buccal swabs, reference pubic hair cuttings, and pubic hair combings. Subsequently, 25 sample sets were prepared such that references and pubic hair combings originated from the same donor, and the remaining 25 sets included references and combings from two different donors. Qualified analysts characterized the hairs in the sample sets both macroscopically and microscopically. The questioned hair and the buccal swab were then analyzed using nuclear DNA and mtDNA typing methodologies. Analysts had a variable success rate for visually identifying the source of the questioned hairs, ranging from 48% to 70%. The microscopic hair examinations were successful in characterizing 72% of the hairs; however, false inclusions/exclusions did occur. Nuclear DNA typing allowed for positive identification of the hair donor in 38% of sample sets. MtDNA analysis resulted in 25 accurate exclusions, 23 correct inclusions, and two false inclusions. The results indicate that no one method can consistently provide discriminatory information in regards to hair characterization, but by coupling different types of examination in a logical order, the most valuable conclusions may be drawn.

Acknowledgements

I would like to express my utmost gratitude to several individuals who made this project possible: Dr. David Foran, graduate advisor; Lisa Brewer and Dr. Homer Hawkins, committee members; Grady Goldman, mentor and former Assistant Laboratory Director; Benny Del Re, Laboratory Director; Nancy Marte, Mark Moriyama, Denise Wong, Brooke Barloewen, Mark Powell, Alice Neumann, Diana Grappasonno, Opritsa Tudoriu, Jeremiah Garrido, Cassandra Musgrave-Nelson, Amanda Cardenas, Gentry Roth, Cathleen Trowbridge, and Cordelia Willis, Criminalists at the Santa Clara County Crime Laboratory; Matthew Gabriel, San Francisco Police Department Crime Laboratory; all sample donors; David and Joann Ziech; John and Barbara Burley; and finally, John Christian Burley, for his support, love, and patience.

TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Note: Images in this thesis are presented in color

Introduction

Hair transfer evidence is a critical tool for the forensic scientist as it can associate assailants and victims of various types of crimes, place individuals at the scene of a crime, or exclude innocent persons. Pubic hair transfer from victim to suspect, or vice versa, can be the best and only evidence providing proof of an intimate act resulting from a sexual assault. Following a sexual assault, the victim often receives an examination at a hospital to treat injuries, provide medication for birth control and sexually transmitted diseases, and to collect evidence. Pubic hair combings are taken to obtain potentially foreign hairs, but will often contain the victim's own pubic hairs (Linch *et al.*, 2001). Therefore, a forensic examination is necessary to differentiate the hairs present in a combing. Various methods are currently utilized for the characterization of pubic hairs, including visual (macroscopic) examinations without the use of magnification, microscopic examinations, nuclear DNA typing, and mitochondrial DNA (mtDNA) typing. A recent survey of 14 laboratories across United States by the author of this paper (2004) indicated that they rely on a variety of these methods for the characterization of pubic hairs. Seven of the laboratories perform a complete microscopic hair comparison first and then nuclear or mtDNA analysis when appropriate. Four laboratories rely on a macroscopic examination to identify potentially probative hairs which are then analyzed at the nuclear or mitochondrial level. Only one laboratory surveyed has the ability to perform mtDNA analysis in-house, and the remaining laboratories outsource the evidence for mtDNA testing if necessary. Finally, three laboratories no longer look at hairs in pubic hair combings. Clearly, there is no consensus as to the most appropriate way to analyze hairs in the forensic community.

Each of the approaches for examining pubic hairs has advantages and disadvantages based on time, cost, facility structure, sample size and condition, and the ultimate value of the conclusion resulting from the analysis. Any single approach is frequently strengthened when used in conjunction with an additional type of analysis, for example, microscopic examination followed by mtDNA sequencing. Both are more powerful together than they are separately. A logical progression of examinations may differ depending on the type and condition of hair evidence available, background of the case, and laboratory protocol, as some types of analysis may have a deleterious effect on others. Although each method for hair examination has been investigated individually or in conjunction with one additional technique (Soules *et al.*, 1978; Keating, 1982; Mann 1990; Exline *et al.*, 1998; Linch *et al.*, 1998, 2001; Houck and Budowle, 2001), a strategy for examination taking into account all types of analysis has not been well-defined. The purpose of this research was to address this need and establish a logical and strategic manner in which to assess hairs, based on the accuracy and discriminating power of results, sample availability, and time and cost of analysis. To establish an approach for the examination of pubic hairs, it is necessary to review the findings of past research on this subject, and analyze the advantages and disadvantages of the different analysis methods used by laboratories.

Macroscopic Examination of Hairs

The most basic technique for analyzing hairs is the macroscopic, or visual, examination. This type of examination takes into account the physical aspects of hair, such as color, length, and convolution. Visual examinations are often implemented by crime laboratories as an inexpensive, quick, and nondestructive screening tool to

determine the similarity or dissimilarity of evidentiary hairs as compared to exemplar hairs. The comparison only takes a few minutes, and the only cost to the laboratory is labor—no reagents or consumables are necessary.

In some cases, color, length, and convolution may be enough to differentiate obviously foreign hairs. An example of this would be when a black hair was found in the pubic hair combings of a blond-haired individual. However, this type of examination can be quite subjective due to the limited number of characteristics that can be differentiated by the naked eye. In addition, several aspects of pubic and head hairs, such as color and convolution, are not discrete. These characteristics are often shared by many individuals, in particular those individuals of similar ethnic backgrounds. For example, African-American individuals regularly have curly and kinky hairs that are heavily pigmented, while Asian individuals have relatively straight, coarse, and heavily pigmented hairs (Deedrick, 2000). The subjective nature of this technique introduces the potential for an incorrect assessment.

Soules *et al.* (1978) first evaluated the transfer of pubic hair following a sexual assault as well as the persistence of acid phosphatase (abundant in semen) and spermatozoa. In this study, pubic hair combings were collected from each of fifteen couples following one episode of sexual intercourse, resulting in no visual observation of hair transfer to the fifteen females. The hairs collected from the female participants were simply examined at the macroscopic level. In addition, time of collection since intercourse, and general activity (showering or exercising) after intercourse were not evaluated, factors that may explain the presence or absence of foreign hairs. Finally,

transference to the male subjects was not measured, which is equally important in establishing contact between two individuals.

Keating (1982) also addressed the subject of cross transference of pubic hairs during sexual intercourse. His research evaluated hair transfer occurring between one couple during twenty occasions of sexual intercourse. A total of forty pubic hair combings were collected, twenty from the female and twenty from the male. Of these forty samples collected, Keating determined that pubic hair was transferred 22.5%, or 9 out of 40 times, exclusively from the male to the female subject. In this evaluation, the pubic hairs collected after intercourse and a set of standard hairs were compared based on macroscopic characteristics. Microscopic techniques were only implemented to identify the growth phase of the root, and no microscopic comparisons were made. The author concluded that, while pubic hair transfer does occur, the results obtained from only one couple were not a basis for meaningful conclusions. Further, like research by Soules *et al.* (1978), post-coital physical activity and time of collection since intercourse were not considered.

The results of these two studies show conflicting frequencies of pubic hair transfer. In the first study, there was no instance of transfer, and in the second study several foreign hairs were identified. This contradiction could be real, or might be due to a limited power of discrimination provided by a macroscopic examination. For instance, if the single couple in the second study had notably different hair qualities, it may have been easier to identify foreign hairs than in the first study involving fifteen couples. Since this type of examination is not highly discriminatory, further testing using

microscopic or molecular analyses could offer additional information as to the source of the hair.

Microscopic Examination of Hairs

The first reported forensic investigation of human hair using microscopy was performed by Rudolf Virchow, a professor and prosecutor of the Dead House of the Berlin Charité Hospital (Inman and Rudin, 2001). Virchow determined through a microscopic comparison that hairs found on a victim originated from the defendant. Microscopic examination of hairs can establish potential hair associations or exclusions based on features of the cuticle, medulla, cortex, and root. Hairs are generally not damaged during microscopic analysis, allowing for a subsequent examination at the molecular level. An analyst can also identify phenotypic, or physical, characteristics of hairs under the microscope, such as damage and color treatment. Trace evidence adhering to hairs, including glass fragments or blood, can also be collected during the examination. Root material is needed for successful nuclear DNA typing, thus a microscopic characterization of root morphology and stage of growth can provide insight as to the likelihood of obtaining a nuclear DNA profile. Furthermore, the microscopic analysis can potentially differentiate siblings or other maternally related individuals who share mitochondrial DNA sequences (see below).

There are several disadvantages to a microscopic examination. The analysis can be very time intensive, cannot provide a positive identification or evidence that can stand alone, necessitates a sufficient exemplar sample set for comparison, and requires extensive staff training and experience. In addition, the hair examiners cannot provide a statistical likelihood that a hair came from a certain individual and not another (Smith and

Linch, 1999). The ability to differentiate hairs is largely dependent on the training and experience of an analyst, but false inclusions and false exclusions can occur regardless. The nature of hairs collected could also hinder a comprehensive examination (Hicks, 1977). Some hairs may be extremely featureless and exhibit little pigmentation, texture, or other details. Other hairs may have an abundance of features that show extreme variation and show similarities to a wide range of compared hairs. In addition, a very heavy accumulation of pigment can make it impossible to discern other features of hairs. Furthermore, because this type of analysis is somewhat subjective and rarely yields a definitive finding, many laboratories are not willing to commit time and resources towards training hair examiners; a lot of time and effort is necessary for a limited result (Taupin, 2004).

A six-year case study by Mann (1990) reported the occurrences of pubic hair transfer based on 112 nonhomicidal sexual assault cases occurring between January 1983 and December 1988. Hairs collected from victim and suspect pubic hair combings were first examined macroscopically and subsequently at the microscopic level by at least one trained and experienced hair examiner. Pubic hair transfers from suspect to victim were found 4% of the time, far lower than the 22.5% rate observed in Keating's casework simulation (1982). However, it is important to remember that only one couple was part of Keating's study, and a single person may not be representative of people in general. It is also possible that the lower rate of occurrence determined from Mann's study correlates with the increased power of discrimination offered by the microscopic examinations utilized in the casework. Mann, like Keating, observed no transfer of pubic hair from female to male.

Exline *et al.* (1998) designed a study in which hairs from pubic hair combings were collected from several individuals immediately following sexual intercourse. These hairs were then compared using standard macroscopic and microscopic techniques to exemplar hairs collected from the participants. Results obtained from 55 instances of sexual intercourse indicated that hairs were transferred 17.3%, or in 19 out of 110 pubic hair combings. Unlike Keating's (1982) and Mann's (1990) studies, transfers from females to males were more prevalent (23.6%, or 13 out of 55) than transfers from males to females (10.9%, or 6 out of 55). Only one instance of simultaneous transfer between partners was observed. An additional finding was that all of the foreign pubic hairs recovered from the combings were identified as being in the catagen (regression phase) or telogen (resting) phase. The author indicated that the contact and forces exercised during sexual intercourse were not sufficient to extract hair in the anagen (active growth) phase from an individual.

Prior to the research performed by Exline *et al.* (1998), studies concerning the transfer frequency of pubic hair were based on forensic casework or limited human subject data, both lacking information on situational variables. Exline *et al.* addressed situational variables such as duration of intercourse, hours between intercourse and sample collection, position of subjects during intercourse, and interval from bathing prior to intercourse; however, no correlation was found between the prevalence of pubic hair transfer and these variables. The authors concluded that their research most likely overestimates the frequency of pubic hair transfer recovery, as optimal collection conditions were implemented. Consequently, the actual frequency of recovery encountered in casework would likely be lower.

Macroscopic and microscopic examinations can be subjective techniques and do not provide positive identification, so it is possible that some of the frequencies reported by Keating (1982), Mann (1990), and Exline *et al.* (1998) are inaccurate. Good examples of the inaccuracies that occur using microscopic hair analysis have been seen in post-conviction testing with DNA analysis. Several suspects previously convicted based on hair examinations have been found to be innocent (Giannelli, 2001; Saferstein, 2004).

Nuclear DNA Analysis of Hairs

The most discriminating method for the analysis of pubic hairs lies at the nuclear level, examining nuclear DNA markers called short tandem repeats (STRs). STR analysis is a valid and reliable tool for the genetic characterization of forensic biological specimens (Moretti *et al.*, 2001) that can be performed in just a few day's time. All individuals, except identical twins, are presumed to have a unique nuclear DNA type, allowing for positive identification. In the United States this uniqueness is frequently determined by examining up to 15 nuclear DNA markers (STR loci) and comparing the frequency of the observed ratios to a database of random individuals of the same ethnicity. The likelihood of two people sharing the same STR profile by chance can be one in several trillion, far exceeding the number of people on Earth, functionally individualizing the biological evidence.

While this technique can be highly effective in differentiating hairs, it does have some disadvantages. Nuclear DNA analysis is somewhat expensive, costing at least $75 per sample. In addition, a portion of the sample used for nuclear DNA typing will be consumed, possibly eliminating the opportunity for subsequent retesting. Furthermore, nuclear DNA profiles cannot be obtained from the shaft portion of a hair. Nuclear DNA

analysis requires that the hairs have root material present and in suitable condition. Only roots in certain stages of growth will yield nuclear DNA typing results (Linch *et al.*, 1998).

Linch *et al.* (1998) examined the importance of microscopic hair root morphology in selecting hairs for nuclear DNA typing and the likelihood of obtaining DNA typing results from hairs in different stages of growth. The results from this research stressed that hairs in the resting, telogen phase should not be submitted for nuclear DNA typing attempts. Only hairs with anagen/catagen hair bulbs without translucent sheath tissue (often referred to as "plucked" or "pulled" hairs), or telogen clubs with a follicular tag ("shed" or "combed" hairs) are suitable for nuclear DNA typing.

Higuchi, *et al.* (1988) reported that the amount of purified DNA from freshly plucked hairs was 200 ng or less, and from shed hairs was less than 10 ng. These quantities are adequate to develop fifteen-locus STR profiles from pulled hairs and most shed hairs, supporting the assessment made by Linch *et al.* (1998) of hair morphology as a predictor of successful nuclear DNA typing. When the yield obtained from a hair falls below the necessary quantity, or if chemical inhibitors are present, partial DNA profiles (data at less than fifteen loci) may result, greatly lowering the level of discrimination.

Mitochondrial DNA Analysis of Hairs

Genetic information can also be obtained from hairs by examining the mitochondrial genome, which was completely sequenced and published in 1981 by Anderson *et al.* The sequence obtained by Anderson *et al.* (1981) is frequently used as a reference sequence and is referred to as the Anderson sequence. Like nuclear DNA analysis, mtDNA analysis has proven to be a viable technique for human identification

testing (Wilson *et al.*, 1993; Holland and Parsons, 1999). More pertinent to this study, mtDNA analysis has been established as a practical method for the analysis of shed hairs (Wilson *et al.*, 1995). Human mtDNA is an extrachromosomal, circular genome found in the mitochondria of cells consisting of approximately 16,569 base pairs. MtDNA is maternally inherited and exists in hundreds to thousands of copies per cell. Also, due to its unique mode of inheritance, mtDNA does not undergo recombination (Wilson, 1993).

In a forensic examination, when it becomes apparent that a questioned hair does not have suitable root morphology for nuclear DNA analysis, then mtDNA analysis can be an alternative method. Nuclear material is apparently degraded during the keratinization process of the human hair shaft. In contrast, mitochondria can survive the keratinization process and are commonly observed components of the shaft (Linch *et al.*, 2001). Since there are many copies of mtDNA in the hair shaft, only a small portion of hair (~1cm) is necessary to obtain mtDNA typing results (Saferstein, 2004). The remaining portion of the hair can then be retained should further analysis be required.

An additional difference between mtDNA and nuclear DNA lies in their respective modes of inheritance. MtDNA is inherited maternally, while with nuclear DNA one copy from each parent is transmitted to the offspring. The mtDNA sequence for siblings and all their maternal relatives should be identical, barring mutation. This characteristic can be helpful in forensic cases where known maternal relatives can provide references for comparison to missing persons. On the other hand, this aspect of mtDNA prevents positive identification of biological samples, which is possible with nuclear DNA.

In addition to the potential for matching other individuals of the same maternal lineage, matches will also occur to other lineages that either have the same DNA sequence by chance or that have mutated independently to constitute the same mtDNA sequence. Some mtDNA sequences occur more frequently than others, an aspect restricting discriminatory power for common types while increasing discriminatory power for rare or unique types. Holland and Parsons (1999) determined that, on average, two randomly chosen individuals will have the same mtDNA sequence once outs of ~270 times, or 0.37%. It has therefore been important to establish databases to assess the frequency of particular mtDNA sequences. To convey the rarity of a mtDNA type among unrelated individuals, the number of times a particular sequence is observed in a database is assessed. Several databases have been compiled for these purposes, including data from forensic studies (e.g. Budowle *et al.*, 1999) and anonymous profiles contributed by collaborating forensic laboratories (Monson *et al.*, 2002).

Several additional disadvantages of mtDNA typing exist that should be noted. MtDNA analysis is extremely costly for state and local laboratories that are unable to perform mtDNA testing in-house. Outsourcing evidence to mtDNA laboratories can be expensive, much more so than nuclear DNA analysis. MtDNA sequencing can cost up to $1,500 per sample when outsourced to a private laboratory (www.serological.com, 2004). Likewise, it is slightly more labor intensive than nuclear DNA analysis, requiring two amplification-like reactions and several post-amplification processing steps (Wilson *et al.*, 1993, 1995). Finally, facility structure and layout are important when conducting mtDNA sequencing to avoid contamination. Samples such as hairs can have small amounts of DNA, and the potential for contamination is higher for these samples than for

rich sources of DNA. The extremely small quantities of mtDNA in some forensic samples can be overpowered by mtDNA from a second source, including analysts, other evidence, or amplified product. Analysts should always take precautions to prevent contamination; however, when processing samples with low quantities of mtDNA, extra precautions need to be taken. To avoid cross-contamination, extraction and amplification of samples with low quantities of mtDNA should take place in an area that is kept extremely clean and, more importantly, isolated from all samples that may contain large quantities of mtDNA.

There are biological aspects of the mitochondrial genome that need to be considered to ensure that mtDNA typing results are interpreted accurately; in particular to this study, the presence of more than one mtDNA sequence within an individual, known as heteroplasmy. Heteroplasmy has been known to occur in several tissue types from the same individual, or in one tissue type and not another. Calloway *et al.* (2000) found that the frequency of heteroplasmy was highest in muscle tissue as compared to blood, heart tissue, and brain tissue. In addition, heteroplasmy can be present in variable proportions within the same tissue. Sekiguchi *et al.* (2004) found that heteroplasmy can occur at different positions in different hairs from the same individual. Heteroplasmy was observed in 3.75% to 8.75% of the hairs from a single individual and the heteroplasmic nucleotide positions were varied. For example, heteroplasmy was found at position 16291 in one hair from an individual, and at positions 229, 189, and 273 in three additional hairs from the same individual. Most commonly, there is a predominant sequence at the heteroplasmic position; however, heteroplasmy can also occur in equal ratios of the polymorphic base (Calloway *et al.*, 2000; Sekiguchi *et al.*, 2004). Holland

and Parsons (1999) reported rates of heteroplasmy ranging from 2–8%, and rates as high as 11.6% have been more recently described (Calloway *et al.*, 2000). Grzybowski (2000) found 24 heteroplasmic positions in 100 single hair roots obtained from 35 individuals. Heteroplasmy was also detected at up to six positions in one region for a single individual in his research. This research has been criticized by some individuals in the forensic community based on the experimental parameters used, and the frequency of heteroplasmy reported by Grzybowski could be overestimated (Budowle *et al.*, 2002). Due to the various ways in which heteroplasmy can manifest (e.g. present in some tissues and not others, and present in varying ratios within the same tissue) analysis of mtDNA typing data can be complicated, and it is important to consider the various ways heteroplasmy can occur when interpreting results.

Typical mtDNA sequencing performed in a forensic laboratory looks at the majority of the non-coding region, which contains two hypervariable regions, HVI and HVII. These two regions are approximately 1,125 base pairs in length (Holland and Parsons, 1999). The hypervariable regions can be sequenced to potentially differentiate two maternally unrelated individuals based on sequence differences, or polymorphisms.

After mtDNA analysis became generally accepted in the forensic community and the courts, Houck and Budowle (2002) used mtDNA sequencing results to assess the performance of microscopic analyses performed at the FBI laboratory. A total of 170 microscopic hair examinations and their respective mtDNA sequencing results were reviewed. For the microscopic examinations, 80 associations were made, 19 exclusions were made, 37 analyses were inconclusive, and 34 hairs were deemed unsuitable for analysis. MtDNA sequencing resulted in 97 associations, 64 exclusions, three

inconclusive analyses, and six hairs unsuitable for analysis. Of the 80 microscopic associations, nine, or 11% were excluded by mtDNA analysis, demonstrating the discriminating power of mtDNA testing when it follows the microscopic examination. However, had the mtDNA typing been performed first, some samples could have shared haplotypes. In this instance, microscopic examinations could potentially differentiate those samples that could not be differentiated by mtDNA typing. The researchers emphasized that neither of these two methods provides an absolute positive identification. However, combining the two methods can provide the most complete evaluation since they rely of independent types of information, genotypes, or genetic information, and phenotypes, or physical information.

One of the more recent developments in mtDNA testing is designed to simplify analysis and targets a subset of the most polymorphic sites within HVI and HVII using sequence-specific oligonucleotide (SSO) probes. In several studies, SSO hybridization assays have been found to perform well as a substitute for or precursor to mtDNA sequencing. Stoneking *et al.* (1991) first developed an alternative method for screening large numbers of samples using SSO probes. This test consisted of 23 SSO probes spanning nine regions within HVI and HVII. The degree of diversity revealed by this panel of probes was reported as being only slightly less than the diversity revealed by direct mtDNA sequencing. However, this assay required 23 individual hybridization reactions, making this technique extremely time-consuming. Melton *et al.* (2001) improved the previous method, utilizing 21 SSO probes within HVI and HVII, when typing 2,282 individuals from various ethnic groups. This study also indicated that SSO hybridization assays could be satisfactory forensic typing methods, as determined by high

diversity estimates, or the level of variation within ethnic groups. The value for Caucasians (922 individuals) was reported at 0.964, for African-Americans (805 individuals), 0.983, and for Hispanics (555 individuals), 0.998. Melton *et al.* (2001) stressed that SSO typing underestimates the variation present in the entire control region—the diversity measures for SSO assays, while high, will be even higher at the mtDNA sequence level.

An additionally fine-tuned SSO assay, the Linear Array Mitochondrial DNA HVI/HVII Region-Sequence Typing Kit™, developed by Roche Applied Science (Indianapolis, IN), is now commercially available for forensic mtDNA testing. The kit was designed to reduce the cost and time needed for analysis of samples, while maintaining the highest discriminatory power possible with an SSO assay. This technology is based on a reverse dot blot technology, used for a long time in forensics, with the exception that the probes are arranged in a linear fashion on a strip versus being arranged as dots on a strip. Figure 1 illustrates the general foundation for the assay and the detection chemistry used. The assay uses an array of thirty-three SSO probes, which target nineteen polymorphisms within ten regions of HVI and HVII. Figure 2 shows the probe designations in each region and polymorphisms targeted in those regions by the Linear Array™ assay. The sites chosen for this assay were selected to maximize the discriminatory power of the test while minimizing the number of probes. This assay is relatively inexpensive as compared to nuclear DNA typing or mtDNA sequencing, costing approximately $50 per sample, is less labor-intensive than mtDNA sequencing, and takes only 2 hours to process 24 samples post-amplification. This system can be useful for making quick exclusions, or for directing an analysis towards those specimens

Figure 1. Linear Array™ Detection Chemistry

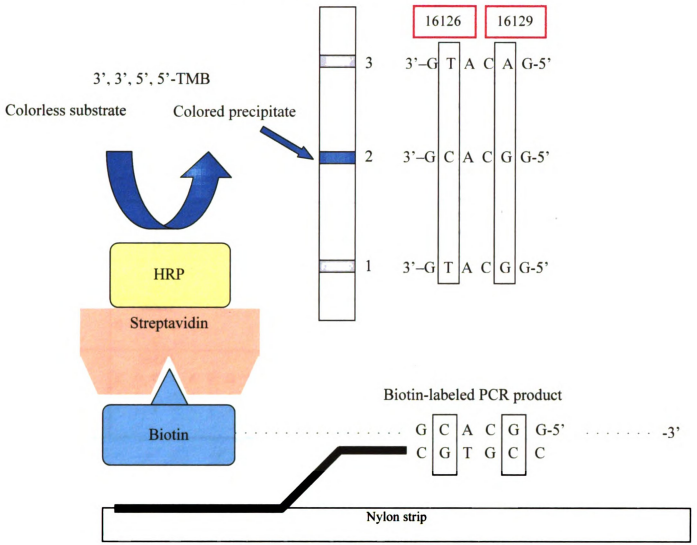


Figure 2. Probe designations and sequence polymorphisms targeted by the Linear Array™ assay

	Probe Designations	Sequence Variation Detected
HV I		**16093**
	16093 1	A T **T** T C
	16093 2	. . **C** . .
		16126 **16129**
	IA1	T G **T** A C **G** G T
	IA2	. . **C** . . . . .
	IA3	. . . . . **A** . .
		16304 **16309** **16311**
	IC1	A G **T** A C A T **A** G **T** A C
	IC2	. . **C** . . . .
	IC3	. . . . **C** . .
	IC4	. . . . **G** . . . .
	ICw2/w3	. . **C** . . . . . . **C** . .
		16362
	ID1	C G **T** C C
	ID2	. . **C** . .
		16270 **16278**
	IE1	C A **C** T A G G A T A **C** C A
	IE2	. . . . . **T** . .
	IE3	. . **T** . . . . .

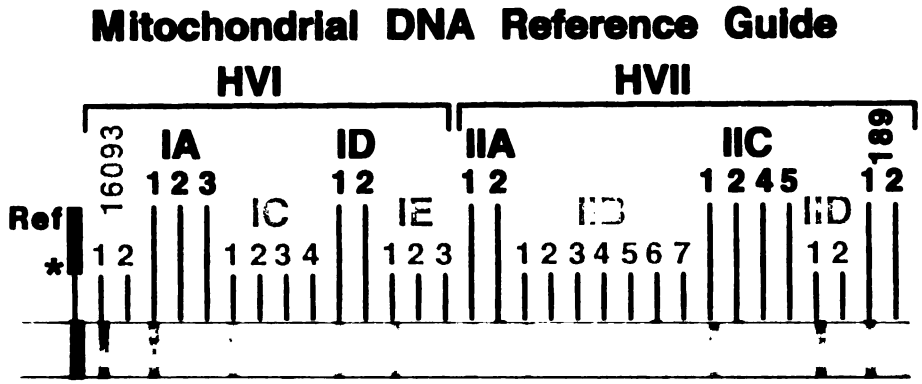
	Probe Designations	Sequence Variation Detected
HV II		**73**
	IIA 1	G T **A** G T
	IIA 2	. . **G** . .
		146 **150** **152**
	IIB1	C C **T** C A T **C** C **T** A T
	IIB2	. . **C** . . . . .
	IIB3	. . . . . **C** . .
	IIB4	. . **C** . . . . **C** . .
	IIB5	. . . . . **T** . . . .
	IIB6	. . . . . **T** . **C** . .
	IIB7	. . **C** . . . **T** . **C** . .
		189 **195** **198** **200**
	IIC1	G A **A** C A T A C **T** T A **C** T **A** A A
	IIC2	. . . . . **C** . . . . .
	IIC4	. . . . . **C** . . **T** . . . .
	IIC5	. . **G** . . . . . **G** . .
		247
	IID1	T T **G** A A
	IID2	. . **A** . .
		189
	189 1	G A **A** C A
	189 2	. . **G** . .

that possess the greatest evidentiary value, especially when there are large numbers of evidentiary samples to examine.

When using normal mtDNA sequencing, the DNA sequence is termed that individual's haplotype. However, since the Linear Array™ only targets a small subset of sequences within HVI and HVII, the result will be referred to as a Linear Array™ type. To determine a particular Linear Array™ type, the banding pattern on a strip is compared to a reference guide provided with the product. Numbers on the guide refer to the sequence variations detected in the region. For example, a band in the IA region can be assigned a '1', a '2', or '3'. A '1' represents a 'T' at DNA position 16126 and a 'G' at 16129, a '2' represents a 'C' at 16126 and a 'G' at 16129, and a '3' means that there is a 'T' at 16126 and an 'A' at 16129. Each of the ten regions is scored in this manner and the overall Linear Array™ type is represented by listing the ten numbers consecutively. For example, in all regions, the probe '1' sequence corresponds to the Anderson sequence, and the Linear Array ™ typing result is designated as '1111111111'. Figure 3 shows how the sequence can be determined for a sample from the bands detected on a strip. The manufacturer has used the term 'probe signals' interchangeably with 'bands'.

Using the Linear Array mtDNA Typing Kit™, Reynolds *et al.* (2000) conducted a study of 689 individuals from four ethnic groups (200 African American, 200 U.S. Caucasian, 200 U.S. Hispanic, and 89 Japanese) and established genetic diversity values of 0.993 for African-Americans, 0.9768 for U.S. Caucasians, 0.9449 for U.S. Hispanics, and 0.9806 for Japanese. Reynolds *et al.* (2000) reported that the most common Linear Array™ type in the Caucasian group occurred in 10.5% of individuals. The most common Linear Array™ type in the African-American group occurred in 8% of

Figure 3. Sequence determination using the Linear Array™ assay (HVI sequence determination shown, HVII sequence determinations not shown)



16093	IA	IC	ID	IE	IIA	IIB	IIC	IID	189
1	1	1	1	1	1	1	1	1	1

	Probe Designations	Sequence Variation Detected
HVI	16093 1 16093 2	16093 A T T T C • • C • •
	IA1 IA2 IA3	16126 16129 T G T A C G G T • • C • • • • • • • • • • A • •
	IC1 IC2 IC3 IC4 ICw2/w3	16304 16309 16311 A G T A C A T A G T A C • • C • • • • • • • • • • • • • • • • • • C • • • • • • • • • G • • • • • • C • • • • • • C • •
	ID1 ID2	16362 C G T C C • • C • •
	IE1 IE2 IE3	16270 16278 C A C T A G G A T A C C A • • • • • • • • • • T • • • • T • • • • • • • • • •

(Linear Array™ Package Insert, 2003)

individuals, and was unique to that group. The most frequently occurring Linear Array™ type within a distinct group was observed in Japanese individuals, at 15.7%. Of the 689 individuals typed, five, or 0.7%, had detectable heteroplasmy, or the occurrence of more than one band in a region on these strips.

Forensic studies performed by the National Institute for Standards and Technology (NIST) using the Linear Array mtDNA Typing Kit™ (Kline *et al.*, 2004) shadowed the work done by Reynolds *et al.* (2000), with a population size of 666 individuals (266 Caucasians, 252 African-Americans, and 128 Hispanics). Genetic diversity values of 0.0960 for Caucasians, 0.977 for African-Americans, and 0.954 for Hispanics were reported. Of the 666 individuals in this study, seven, or 1%, showed an instance of heteroplasmy.

The Linear Array mtDNA Typing Kit™ has also been used for the identification of eighteen human skeletal remains from mass graves in Croatia. The Linear Array™ types from these remains were then compared to a database of Linear Array™ types of 105 Croatian individuals and a set of four putative maternal references (Gabriel *et al.*, 2001 and 2003). In this database, fifty different Linear Array™ types were identified, 33 of which were unique. The most frequent types occurred 18 times, or 17.1% and 11 times, or 10.5%; all other profiles occurred 5% or less. The corresponding genetic diversity value for this database was 0.952. Results were obtained for fourteen out of the eighteen bone samples, all with unique Linear Array™ types. One of the bone samples and one reference from a putative mother gave a preliminary match with the Linear Array™ kit which could not be further differentiated using direct sequencing. Further, the Linear Array™ type shared by these two individuals was unique in the database of

105 Croatian individuals. Three out of the 105 individuals (2.9%) in the database showed heteroplasmy, all at different positions.

Regardless of the method of testing utilized, sequencing, or SSO typing, mtDNA is not a unique identifier. However, attempts have recently been made to reach the maximum discriminating power through examination of single nucleotide polymorphisms (SNPs) in the mtDNA coding region in conjunction with sequences found in the mtDNA control region (Brandstätter *et al.,* 2004; Branicki *et al.*, 2004, Coble *et al.*, 2004; Coene *et al.,* 2004; Vallone *et al.*, 2004). The SNPs selected in these studies showed a high degree of variation among individuals and were not linked to any genetic diseases or phenotype. Overall, the results of these studies show that the addition of these positions expectedly increases the discriminating power of mtDNA analysis and differentiates several sequences that remained unresolved using standard mtDNA typing procedures. For instance, Coble *et al.* (2004) utilized 8 panels of SNPS, reducing the frequency of the most common type in the European Caucasian group from 7% to 2% and the increasing eighteen most common types to 105 different types, 55 of which were seen only once. The utility of markers outside the control region will not be addressed in this study but may provide additional options for increasing discriminatory power in future studies.

Research Goals

The goal of this research was to establish a logical and strategic manner to assess pubic hairs considering such variables as cost, time of analysis, facility structure, and discriminating power. Unlike previous research where the methods were assessed individually or in conjunction with one other type of analysis, this study investigated four different examinations (macroscopic evaluations, microscopic comparisons, nuclear

DNA typing, and mtDNA typing using a SSO assay) that are used in the forensic community. By identifying the advantages and disadvantages of each method, and the success associated with these methods, time and money spent on analysis would be minimized while maximizing the efficiency and overall evidentiary value of examinations.

Materials and Methods

Summary of Samples

Fifty participants were recruited to donate three types of specimens each: pubic hair combings, pubic hair standards (cuttings), and reference buccal swabs. A majority of participants were not related; however, one mother and her son, one mother and her two daughters, and one set of fraternal twins donated samples for this study. Pubic hair standards were collected by cutting at least fifteen pubic hairs from the root end at skin level. Pubic hair combings were obtained by combing or gently pulling hairs from the pubic region, collecting at least five hairs. Two reference buccal swabs were also collected by rubbing the inside of the cheek with sterile swabs. In addition to specimen collection, the participants provided information on their age, sex, and ethnicity. The identity of the donor and their personal information were kept confidential throughout the course of this study. This research was approved by the University Committee for Research Involving Human Subjects (UCRIHS), and all volunteers agreed to participate by signing an informed consent document approved by this committee.

After sample collection was completed, fifty sample sets were prepared, intended to simulate an actual case in which a reference buccal swab, pubic hair standards and a questioned hair from pubic combings were collected from a victim or suspect following a sexual assault. Sample set preparation was performed by an individual uninvolved in the analyses or characterization of the hairs, thus making the study a blind test. Each sample set included pubic hair standards, two reference buccal swabs, and one hair from the pubic hair combings. Information regarding sample set preparation was recorded in a master key, which was undisclosed until all samples were analyzed. The reference pubic

hairs and reference buccal swab in a sample set always originated from the same individual. Twenty-five sample sets were prepared such that the pubic hair from the combings came from the same individual as the reference standards, and the remaining twenty-five sample sets were prepared such that the pubic hair from the combings originated from a different individual than the reference standards. These will be referred to as 'same source sets', or 'matching sets', and 'different source sets', or 'non-matching sets'.

Macroscopic Examinations

Ten analysts in the serology/DNA unit of the Santa Clara County District Attorney's Crime Laboratory participated in the macroscopic examination of hairs in the sample sets. Each analyst was asked to examine the hairs in all 50 sample sets as they would for a typical sexual assault case, looking at such macroscopic characteristics as color, length, and convolution. Based on the observed characteristics of the hairs, a determination as to the macroscopic similarity or dissimilarity of the questioned hair and exemplar hairs was then made and recorded. The analysts were instructed to make a definitive conclusion and to avoid reporting inconclusive results.

Microscopic Examinations

Following the macroscopic examinations, four trained and experienced hair examiners were asked to characterize a subset of the fifty sample sets using microscopy. Due to the labor-intensive process of a microscopic hair examination and the limited number of experienced hair examiners available, only eighteen sets were selected for this portion of the study. Nine sample sets in which the pubic hair combings and standards were from the same donor and nine sample sets in which the reference and questioned

hairs were from different sources were chosen. Three examiners looked at four sets and the fourth examiner looked at six sets. Each analyst received an equal number of same source sets and different source sets. The hair examiners chosen had a wide range of experience levels, ranging from two years to over twenty years. The similarity or dissimilarity of the questioned and exemplar hairs in the sample sets was unknown to all participants, as they were selected and prepared by an individual uninvolved in the analyses or comparison of the hairs.

Each analyst was asked to microscopically characterize the hairs as they would for a typical criminal case. All hairs to be examined were mounted on glass slides using Permount mounting medium, ensuring that the entire length of the hair, proximal to distal end, was under the coverslip and uninterrupted by bubbles or other artifacts. The hairs were subsequently examined using a comparison microscope, consisting of two compound light microscopes connected by an optical bridge. This type of microscope allows the analyst to view exemplar and questioned hairs simultaneously. During the microscopic examinations, characteristics of the cuticle, medulla, cortex, and root (if present) were noted. A range of characteristics was first established for the pubic hair standards, or the upper and lower limits of variation of a particular hair characteristic (Ogle and Fox, 1999). After this was determined, the single pubic hair from the combing was examined alongside the standards and specific internal and external morphologies were noted. Finally, a determination as to the similarity or dissimilarity of the two types of hairs in the set was made and recorded. The analysts were instructed to report their results as they would for casework.

The results of the microscopic examination generally fall into three categories: associations, inconclusive results, or exclusions.

- Association: the questioned hair was determined to exhibit the same microscopic characteristics as the known hair samples. Given this result, the questioned hair cannot be excluded and could have originated from the person who supplied the known reference.
- Inconclusive: the questioned hair may exhibit similarities to the known hair samples, but unexplainable differences also are observed. In this instance, no conclusion can be drawn about the origin of the hair. Inconclusive results may result from additional variables, including a limited exemplar set, or when the hairs to be examined are extremely featureless.
- Exclusion: the questioned hair was determined to be dissimilar to the known hairs and therefore could not be associated with the person who supplied the reference hairs.

DNA Extraction

One half of a buccal swab from each set was excised into a sterile 1.5 μL microcentrifuge tube containing 500 μL of digestion buffer (10mM Tris-HCl, 10 mM EDTA, 50 mM NaCl, 2% SDS, pH 7.5) and 15 μL of 10 mg/mL proteinase K (Gibco BRL® Life Technologies, Gaithersburg, MD). The fifty reference buccal samples were separated into four extraction sets (three sets of thirteen samples and one set of eleven samples), each with a reagent blank control. Each sample was incubated at 56°C for 2–20 hours. After incubation, 500 μL of phenol:chloroform:isoamyl alcohol (25:24:1, biotech grade) (Shelton Scientific, Inc. Shelton, CT) was then added to each sample. The tubes were vortexed and centrifuged at 7,500 rpm for five minutes. The aqueous layer was

removed and transferred to a Centricon® Centrifugal Filter Device (Millipore Corporation, Bedford, MA) with a 1.5 mL of TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) and centrifuged at 2,800 rpms for fifteen minutes. The DNA in the Centricon® was then washed with 3 mL of TE buffer and centrifuged two more times. After the third wash step, the Centricon® devices were inverted and centrifuged at 1,800 rpms for two minutes to collect the purified DNA. The volume of each retentate was measured and then transferred to sterile 0.6 mL microcentrifuge tubes. To achieve the recommended target ranges of input DNA for subsequent steps, a 1/10 dilution was made from each reference sample using TE buffer as the diluent, as recommended by laboratory protocol. All DNA samples were stored at -20°C.

The 32 hairs that were not microscopically examined were extracted following the macroscopic examinations. The single hair representing the pubic hair combing from each of these sets was rinsed thoroughly with sterile water and then blotted dry with a sterile lab wipe. The 18 hairs, which were microscopically examined, had been mounted under a coverslip on a glass slide using Permount. Several drops of xylene were placed on the edges of the slide until the coverslip could be removed without force. The hairs were rinsed with xylene, then with sterile water, and finally blotted dry with a sterile lab wipe.

Approximately one centimeter from the root end of each questioned hair was cut and transferred to a sterile 1.5 mL microcentrifuge tube containing 500 µL of digestion buffer, 15 µL of 10 mg/mL proteinase K, and 20 µL of 1M DTT (Shelton Scientific, Inc.). The fifty hairs were separated into five extraction sets (10 hairs per set), each with a reagent blank control. Hair samples were incubated at 56°C overnight. DNA isolation

and purification proceeded as described above. A dilution was not made from the recovered hair samples due to the low level of DNA expected. These samples, like the reference samples, were stored at -20°C.

DNA Quantitation Using QuantiBlot®

The QuantiBlot® Human DNA Quantitation Kit (Applied Biosystems, Foster City, CA) was used to determine the quantity of nuclear DNA recovered from each sample. All steps were followed as described in the QuantiBlot® kit protocol, and the colorimetric detection method was used. One microliter of each reference sample, diluted 1/10, 1 μL of each undiluted hair sample, and 1 μL of each undiluted reagent blank control was added to a Biodyne B membrane (Gibco BRL® Life Technologies). Following hybridization and color development, the DNA concentration of each sample was determined by comparing the intensity of the sample band to a set of bands produced using human standards (Human Genomic DNA Standard, 240.4 ng/μL, Promega Corporation, Madison, WI) ranging from 10 ng down to 0.15625 ng.

Amplification of Nuclear DNA

Samples and controls were amplified using the AmpF*l*STR® Identifiler™ Amplification Kit (Applied Biosystems) using a 25 μL volume reaction. This multiplex reaction co-amplifies fifteen STR loci (CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, THO1, TPOX, and vWA) and amelogenin, a gender-typing locus. All samples were amplified in 0.5 mL thin-walled tubes (Applied Biosystems). Each reaction consisted of 10 μL Reaction Mix (provided in the Identifiler™ kit), 5 μL of locus-specific dye-labeled and unlabeled primers, 0.5 μL AmpliTaq Gold® DNA Polymerase (5 U/μL), and 0.75 ng to 1.0 ng of

total nuclear input DNA. The volume was raised to 25 µL with TE buffer. If the nuclear DNA concentration was unknown, as was the case for 36 hairs, 10 µL of the sample were added. A positive control, AmpF*l*STR® Control DNA 9947A (provided in the Identifiler™ kit), was included with each amplification set at a concentration of 0.8 ng, as was a negative control (no added DNA). GeneAmp® PCR System 9700 thermal cyclers (Applied Biosystems) were used to amplify the DNA. The amplification parameters employed were as follows: activation at 95°C for 11 minutes, followed by 28 cycles of denaturation at 94°C for 1 minute, annealing at 59°C for 1 minute, and extension at 72°C for 1 minute. The last cycle was followed by a final extension at 60°C for 90 minutes. An infinite hold at 10°C was implemented for those samples that would remain in the thermal cycler for extended periods of time. The reference samples were amplified in two sets, and the hairs in four sets. Following amplification, all samples were stored at -20°C.

Amplification of mtDNA

DNA was amplified with biotinylated primers to generate two biotinylated PCR products ~444 bp and ~416 bp in size using the Linear Array™ Mitochondrial DNA HVI/HVII Region-Sequence Typing Kit (Roche Applied Science, Indianapolis, IN). All samples were amplified using a 50 µL reaction in 0.5 mL thin-walled tubes. Each reaction consisted of 20 µL Reaction Mix (AmpliTaq Gold® DNA Polymerase, PCR Buffer II, $MgCl_2$, dATP, dGTP, dCTP, and dTTP), 10 µL Primer Mix (HVI primers: F15975-93B, R16418-01B, HVII primers: F15-34B, R429-10B), and up to 20 µL of sample, targeting 5 to 10 pg of nuclear DNA, not to exceed 100 pg. Dilutions were made using TE buffer as the diluent to fall in this target range, as recommended by the manufacturer. If the nuclear DNA concentration was unknown, 10–25% of the remaining

volume was added. The reaction volume was brought up to 50 µL with TE buffer. Ten picograms of a positive control DNA (AmpF*l*STR® Control DNA 9947A, provided in the Identifiler™ kit) was included with each amplification set, as was a negative control (no added DNA). GeneAmp® PCR System 9700 thermal cyclers were used to amplify the DNA. The amplification parameters employed were as follows: activation at 94°C for 14 minutes, followed by 34 cycles of denaturation at 92°C for 15 seconds, annealing at 59°C for 30 seconds, and extension at 72°C for 30 seconds. The last cycle was followed by a final extension at 72°C for 10 minutes. An infinite hold at 4°C was implemented for those samples that would remain in the thermal cycler for extended periods of time. The reference samples were amplified in two sets, and the hairs in four sets. Following amplification, all samples were stored at -20°C.

Nuclear DNA Typing Using Capillary Electrophoresis

Amplified hair samples and corresponding controls were analyzed on the ABI PRISM® 3100 Genetic Analyzer capillary electrophoresis instrument (Applied Biosystems). Performance Optimized Polymer (POP-4) and 1X Genetic Analyzer Buffer (Applied Biosystems) were used during capillary electrophoresis. In a 96-well plate, 1 µL of sample was added to 10 µL of deionized formamide (Amresco, Solon, OH) and 1 µL of LIZ-500™ size standard (Applied Biosystems), which contains DNA fragments ranging from 75 bp to 500 bp in size. An allelic ladder (provided with the Identifiler™ kit) containing the most common alleles for each STR locus was included for genotyping samples. Samples were denatured at 95°C for 3 minutes and snap-cooled at 4°C for approximately 5 minutes. The samples were then injected on the instrument as per the manufacturer's instructions. Samples producing low relative fluorescent units (rfus) were

prepared a second time using 3 uL of amplified product. Samples exhibiting high rfus, split peaks or shoulders, or spectral pull-up, were either diluted or extended in the thermal cycler for an additional 45 minutes at 60°C (to complete nucleotide addition) and diluted as appropriate. Following electrophoresis, all samples were analyzed using GeneScan® version 3.1.2 and Genotyper® version 2.5.2 (Applied Biosystems) for the NT platform. This software characterizes the signals detected by the instrument as a series of peaks on a graph, called an electropherogram. The threshold of detection for peaks was initially set at 150 rfus, and was lowered to 50 rfus where appropriate.

The criteria for positive identification used by the Santa Clara County Crime Laboratory is a likelihood ratio above 1 in 260 billion, meaning that the chance of the results randomly matching another person from the same ethnic group is at least 1 in 260 billion. This positive identification cut-off has also been implemented by the FBI (Budowle, 2000). This is a highly conservative value considering that it is approximately 900 times the number of people living in the U.S., and 40 times the inhabitants on Earth (U.S. Census Bureau, 2004). The likelihood ratio for a fifteen-locus match will always exceed an identification criteria of 1 in 260 billion according to statistical calculations performed by the Santa Clara County Crime Laboratory. When the most common allele frequencies at each of the fifteen loci were included in a frequency calculation, the result exceeded the set identification value of 1 in 260 billion. Likelihood ratios for partial profiles (profiles of fewer than fifteen loci) were calculated using guidelines as stated in the National Research Council's recommendation 4.1 (1996) and allele frequencies provided in the AmpF*l*STR® Profiler Plus™, COfiler™, and Identifiler™ User's Manuals (1997, 1998, and 2001) and by Wraxall (rev. 2002 and 1999). Exclusions were

made when multiple differences existed between the known and questioned sample profiles.

MtDNA Typing Using the Linear Array™ Hybridization Assay

To begin, the temperature of the heated water bath and the pH of reagents were measured to ensure they were within the manufacturer's recommended values. Next, 15 µL microliters of amplified product was added to 15 µL of denaturation solution (0.4 M NaOH, 20 mM EDTA) in a sterile 0.5 mL tube. A positive control (AmpF*l*STR® Control DNA 9947A) and negative control (no added DNA) were included with each set of hybridizations. While samples were denaturing, the Linear Array™ strips were labeled with the appropriate sample number and placed in the 24-well tray. The amplified products were then added and hybridized to the strips. Subsequently, an enzyme conjugate solution (streptavidin-horseradish peroxidase) was added to bind to the biotin-labeled DNA hybridized on the strip, and finally the conjugate was visualized using a colorimetric development technique. The mechanism for detection was illustrated in Figure 1. The bands developed on the strip were then compared to the Linear Array™ mtDNA reference guide provided in the kit, and the sequence determination for nineteen positions was recorded when possible. Four different designations were assigned depending on the banding pattern observed on the strips. When a single band was observed, it was scored with the corresponding number on the guide. Weak signals, appearing lighter than the other bands on the strip, were designated with a 'w' followed by the corresponding number from the guide. When no signal was detected in a particular position, it was scored as '0'. Finally, when more than one band was seen at a certain position, all corresponding numbers on the guide were recorded.

Inclusions were made if the exemplar and questioned samples shared the same Linear Array™ type, and exclusions were made when differences were observed.

Samples with strong probe signals, or non-specific binding, were diluted and hybridized to the strips a second time. Samples with no or very low signals across the entire strip were also hybridized again, with addition of a higher volume of amplified product, or amplified and hybridized a second time. The hybridization assay was repeated for all samples exhibiting two bands in one region. If the same banding pattern was seen the second time, an additional sample was cut from the buccal swab or hair, and the entire process was repeated.

Frequency calculations for the Linear Array™ types were made using the FBI's mtDNA Population Database version 1.2, which is a tool used for forensic comparison purposes (Monson *et al.*, 2002). The Anderson sequence is used as the reference in this database, and only those sequences that differ from this reference are queried. Sequences in a Linear Array™ type that differ from the Anderson sequence were entered, which will include all individuals having the same sequences in these positions, regardless of sequence variation at any other position not tested. All positions having a 'w' or a '0' were queried such that any sequence could be included at that location. For example, the Linear Array™ type '1w101120111' would be entered such that any sequence could be present at positions 16126, 16129, 16304, 16309, 16311, 146, 150, and 152, but a G had to be present at position 73. The forensic database searched had profiles from individuals of varying ethnic origins including Caucasians, African-Americans, Hispanics, Asians, and Native Americans. The total number of profiles in the forensic database was 4,839.

Upon completion of the analyses, a quality control check was performed to ensure that samples were not switched during the course of the study. As described above, twenty-five sets contained samples from the same individual. For these sets, the nuclear DNA results (when available) and mtDNA results from the exemplar and questioned samples were compared to make sure that the profiles matched. In the remaining 25 sample sets where the questioned hair did not originate from the same individual as the exemplar hairs and buccal swab, DNA results from the questioned hair were compared to the correct buccal swab results to check for concordance.

Results

Sample collection

As instructed, all fifty participants collected and returned two buccal swabs, and pubic hair combings and pubic hair standards. Each type of sample was packaged properly in the appropriately labeled plastic bags and packaging sleeves provided. Usually five hairs were present in the pubic hair combings bag, but on one occasion, only one hair was present. Fifteen hairs were included in the most of the pubic hair standards bag, but on at least two occasions there were less than fifteen.

Macroscopic examinations

The results reported by ten analysts examining the hairs in all fifty sample sets were both varied in the accuracy of characterization, as well as in the number of sets characterized as similar or dissimilar from the reference samples. The number of hairs out of fifty described as dissimilar from the known hairs ranged from 4 to 24, and the number reported as consistent with the knowns ranged from 26 to 46. Rates of accuracy were determined at three levels: percent correctly characterized as consistent with the reference, percent correctly characterized as dissimilar from the reference, and total percent correct. Table 1 summarizes these results. Accuracy rates for hairs reported as from the same source varied from 72% to 100%, and for those described as from different sources from 16% to 68%. The totals for all hairs correctly characterized ranged from 48% to 70% correct, with an average accuracy rate of 58%. On average, each screening analyst spent approximately two hours characterizing the fifty sets of hairs, and there were no material costs associated with these examinations.

Table 1. Results of macroscopic examinations

Analyst	Number reported similar	Number reported dissimlar	Number correct from same source	Number correct different sources	% correct from same source	% correct from different sources	% correct total
1	46	4	25	4	100%	16%	58%
2	45	5	24	4	96%	16%	56%
3	43	7	24	6	96%	24%	60%
4	42	8	23	6	92%	12%	58%
5	41	9	20	4	88%	16%	48%
6	40	10	22	7	88%	28%	58%
7	36	14	23	10	92%	40%	62%
8	30	20	17	12	68%	48%	58%
9	28	22	18	15	72%	60%	66%
10	26	24	18	17	72%	68%	70%

Microscopic Examinations

Four qualified hair analysts examined a total of eighteen sets of hairs at the microscopic level. The results of each analyst's findings are represented in Table 2. Analyst #1 spent approximately ten hours examining four sample sets, Analysts #2 and #4 both spent fifteen hours examining four sets, and Analyst #3 spent twenty hours to characterize six sets. On average, it took 3.3 hours to examine the hairs in a single set, and the material cost per microscopic comparison was less than five dollars.

Overall, the analysts were successful in characterizing 72% of sets accurately and incorrectly characterized 28%. Ten associations were made and 80% of these were accurate. The hairs in two non-matching sets were incorrectly associated. Five of the six exclusions reported, or 83%, were correctly differentiated. One incorrect exclusion was reported for a matching set. Inconclusive results were given for two same source sets.

Analyst #1 examined four hair sets, making two correct associations, one correct exclusion, and incorrectly associating the hairs in one set. The overall success rate for accurately discriminating hairs was 75% for this analyst. Analyst #2 had an overall success rate of 50%, reporting one correct association, one correct exclusion, one incorrect association, and one inconclusive result for a matching set. The inconclusive result reported by this analyst was based on a stated inadequate exemplar set of only five hairs. Analyst #3 made two correct associations, two correct exclusions, one incorrect association, and reported one inconclusive result for a same source set. Again, the reason for the inconclusive result was based on lack of an adequate number of exemplar hairs. This analyst was able to correctly identify the source of 66% of the hairs.

Table 2. Summary of Microscopic Examinations

Analyst	Sample set	Questioned hair donor	Exemplar hair donor	Similar or dissimilar	Conclusion	Years of experience	Average length of examination/set (hrs)	Success rate
1	2	31	40	Dissimilar	Association	10	2.5	75%
1	5	20	20	Similar	Association			
1	17	37	37	Similar	Association			
1	36	5	17	Dissimilar	Exclusion			
2	8	24	11	Dissimilar	Association	20	3.75	50%
2	23	35	35	Similar	Inconclusive			
2	30	2	2	Similar	Exclusion			
2	31	38	14	Dissimilar	Association			
3	4	45	6	Dissimilar	Association	2	3.3	66%
3	21	55	55	Similar	Inconclusive			
3	25	16	13	Dissimilar	Exclusion			
3	33	47	47	Similar	Association			
3	37	56	56	Similar	Association			
3	43	14	5	Dissimilar	Exclusion			
4	16	1	1	Similar	Association	15	3.75	100%
4	18	31	32	Dissimilar	Exclusion			
4	40	18	18	Dissimilar	Exclusion			
4	46	13	46	Similar	Association			

Analyst #4 was successful in correctly characterizing all four sets analyzed. This individual reported two associations and two exclusions.

Nuclear DNA Analysis

The nuclear DNA analysis for 50 hairs, 50 references, and controls took almost 80 hours, and cost over $7,500. Therefore, almost one hour and $75 were spent on the analysis of a single sample. Nuclear DNA analysis of an item takes much longer than one hour, but samples are batched together during the process to save time.

Fifteen-locus profiles were obtained from all reference buccal swabs. The DNA profiles were deduced from peaks on a graph, called an electropherogram. All negative amplification controls and reagent blank controls showed no peaks in the electropherograms, and a correct fifteen-locus profile was obtained from all positive controls. Repeating the PCR final extension step and/or diluting was necessary to eliminate shoulder peaks, pull-up, and off-scale peaks present in eight DNA samples from buccal swabs. One DNA sample had to be reinjected using 3 μL of amplified product to obtain a full profile.

Thirty-six hairs, or 72%, did not produce a signal using QuantiBlot®, meaning that the concentration of nuclear DNA present was below the detection limit of this system. Concentrations for the remaining fourteen hairs were estimated from the 0.15625 ng and 1.25 ng standards.

A fifteen-locus DNA profile was obtained for seventeen of the 50 hair samples, or 36%, including eight hairs mounted in Permount prior to DNA analysis. Full DNA profiles were generated for all 14 hairs with quantitation information, and from 3 hairs with negative quantitation results. Partial profiles were obtained from five samples, or

10%. Finally, DNA typing results were not obtained from 28 hairs, or 56%, including ten hairs mounted in Permount prior to DNA testing. Partial or no DNA profiles were obtained those hairs with non-detectable quantitation results.

Comparisons of the STR typing results between the reference buccal swab and hair sample in each set were made (Table 3). Because no comparison could be made between a fifteen-locus profile from an exemplar and questioned sample that gave no results, these twenty-eight sets were deemed inconclusive. Full profiles were obtained from both the buccal swab and hair in seventeen sets. Nine exclusions and eight positive identifications were made in these sets. Comparisons were also made between five hairs showing partial profiles and their corresponding reference samples. Two partial profiles from hairs (one with alleles at 13 loci, and the other with alleles at 11 loci) resulted in exclusion when compared to the reference sample, based on the different alleles present in each profile. The remaining three hairs could not be eliminated as originating from the source of the buccal swab. One of these partial profiles had results at 4 loci (4 alleles total), the second had results at 5 loci (six alleles total), and the third had results at 7 loci (ten alleles total). The alleles detected in the partial profiles from these hairs were also present in the profiles from the corresponding buccal swabs, and were therefore characterized as partial inclusions. The likelihood ratios calculated for the three partial inclusions are as follows:

- Hair sample set 7 (four alleles total), 1 in 2,000 African-Americans, 1 in 140 Caucasians, and 1 in 200 Hispanics
- Hair sample set 20 (ten alleles total), 1 in 9 million African-Americans, 1 in 1 million Caucasians, and 1 in 1.3 million Hispanics

- Hair sample set 32 (six alleles total), 1 in 13,000 African-Americans, 1 in 1,900 Caucasians, and 1 in 5,400 Hispanics

MtDNA Analysis

The mtDNA analysis for 50 hairs, 50 references, and controls took about 60 hours, and cost over $4,500. Therefore, just over 30 minutes and $45 were spent on analysis per sample. MtDNA analysis of an individual item takes much longer than one hour, but as in nuclear DNA analysis, samples are batched together during the process to save time.

Results were obtained from all 100 buccal and hair samples using the Linear Array™ typing kit. A correct result was obtained from all positive controls, and no bands were detected on the strips for all negative controls. A Linear Array™ type for any sample was determined by comparing the bands in each region of the strip to a standard reference guide.

There were four types of banding patterns detected on the strips. Most commonly, single probe signals were observed. Weak signals, lighter than any other bands on the strip, were observed in 13, or 26%, of Linear Array™ types. An example of a weak signal in region IIC is shown in Figure 4. No band was detected in certain positions of the strip for 17, or 34% of Linear Array™ types. The sample in Figure 4 also lacks a band at position 189. Finally, multiple bands in a single region were observed in 6% of samples.

The mtDNA results for the fifty sample sets are shown in Table 4. Examples of inclusions and exclusions can be seen in Figures 4 and 5. Upon comparison of mtDNA results from the reference sample and hair sample in each set, twenty-three correct

Table 3. Summary of STR typing results

Sample No.	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	AMEL	D5S818	FGA	Comments
R1	11, 13	30, 30	8, 9	10, 11	15, 17	6, 7	12, 14	9, 11	17, 24	14, 15	17, 19	9, 10	15, 16	XY	11, 13	23, 24	Inconclusive/
H1	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	no hair data
R2	12, 13	29.2, 30	9, 10	10, 12	16, 17	6, 6	12, 12	11, 11	17, 17	14, 15	17, 19	8, 11	13, 14	XY	10, 11	20, 20	Inconclusive/
H2	inc	inc	inc	inc, 12	inc	inc	inc	NR	NR	NR	NR	NR	NR	NR	NR	NR	no hair data
R3	13, 15	29, 3.2	8, 12	11, 12	15, 16	6, 7	11, 13	9, 11	18, 25	13, 15	17, 19	9, 9	13, 15	XX	11, 13	20, 24	Inconclusive/
H3	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	no hair data
R4	10, 15	31.2, 31.2	9, 12	11, 12	16, 17	7, 9	10, 12	9, 11	17, 25	15, 15	14, 18	8, 12	14, 14	XX	11, 12	23, 23	Exclusion
H4	11, 13	28, 30	8, 10	11, 12	16, 18	7, 9	9, 12	9, 11	17, 25	13, 14	17, 17	8, 8	13, 15	XX	9, 12	22, 23	
R5	13, 16	31, 32	8, 12	10, 11	15, 16	6, 6	9, 11	11, 13	21, 21	13, 16.2	16, 18	8, 9	14, 17	XX	10, 13	21, 26	Inconclusive/
H5	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	no hair data
R6	13, 14	29, 29	10, 10	10, 12	15, 17	7, 9	11, 11	9, 13	17, 23	14, 15.2	14, 17	8, 10	13, 15	XY	9, 9	21, 21	Inconclusive/
H6	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	no hair data
R7	11, 14	29, 32.2	8, 12	10, 11	14, 15	9, 9.3	8, 11	11, 12	20, 23	14, 14	19, 19	8, 10	14, 17	XY	11, 13	20, 21	Inclusion/
H7	11, inc	inc	NR	NR	inc	9, inc	8, inc	12, inc	inc	14, inc	inc	inc	NR	NR	NR	NR	partial profile
R8	12, 14	29, 30.2	12, 13	10, 12	14, 18	6, 6	12, 14	10, 12	17, 24	13, 15	17, 17	8, 8	12, 14	XY	13, 13	21, 22	Inconclusive/
H8	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	no hair data
R9	13, 13	30, 31.2	7, 10	10, 12	16, 17	7, 8	9, 14	11, 12	20, 24	14, 16	14, 17	10, 11	17, 21	XX	12, 12	23, 23	Inconclusive/
H9	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	no hair data
R10	12, 13	28, 29	8, 8	11, 12	14, 17	9.3, 9.3	9, 10	10, 10	23, 25	13, 16	18, 18	10, 11	12, 16	XY	12, 12	23.2, 25	Inconclusive/
H10	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	no hair data
R11	14, 16	30, 31	10, 10	11, 12	15, 18	6, 9.3	8, 9	10, 11	20, 26	13, 14.2	17, 17	8, 11	13, 17	XX	11, 12	24, 24	Exclusion
H11	13, 14	29, 29	10, 10	10, 12	15, 17	7, 9	11, 11	9, 13	17, 23	14, 15.2	14, 17	8, 10	13, 15	XY	9, 9	21, 21	
R12	13, 15	28, 31.2	9, 9	11, 11	16, 17	6, 7	11, 13	12, 12	16, 17	13, 15	17, 17	8, 11	12, 22	XX	11, 12	22, 26	Inconclusive/
H12	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	no hair data
R13	14, 16	30, 31	10, 12	11, 11	17, 17	7, 9.3	11, 11	10, 11	19, 25	13, 14	19, 19	11, 11	14, 14	XX	12, 12	22, 22	Inconclusive/
H13	14, inc	NR	NR	NR	inc	NR	NR	NR	NR	NR	NR	NR	NR	X, inc	NR	NR	no hair data
R14	12, 14	27, 29	8, 10	10, 13	16, 16	8, 9	12, 13	9, 12	20, 22	11, 14	15, 16	9, 9	18, 21	XY	8, 12	23, 23	Inclusion
H14	12, 14	27, 29	8, 10	10, 13	16, 16	8, 9	12, 13	9, 12	20, 22	11, 14	15, 16	9, 9	18, 21	XY	8, 12	23, 23	
R15	10, 13	28, 31.2	9, 10	10, 12	16, 17	9, 9.3	10, 12	9, 10	19, 21	13, 15	15, 15	8, 8	14, 15	XY	11, 11	19, 24	Inconclusive/
H15	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	no hair data
R16	10, 14	30, 30	10, 11	10, 11	15, 18	9, 9.3	9, 12	12, 13	17, 24	13, 16	15, 16	8, 8	14, 15	XY	11, 13	18, 21	Inclusion
H16	10, 14	30, 30	10, 11	10, 11	15, 18	9, 9.3	9, 12	12, 13	17, 24	13, 16	15, 16	8, 8	14, 15	XY	11, 13	18, 21	
R17	11, 12	31, 32.2	9, 10	11, 14	15, 16	8, 9.3	11, 11	11, 12	21, 23	14, 14	17, 20	8, 8	12, 18	XY	9, 11	21, 22	Inclusion
H17	11, 12	31, 32.2	9, 10	11, 14	15, 16	8, 9.3	11, 11	11, 12	21, 23	14, 14	17, 20	8, 8	12, 18	XY	9, 11	21, 22	
R18	13, 15	27, 28	9, 12	11, 12	15, 16	6, 7	12, 13	11, 12	16, 17	15, 15.2	15, 17	8, 11	16, 22	XY	11, 11	22, 25	Inconclusive/
H18	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	no hair data
R19	11, 12	31, 33.2	10, 12	10, 12	15, 18	6, 6	12, 12	11, 12	16, 19	13, 13.2	16, 17	8, 8	12, 16	XY	11, 11	23, 25	Inconclusive/
H19	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	no hair data
R20	11, 14	27, 29	9, 10	10, 11	16, 17	6, 9.3	8, 11	10, 13	17, 18	14, 17	16, 17	8, 8	12, 17	XY	11, 12	21, 24	Inclusion/
H20	11, 14	inc	inc	inc	16, inc	6, 9.3	inc	inc	18, inc	14, 17	16, inc	8, inc	inc	NR	NR	NR	partial profile
R21	12, 14	28, 30	12, 14	11, 11	18, 18	7, 7	8, 13	12, 13	19, 24	12, 14	15, 17	8, 9	15, 17	XY	12, 14	18, 26	Inclusion
H21	12, 14	28, 30	12, 14	11, 11	18, 18	7, 7	8, 13	12, 13	19, 24	12, 14	15, 17	8, 9	15, 17	XY	12, 14	18, 26	

NR-no results obtained inc-inconclusive results R-reference sample H-hair sample

Table 3 (continued). Summary of STR typing results

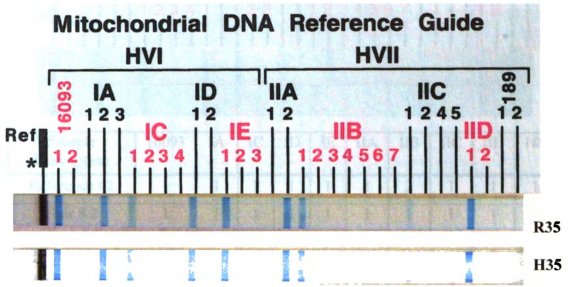
Sample No.	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	AMEL	D5S818	FGA	Comments
R22	14, 14	28, 30	9, 10	12, 12	17, 18	9.3, 9.3	8, 12	11, 12	23, 24	13.2, 14	16, 16	8, 11	17, 17	XX	12, 13	23, 24	Inconclusive/
H22	NR	NR	NR	NR	NR	NR	inc	13, inc	NR	NR	NR	NR	NR	NR	NR	NR	no hair data
R23	13, 14	29, 30	9, 10	11, 11	14, 16	8, 9	8, 9	9, 13	19, 19	13, 14	16, 17	8, 9	12, 15	XY	11, 12	20, 24	Inconclusive/
H23	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	no hair data
R24	10, 13	30, 30	10, 12	10, 11	15, 15	6, 9.3	11, 12	11, 12	18, 27	13, 15	16, 18	11, 11	13, 14	XX	12, 12	22, 23	Exclusion
H24	14, 14	28, 30	9, 10	12, 12	17, 18	9.3, 9.3	8, 12	11, 12	23, 24	13.2, 14	16, 16	8, 11	17, 17	XX	12, 13	23, 24	
R25	13, 15	27, 30	9, 9	12, 12	15, 17	6, 7	13, 13	11, 12	16, 18	14, 15	15, 17	8, 8	12, 22	XX	11, 13	22, 26	Exclusion/
H25	14, 14	29, 30	10, inc	10, 12	14, 15	6, 7	12, 13	10, 12	16, 21	13, 14	15, 18	8, 11	13, 17	inc	11, 12	inc	partial profile
R26	12, 13	28, 29	8, 10	10, 11	16, 16	8, 9.3	8, 11	11, 12	20, 24	14, 14	14, 17	8, 8	13, 21	XX	11, 12	19, 20	Exclusion
H26	14, 15	29, 30	10, 10	11, 11	16, 18	6, 9	12, 12	9, 12	18, 20	14, 14.2	15, 17	8, 9	14, 19	XX	11, 11	22, 24	
R27	11, 13	28, 30	8, 10	11, 12	16, 18	7, 9	9, 12	9, 11	17, 25	13, 14	17, 17	8, 8	13, 15	XX	9, 12	22, 23	Exclusion
H27	11, 13	31.2, 33.2	10, 12	11, 12	17, 17	6, 7	9, 12	9, 12	23, 24	15.2, 15.2	14, 16	8, 8	18, 19	XX	10, 11	18, 22	
R28	11, 14	29, 31.2	9, 11	11, 12	14, 18	6, 8	12, 14	13, 13	23, 25	10.2, 14	15, 16	9, 11	12, 17	XX	12, 13	20, 24	Inconclusive/
H28	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	no hair data
R29	12, 15	30, 30	9, 11	11, 12	15, 15	6, 9.3	11, 13	11, 13	17, 17	15, 15	16, 17	8, 10	14, 17	XX	11, 13	22, 24	Inconclusive/
H29	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	no hair data
R30	13, 15	29, 33.2	11, 12	12, 12	14, 17	7, 8	11, 12	9, 12	17, 17	16, 16.2	17, 18	8, 8	12, 14	XX	11, 12	25, 27	Inconclusive/
H30	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	no hair data
R31	13, 13	30, 31.2	9, 10	11, 12	15, 17	6, 9.3	11, 13	12, 12	17, 18	13, 14	17, 18	8, 8	12, 14	XX	12, 13	22, 26	Exclsuion/
H31	13, 14	30, 33.2	11, 12	11, inc	17, 18	7, 9.3	9, 12	9, 10	20, 25	13, 14.2	18, inc	8, 8	13, 14	inc	12, inc	inc	partial profile
R32	12, 13	28, 30	9, 10	12, 13	14, 15	6, 7	12, 12	11, 13	17, 24	14, 16	14, 19	8, 11	12, 17	XY	11, 12	22, 23	Inclusion/
H32	12, 13	inc	inc	inc	inc	6, inc	12, inc	inc	NR	16, inc	19, inc	NR	NR	NR	NR	NR	partial profile
R33	12, 13	28, 32.2	9, 11	12, 14	15, 16	7, 9.3	11, 12	9, 12	17, 18	14, 14	16, 20	8, 11	14, 14	XX	7, 11	19, 25	Inclusion
H33	12, 13	28, 32.2	9, 11	12, 14	15, 16	7, 9.3	11, 12	9, 12	17, 18	14, 14	16, 20	8, 11	14, 14	XX	7, 11	19, 25	
R34	11, 14	27, 29	9, 12	10, 12	14, 16	9, 9	11, 12	11, 12	19, 23	14, 15	16, 19	8, 11	14, 17	XX	11, 11	21, 24	Inclusion
H34	11, 14	27, 29	9, 12	10, 12	14, 16	9, 9	11, 12	11, 12	19, 23	14, 15	16, 19	8, 11	14, 17	XX	11, 11	21, 24	
R35	13, 15	31, 31.2	8, 11	10, 11	14, 18	6, 6	10, 12	11, 11	16, 18	14, 15	17, 18	8, 11	12, 15	XX	13, 13	21, 23	Inclusion
H35	13, 15	31, 31.2	8, 11	10, 11	14, 18	6, 6	10, 12	11, 11	16, 18	14, 15	17, 18	8, 11	12, 15	XX	13, 13	21, 23	
R36	12, 13	28, 29	10, 10	8, 11	15, 18	7, 9.3	8, 13	12, 12	20, 23	13, 13.2	16, 16	8, 11	15, 15	XX	11, 12	21, 23	Inconclusive/
H36	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	no hair data
R37	12, 13	29, 31	8, 8	12, 12	15, 18	8, 9.3	11, 13	11, 12	18, 18	13, 14	15, 18	8, 8	15, 17	XY	10, 13	22, 23	Inconclusive/
H37	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	no hair data
R38	11, 13	28, 29	8, 12	10, 11	16, 18	9.3, 9.3	10, 11	12, 13	16, 17	14, 16	16, 16	8, 8	14, 15	XY	11, 12	22, 25	Inclusion
H38	11, 13	28, 29	8, 12	10, 11	16, 18	9.3, 9.3	10, 11	12, 13	16, 17	14, 16	16, 16	8, 8	14, 15	XY	11, 12	22, 25	
R39	11, 11	30.2, 32.2	11, 11	12, 14	15, 18	9, 9	8, 12	9, 13	18, 18	13, 15.2	17, 18	8, 8	13, 13	XX	11, 13	22, 24	Inconclusive/
H39	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	no hair data
R40	14, 15	29, 32.2	10, 10	11, 12	16, 19	8, 9.3	12, 14	9, 12	19, 25	13, 16.2	16, 16	8, 9	12, 14	XY	12, 12	20, 21	Inclusion
H40	14, 15	29, 32.2	10, 10	11, 12	16, 19	8, 9.3	12, 14	9, 12	19, 25	13, 16.2	16, 16	8, 9	12, 14	XY	12, 12	20, 21	
R41	10, 16	29, 30.3	11, 13	10, 11	16, 16	7, 9	11, 11	11, 12	18, 18	13, 16	16, 17	8, 8	14, 16	XY	11, 13	20, 21	Inconclusive/
H41	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	no hair data
R42	14, 14	30, 30	10, 11	11, 11	15, 18	8, 9.3	9, 11	10, 11	17, 23	14, 14	15, 16	8, 10	12, 19	XY	12, 12	21, 28	Inconclusive/
H42	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	no hair data
R43	13, 14	30, 33.2	11, 12	11, 12	17, 18	7, 9.3	9, 12	9, 10	20, 25	13, 14.2	18, 19	8, 8	13, 14	XY	12, 12	22, 26	Exclusion
H43	12, 13	28, 29	10, 10	8, 11	15, 18	7, 9.3	8, 13	12, 12	20, 23	13, 13.2	16, 16	8, 11	15, 15	XX	11, 12	21, 23	

NR-no results obtained inc-inconclusive results R-reference sample H-hair sample

Table 3 (continued). Summary of STR typing results

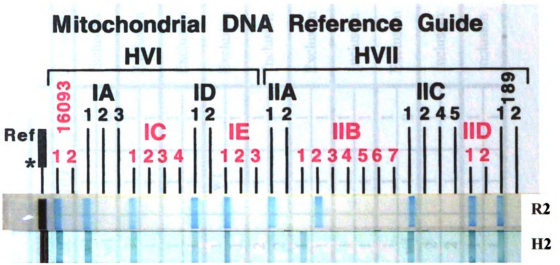
Sample No.	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	AMEL	D5S818	FGA	Comments
R44	11, 13	31.2, 33.2	10, 12	11, 12	17, 17	6, 7	9, 12	9, 12	23, 24	15.2, 15.2	14, 16	8, 8	18, 19	XX	10, 11	18, 22	Exclusion
H44	10, 15	31.2, 31.2	9, 12	11, 12	16, 17	7, 9	10, 12	9, 11	17, 25	15, 15	14, 18	8, 12	14, 14	XX	11, 12	23, 23	
R45	14, 15	29, 30	10, 10	11, 11	16, 18	6, 9	12, 12	9, 12	18, 20	14, 14.2	15, 17	8, 9	14, 19	XX	11, 11	22, 24	Exclusion
H45	13, 13	30, 31.2	9, 10	11, 12	16, 17	6, 9.3	11, 13	12, 12	17, 18	13, 14	17, 19	8, 8	12, 14	XX	12, 13	22, 26	
R46	14, 14	29, 30	8, 10	10, 12	14, 15	6, 7	12, 13	10, 12	16, 21	13, 14	15, 18	8, 11	13, 17	XY	11, 12	20, 21	Inconclusive/ no hair data
H46	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	
R47	12, 13	28, 30	11, 12	10, 12	15, 15	8, 9.3	11, 12	12, 13	17, 25	13, 13	17, 18	8, 8	14, 14	XX	9, 12	24, 24	Inconclusive/ no hair data
H47	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	
R48	14, 14	32.2, 32.2	8, 10	10, 11	15, 17	7, 9.3	8, 11	12, 13	20, 23	14, 14	17, 19	9, 10	14, 18	XY	11, 13	19, 20	Inconclusive/ no hair data
H48	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	
R49	11, 13	28, 31.2	8, 9	10, 10	15, 18	6, 6	12, 14	11, 12	17, 19	14, 15	17, 18	8, 11	15, 16	XX	11, 13	23, 24	Inconclusive/ no hair data
H49	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	
R50	11, 15	30, 31.2	9, 13	10, 10	14, 18	9, 9.3	8, 14	9, 12	18, 26	14, 14.2	16, 17	8, 8	13, 13	XY	11, 12	22, 22	Exclusion
H50	13, 15	29, 32.2	8, 12	11, 12	15, 16	6, 7	11, 13	9, 11	18, 25	13, 15	17, 19	9, 9	13, 15	XX	11, 13	20, 24	
R ext controls	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Inconclusive/ no hair data
H ext controls	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	
R(+) amp controls	13, 13	30, 30	10, 11	10, 12	14, 15	8, 9.3	11, 11	11, 12	19, 23	14, 15	17, 18	8, 11	12, 15	XX	11, 11	23, 24	Expected type
R (+) amp controls	13, 13	30, 30	10, 11	10, 12	14, 15	8, 9.3	11, 11	11, 12	19, 23	14, 15	17, 18	8, 11	12, 15	XX	11, 11	23, 24	
R(-) amp controls	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Inconclusive/ no hair data
H (-) amp controls	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	

NR-no results obtained inc-inconclusive results R-reference sample H-hair sample ext-extraction amp-amplification



Sample Description	16093	IA	IC	ID	IE	IIA	IIB	IIC	IID	189
Reference # 35	1	2	1	1	1	2	1	wl	1	0
Hair # 35	1	2	1	1	1	2	1	wl	1	0

Figure 4. Example of an inclusion with a weak signal in region IIC and no band in position 189.



Sample Description	16093	IA	IC	ID	IE	IIA	IIB	IIC	IID	189
Reference #2	1	1	1	1	1	1	2	1	1	1
Hair # 2	1	1	1	1	1	1	1	1	1	1

Figure 5. Example of an exclusion.

Table 4. Summary of mtDNA typing results using the Linear Array™ hybridization assay

	HVI					HVII					
Sample No.	16093	IA	IC	ID	IE	IIA	IIB	IIC	IID	189	Comments
R1	1	2	1	1	1	2	1	w1	1	0	Inclusion
H1	1	2	1	1	1	2	1	w1	1	0	
R2	1	1	1	1	1	1	2	1	1	1	Exclusion
H2	1	1	1	1	1	1	1	1	1	1	
R3	1	1	1	2	1	1	1	1	1	1	Exclusion
H3	1	1	1	1	1	1	1	1	1	1	
R4	1	1	w2, w3	1	1	2	1	1	w1	1	Exclusion
H4	1	1	1	2	1	2	2	1	1	1	
R5	1	1	1	1	0	2	0	2	1	1	Inclusion
H5	1	1	1	1	0	2	0	2	1	1	
R6	1	w1	0	1	1	2	0	1	1	1	Exclusion
H6	1	1	1	1	1	1	2	1	1	1	
R7	1	1	3	1	1	2	1	1	1	1	Inclusion
H7	1	1	3	1	1	2	1	1	1	1	
R8	1	1	1	1	1	w1	3	1	1	1	Exclusion
H8	1	2	1	1	1	2	1	w1	1	0	
R9	1	1	3	1	1	1	3	2	1	1	Inclusion
H9	1	1	3	1	1	1	3	2	1	1	
R10	1/w2	1	1	1	1	w1, w2	1	1	1	1	Inclusion
H10	1/w2	1	1	1	1	w1, w2	1	1	1	1	

w-weak result

Table 4 (continued). Summary of mtDNA typing results using the Linear Array™ hybridization assay

	HVI					HVII					
Sample No.	16093	IA	IC	ID	IE	IIA	IIB	IIC	IID	189	Comments
R11	1	1	1	2	1	2	2	1	1	1	Exclusion
H11	1	w1	0	1	1	2	0	1	1	1	
R12	1	1	1	1	1	1	2	1	1	1	Inclusion
H12	1	1	1	1	1	1	2	1	1	1	
R13	1	1	1	1	0	2	w1	2	1	1	Inclusion
H13	1	1	1	1	0	2	w1	2	1	1	
R14	1	0	1	2	2	2	1	1	1	1	Inclusion
H14	1	0	1	2	2	2	1	1	1	1	
R15	1	2	1	1	1	2	1	1	1	1	Inclusion
H15	1	2	1	1	1	2	1	1	1	1	
R16	1	1	1	1	3	2	1	1	1	1	Inclusion
H16	1	1	1	1	3	2	1	1	1	1	
R17	1	2	1	1	1	2	1	1	1	1	Inclusion
H17	1	2	1	1	1	2	1	1	1	1	
R18	1	1	1	1	1	1	1	1	1	1	Exclusion
H18	1	1	1	2	3	2	1	0	1	0	
R19	1	1	1	2	3	2	1	0	1	0	Exclusion
H19	1	1	1	1	1	1	2	1	1	1	
R20	1	1	1	1	1	1	1	1	1	1	Inclusion
H20	1	1	1	1	1	1	1	1	1	1	

w-weak result

Table 4 (continued). Summary of mtDNA typing results using the Linear Array™ hybridization assay

	HVI					HVII					
Sample No.	16093	IA	IC	ID	IE	IIA	IIB	IIC	IID	189	Comments
R21	1	2	2	1	1	2	0	1	1	1	Inclusion
H21	1	2	2	1	1	2	0	1	1	1	
R22	1	1	1	1	1	2	0	1	1	1	Exclusion
H22	1	1	1	1	1	2	6	2	1	1	
R23	1	2	1	2	1	2	5	2	1	1	Inclusion
H23	1	2	1	2	1	2	5	2	1	1	
R24	1	1	1	1	1	1	1	1	1	1	Exclusion
H24	1	1	1	1	1	2	0	1	1	1	
R25	1	1	1	1	1	1	2	1	1	1	Exclusion
H25	1	1	3	1	1	2	1	1	1	1	
R26	1	2	1	1	1	2	1	w1	1	w1	Exclusion
H26	1	3	1	1	1	2	1	0	w1	1	
R27	1	1	1	2	1	2	2	1	1	1	Exclusion
H27	2	1	1	1	1	2	0	1	0	1	
R28	1	1	3	1	1	2	1	1	1	1	Inclusion
H28	1	1	3	1	1	2	1	1	1	1	
R29	1	1	3	1	1	2	1	1	1	1	Exclusion
H29	1	1	1	1	1	w1	3	1	1	1	
R30	1	2	1	1	1	2	1	0	1	0	Inclusion
H30	1	2	1	1	1	2	1	0	1	0	

w-weak result

Table 4 (continued). Summary of mtDNA typing results using the Linear Array™ hybridization assay

	HVI					HVII					
Sample No.	16093	IA	IC	ID	IE	IIA	IIB	IIC	IID	189	Comments
R31	1	1	1	1	1	1	2	1	1	1	Exclusion
H31	1	1	3	1	1	2	4	1	1	1	
R32	1	1	1	1	3	2	5	1	1	1	Inclusion
H32	1	1	1	1	3	2	5	1	1	1	
R33	1	1	1	1	1	2	3	1	0	1	Inclusion
H33	1	1	1	1	1	2	3	1	0	1	
R34	1	1	3	1	1	2	1	1	1	1	Inclusion
H34	1	1	3	1	1	2	1	1	1	1	
R35	1	2	1	1	1	2	1	w1	1	0	Inclusion
H35	1	2	1	1	1	2	1	w1	1	0	
R36	1	1	3	1	1	1	5	1	1	1	Exclusion
H36	1	1	3	1	1	2	4	1	1	1	
R37	1	2	2	1	1	2	1	1	1	1	Inclusion
H37	1	2	2	1	1	2	1	1	1	1	
R38	1	1	3	1	1	2	1	1	0	1	Inclusion
H38	1	1	3	1	1	2	1	1	0	1	
R39	1	1	1	2	1	2	1	1	0	1	Inclusion
H39	1	1	1	2	1	2	1	1	0	1	
R40	1	1	1	1	1	1	3	1	1	1	Inclusion
H40	1	1	1	1	1	1	3	1	1	1	

w-weak result

Table 4 (continued). Summary of mtDNA typing results using the Linear Array™ hybridization assay

	HVI					HVII					
Sample No.	16093	IA	IC	ID	IE	IIA	IIB	IIC	IID	189	Comments
R41	1	3	w2, w3	1	1	2	1	w1	w1	w1	Inclusion
H41	1	3	w2, w3	1	1	2	1	w1	w1	w1	
R42	1	1	0	1	3	2	1	1	1	1	Inclusion
H42	1	1	0	1	3	2	1	1	1	1	
R43	1	1	3	1	1	2	4	1	1	1	Exclusion
H43	1	1	3	1	1	1	5	1	1	1	
R44	2	1	1	1	1	2	w1	1	0	1	Exclusion
H44	1	1	w2, w3	1	1	2	1	1	w1	1	
R45	1	3	1	1	1	2	1	0	w1	1	Exclusion
H45	1	1	1	1	1	1	2	1	1	1	
R46	1	1	3	1	1	2	1	1	1	1	Inclusion
H46	1	1	3	1	1	2	1	1	1	1	
R47	1	1	w1	1	1	2	6	2	1	1	Exclusion
H47	1	2	1	1	1	2	1	w1	1	w1	
R48	1	1	3	1	1	2	4	1	1	1	Exclusion
H48	1	1	1	1	1	1	1	1	1	1	
R49	1	2	1	1	1	2	1	w1	1	0	Exclusion
H49	1	1	1	2	1	2	2	1	1	1	
R50	1	1	1	1	1	1	1	1	1	1	Exclusion
H50	1	1	1	2	1	1	1	1	1	1	

w-weak result

Table 4 (continued). Summary of mtDNA typing results using the Linear Array™ hybridization assay

	HVI					HVII					
Sample No.	16093	IA	IC	ID	IE	IIA	IIB	IIC	IID	189	Comments
Rctrl 1-4	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	No results
Hctrl 1-5	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	
R(+) 1, 2	1	1	3	1	1	1	1	1	1	1	Expected results
H(+) 1-4	1	1	3	1	1	1	1	1	1	1	
R(-) 1, 2	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	No results
H(-) 1-4	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	

w-weak result

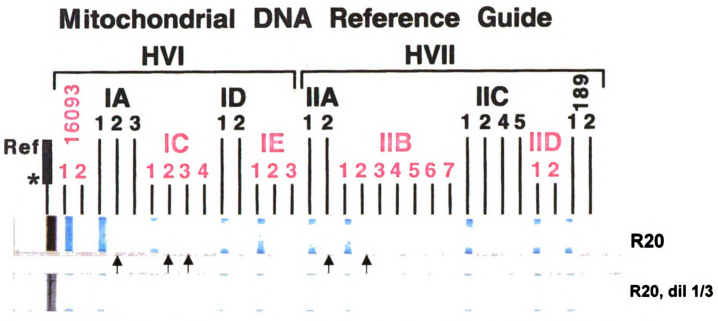
exclusions and twenty-five correct inclusions were made. Two inclusions were observed for non-matching sets.

The mtDNA tests for ten hair samples and three buccal swabs had to be repeated either due to no signals detected, weak signals at all probe regions, strong signals causing ambiguous results (e.g. non-specific binding), or multiple signals present in a single region. An example of non-specific binding is illustrated in Figure 6. Six samples—three references and three questioned hairs—hybridized to more than one location, resulting in a two-banded pattern on the strip. These samples were hybridized a second time with identical results. In addition, the results were the same when the six samples were extracted, quantitated, amplified, and hybridized again. These results are illustrated in Figure 7.

Thirty-five different Linear Array™ types were observed among the fifty participants, and 28 of these were unique. Table 5 lists these Linear Array™ types. One type was observed fives times, two types were observed four times each, one type was observed three times, three type were observed twice each, and the remaining types were unique.

The Linear Array™ types obtained were compared to profiles in the FBI's mtDNA database, and the frequency of occurrence was measured. The lowest frequency observed was 0.0033 for the result '1112321010', and the highest frequency calculated was 0.8153 for the result '1w101120111'.

For each individual, the Linear Array™ type, and when obtained, the nuclear DNA type, from the buccal swab and hair sample were compared and found to be in concordance. The master key utilized for this comparison can be found in Table 6.



Sample Description	16093	IA	IC	ID	IE	IIA	IIB	IIC	IID	189
Reference # 20	1	1, w2	1, w2, w3	1	1	1, w2	1, w2	1	1	1
Reference # 20, dil 1/3	1	1	1	1	1	1	1	1	1	1

Figure 6. Example of non-specific binding. Arrows indicate positions where nonspecific binding has occurred in reference sample 20. This sample was subsequently diluted 1/3 and hybridized on a second strip. As illustrated, the nonspecific binding was resolved. This is also an example of the Anderson Reference Sequence.

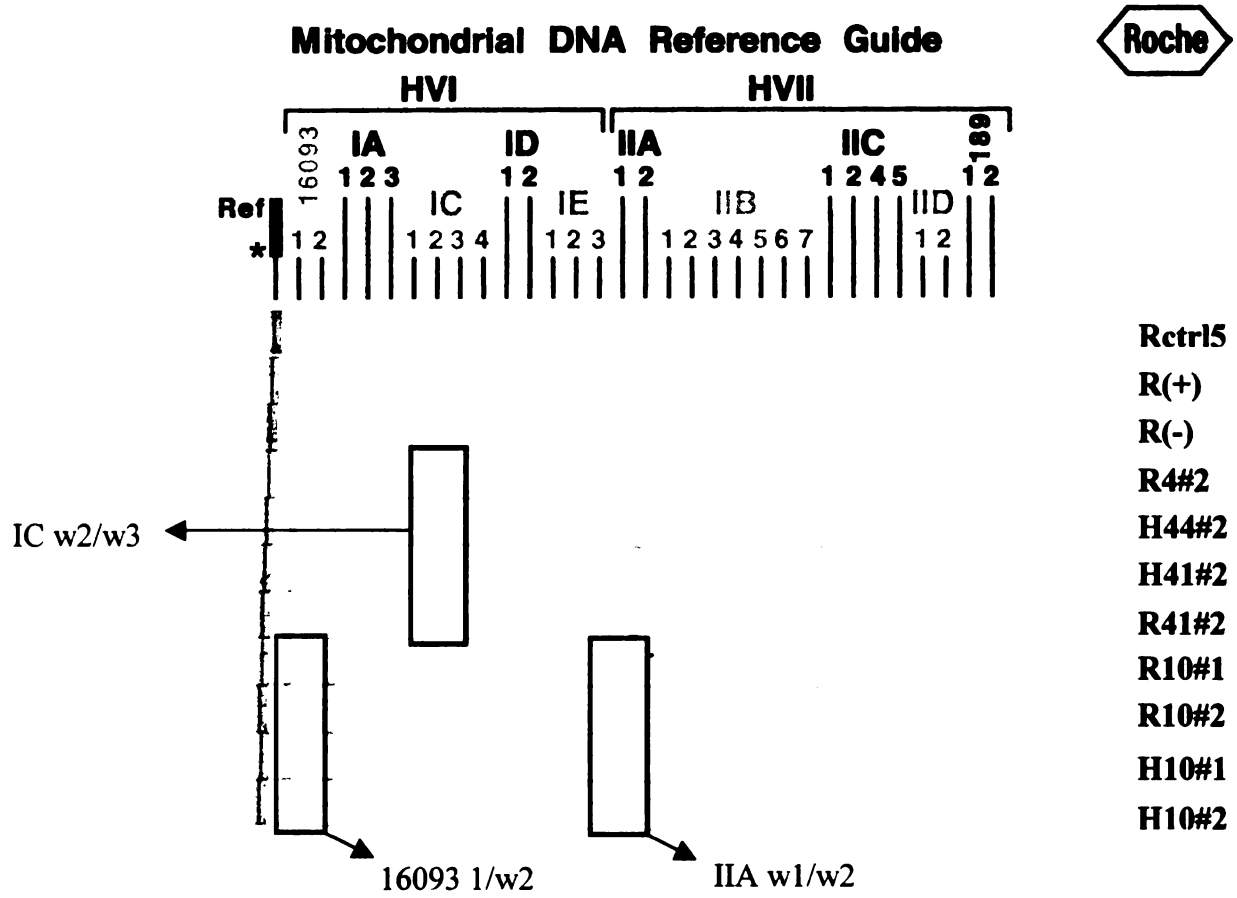


Figure 7. Samples with multiple probe signals in a single region. Two-banded patterns were detected from six samples. R4, H44, H41, and R41 have the type IC w2/w3. R10 and H10 both show two signals in positions 16093 and IIA. The bands in IIA are extremely weak, and the signals were not detected with the digital camera used. These six samples were repeated to confirm the original results and rule out the possibility of contamination. The photo above shows the results of the second analysis of these samples. The original amplified product from R10 and H10 were also run alongside the duplicate samples in this set.

Table 5. Summary of Linear Array™ types observed in fifty individuals

	HVI					HVII				
No. times observed	16093	IA	IC	ID	IE	IIA	IIB	IIC	IID	189
5	1	1	3	1	1	2	1	1	1	1
4	1	1	1	1	1	1	2	1	1	1
4	1	1	1	1	1	1	1	1	1	1
3	1	2	1	1	1	2	1	w1	1	0
2	1	1	1	2	1	2	2	1	1	1
2	1	2	1	1	1	2	1	1	1	1
2	1	1	3	1	1	2	4	1	1	1
1	1	1	1	2	1	1	1	1	1	1
1	1	1	w2, w3	1	1	2	1	1	w1	1
1	1	1	1	1	0	2	0	2	1	1
1	1	w1	0	1	1	2	0	1	1	1
1	1	1	1	1	1	w1	3	1	1	1
1	1	1	3	1	1	1	3	2	1	1
1	1/w2	1	1	1	1	w1, w2	1	1	1	1
1	1	1	1	1	0	2	w1	2	1	1
1	1	0	1	2	2	2	1	1	1	1
1	1	1	1	1	3	2	1	1	1	1
1	1	1	1	2	3	2	1	0	1	0
1	1	2	2	1	1	2	0	1	1	1
1	1	1	1	1	1	2	0	1	1	1
1	1	2	1	2	1	2	5	2	1	1
1	1	2	1	1	1	2	1	w1	1	w1
1	1	2	1	1	1	2	1	0	1	0
1	1	1	1	1	3	2	5	1	1	1
1	1	1	1	1	1	2	3	1	0	1
1	1	1	3	1	1	1	5	1	1	1
1	1	2	2	1	1	2	1	1	1	1
1	1	1	3	1	1	2	1	1	0	1
1	1	1	1	2	1	2	1	1	0	1
1	1	1	1	1	1	1	3	1	1	1
1	1	3	w2, w3	1	1	2	1	w1	w1	w1
1	1	1	0	1	3	2	1	1	1	1
1	2	1	1	1	1	2	w1	1	0	1
1	1	3	1	1	1	2	1	0	w1	1
1	1	1	w1	1	1	2	6	2	1	1

w-weak result

Table 6. Sample preparation masterkey

Sample set	Exemplar donor	Ethnicity, age, sex of exemplar sample donor	Questioned donor	Ethnicity, age, sex of quesioned hair donor
1	9	Caucasian, 26, male	9	Caucasian, 26, male
2	40	Caucasian, 33, male	31	Caucasian, 63, male
3	43	Caucasian, 35, female	12	Caucasian, 26, male
4	45	Asian, 26, female	6	Asian, 29, female
5	20	Asian, 30, female	20	Asian, 30, female
6	15	Hispanic, 42, male	40	Caucasian, 33, male
7	7	Caucasian, 28, male	7	Caucasian, 28, male
8	24	Caucasian, 28, male	11	Caucasian, 26, female
9	23	Caucasian, 25, female	23	Caucasian, 25, female
10	50	Caucasian, 39, male	50	Caucasian, 39, male
11	48	Hispanic, 30, female	15	Hispanic, 42, male
12	39	Caucasian, 29, female	16	Caucasian, 27, female
13	42	Caucasian, 31, female	42	Caucasian, 31, female
14	57	African-American, 48, male	57	African-American, 48, male
15	44	Caucasian, 39, male	44	Caucasian, 39, male
16	1	Caucasian, 28, male	1	Caucasian, 28, male
17	37	Caucasian, 28, male	37	Caucasian, 28, male
18	31	Caucasian, 63, male	32	Caucasian, 27, male
19	32	Caucasian, 27, male	39	Caucasian, 29, female
20	58	Caucasian, 19, male	58	Caucasian, 19, male
21	55	Caucasian, 37, male	55	Caucasian, 37, male
22	30	Hispanic, 35, female	8	Caucasian, 43, female
23	35	Caucasian, 31, male	35	Caucasian, 31, male
24	41	Caucasian, 37, female	30	Hispanic, 35, female
25	16	Caucasian, 27, female	13	Caucasian, 34, male

Table 6 (continued). Sample preparation masterkey

Sample set	Exemplar donor	Ethnicity, age, sex of exemplar sample donor	Questioned donor	Ethnicity, age, sex of quesioned hair donor
26	4	Caucasian, 28, female	21	Caucasian, 32, female
27	6	Asian, 29, female	34	Asian, 27, female
28	29	Caucasian, 30, female	29	Caucasian, 30, female
29	46	Caucasian, 43, female	24	Caucasian, 28, male
30	2	Caucasian, 30, female	2	Caucasian, 30, female
31	38	Caucasian, 57, female	14	Caucasian, 26, male
32	53	Caucasian, 46, male	53	Caucasian, 46, male
33	47	Caucasian/Hispanic, 29, female	47	Caucasian/Hispanic, 29, female
34	3	Caucasian, 56, female	3	Caucasian, 56, female
35	33	Caucasian, 33, female	33	Caucasian, 33, female
36	5	Caucasian, 32, female	17	Caucasian, 32, female
37	56	Caucasian, 41, male	56	Caucasian, 41, male
38	49	Hispanic, 31, male	49	Hispanic, 31, male
39	36	Asian, 52, female	36	Asian, 52, female
40	18	Caucasian, 34, male	18	Caucasian, 34, male
41	10	Caucasian, 26, male	10	Caucasian, 26, male
42	28	Caucasian, 29, male	28	Caucasian, 29, male
43	14	Caucasian, 26, male	5	Caucasian, 32, female
44	34	Asian, 27, female	45	Asian, 26, female
45	21	Caucasian, 32, female	38	Caucasian, 57, female
46	13	Caucasian, 34, male	46	Caucasian, 43, female
47	8	Caucasian, 43, female	4	Caucasian, 28, female
48	17	Caucasian, 32, female	41	Caucasian, 37, female
49	11	Caucasian, 26, female	48	Hispanic, 30, female
50	12	Caucasian, 26, male	43	Caucasian, 35, female

Discussion

Sample Collection

The participants returned buccal swabs, pubic hair cuttings, and pubic hair combings as instructed, with the exception of three participants. The pubic hair standards from two of these individuals contained only five exemplar hairs, and one individual's pubic hair combings contained only one hair. The lack of pubic hair exemplar samples from the two individuals restricted the microscopic hair examinations, as an appropriate range of characteristics could not be established for these sets. The single hair in the pubic hair combings did not hinder sample set preparation or subsequent examinations, as only one hair from the combings was included in each of the sample sets.

Macroscopic examinations

Macroscopic evaluation was the least accurate method for identifying the correct source of a questioned hair. As previously noted, the results of the macroscopic examination varied among analysts in number of hairs characterized as similar or dissimilar from the reference samples, and accuracy of characterization. Several data sets indicate that level of experience also plays role in accuracy rate, as would be expected.

Analyst #1, who has three years of experience in this type of analysis, reported that only four sets out of fifty contained hairs from different sources. Thus, 92% of the sets were characterized as coming from the same source, despite the fact that 50% were not. It is likely that this analyst lacks knowledge and experience in distinguishing hair characteristics, has not been able to create, adapt, or apply a consistent method for analysis, and was therefore unwilling and/or unable to make a pointed decision. Consequently, this analyst had an accuracy rate of 100% for the characterization of same

source hairs, yet a 16% success rate for recognizing hairs from different donors, for a total accuracy rate of 58%. In addition, 21 hairs from non-matching sets were misidentified.

The most accurate interpretations were reported by Analyst #10, who has seven years of experience in this area, and more importantly, is a qualified microscopic hair examiner. This individual accurately characterized 72% of the same source hairs, 68% of the hairs from different donors, for a total accuracy rate of 70%. This analyst's success can likely be correlated to experience level and degree of training in this discipline. This analyst misidentified 7 hairs from non-matching sets, and 7 hairs from matching sets.

Analyst #8 had accuracy rates for identifying same source and different source hairs between those of Analyst #1 and Analyst #10. This analyst's ability to differentiate hairs parallels her average experience level of five years, and basic knowledge of hair variation. Her accuracy rate for identifying hairs from the same source was 68%, and 48% for differentiating hairs from different sources, for an overall accuracy of 58%. This analyst was less stringent in her criteria for concluding that a hair is different than the exemplars, and was likely to characterize known and questioned hairs as dissimilar based on the slightest differences observed. Consequently, these criteria caused the analyst to misinterpret many of the hairs in same source sets as coming from two different individuals. Of the twenty hairs reported by Analyst #8 as dissimilar from the knowns, twelve, or 60%, were correctly described, and the remaining eight, or 40%, were actually hairs from the same donor.

Based on a survey by the author (2004), some laboratories solely rely on macroscopic examinations to identify hairs with the most probative value, which will

then be submitted for further testing using microscopic or molecular analyses. Therefore, to rely on macroscopic examinations for later analyses, the results need to be dependable, accurate, and fairly conservative. From the results of the macroscopic examinations in this study, it is clear that this method is neither accurate nor dependable, with a success rate ranging from 48–70%.

Microscopic examinations

The results of the microscopic examinations illustrate the overall value of performing this comparison. The four microscopic hair examiners were able to characterize 72% of the hairs accurately. However, incorrect assessments were made for three samples, or 17%, and two inconclusive results, or 11%, were reported. The impact of those inaccuracies should be considered. Two associations were reported for two non-matching sets. If these were questioned hairs collected from a victim of sexual assault, the perpetrator could go unnoticed, or an innocent person could go to prison. In addition, one exclusion was reported for a set of hairs from the same individual. If these were questioned hairs collected from the suspect of a sexual assault, the results could potentially cause false allegations, or set a guilty person free. Finally, the two inconclusive assessments were made for sets of matching hairs, allowing for no resolution as to the source. Many individuals have been convicted, and even placed on death row (Gianelli, 2001; Saferstein, 2004), based on the results of microscopic comparisons, so accuracy is essential. If nuclear or mtDNA analysis did not follow these particular microscopic examinations, the true source of 28% of the hairs would remain unknown.

The accuracy of microscopic hair examinations can often be associated with the training and experience of the analysts. However, this did not seem to be the case in this study. Table 2 represents the results of the microscopic examinations. Analyst #1 has over ten years of experience in this area, analyst #2, twenty years, analyst #3, two years, and analyst #4, fifteen years. Time spent analyzing the sample sets also varied among analysts, which seemed to be unrelated to their experience or success rate—Analyst #4 with fifteen years of experience and a 100% accuracy rate, and Analyst #2 with the twenty years of experience and a 50% success rate spent the same amount of time analyzing hairs. Due to the limited number of samples examined by each analyst, a relationship between experience and accuracy could not be determined. Analyst #2 has the most experience in microscopic hair examinations, but had the lowest accuracy rate at 50%. Accuracy rates for the other three analysts increased with their experience level; Analyst #4 has 15 years of experience and was 100% accurate, Analyst #1 has over ten years of experience and was 75% accurate, and Analyst #3 has two years of experience and was 66% accurate. The results of these hair examinations illustrate that false inclusions and false exclusions can occur regardless of experience, or time spent examining samples.

Three limiting factors may have affected the accuracy of the hair comparisons performed by these particular hair examiners. First, Analyst #2 reported she could have made better assessments had the reference samples been pulled, rather that cut, so that the root morphology of the standards could be compared the root present on to the questioned hairs. Second, in two sample sets examined microscopically, the number of exemplar hairs was limited at five hairs; all other sets contained at least 15 exemplars. Analysts #2

and #3, each examining one of these limited sets, stated they could not establish an adequate range of characteristics to compare to the questioned hairs. These analysts therefore chose to report inconclusive results for these sample sets. Finally, due to lack of time for thorough examinations, all analysts felt that their results were best reported as preliminary. The average time spent comparing one set of exemplar and questioned hairs was 3.3 hours. According to these hair analysts, examinations for criminal cases have taken up to 25 hours, depending on the characteristics of the hair in question; the more a questioned hair resembles the reference set of hairs, the longer the examination has taken. If the analysts allotted more time for examination, it is possible that additional characteristics would have been identified, thus allowing the examiner to make a more pointed decision. It is also possible that the results of the microscopic examinations are representative of these analyst's typical success rates.

Although forensic microscopic hair examinations are not always accurate and the examiners cannot state with certainty that a hair originated from a particular individual, this type of examination should not be eliminated as a way to characterize hairs. They can be useful in identifying trace material adhering to hair, such as glass fragments or blood. They are also helpful in identifying phenotypic characteristics of a hair, as well as chemical treatment or personal hygiene. Furthermore, when nuclear or mtDNA analyses fail to produce discriminating results, the microscopic examination may provide the most valuable information. Finally, microscopic examination can also be a good screening tool to identify probative hairs when the number of hairs to characterize is quite large.

Nuclear DNA Analysis

The results of nuclear DNA testing using fifteen STR loci and amelogenin demonstrate the utility of this technique as applied to hair evidence. In this study, full DNA profiles were obtained from all buccal swabs, full profiles were obtained from seventeen out of the fifty hair samples, or 34%, partial profiles were obtained from five out of the fifty samples, or 10%, and no results were obtained from twenty-eight hairs, or 56%. Three types of conclusions could be drawn from the comparison between the STR results from buccal swabs and the questioned hairs—positive identification or exclusion, possible inclusion, or no conclusion based on the lack of information obtained.

By comparing the STR results, a positive match was made between the buccal swab and questioned hair sample in 8 out of the 25 same source sets, or 32%, meaning that the buccal swab and hair originated from the same person. Exclusions were also made, meaning that the source of the buccal swab can be eliminated as being the source of the hair. Nine exclusions out of the 25 sets, or 36%, were made based on multiple differences seen in full nuclear DNA profiles from these sample sets. Two additional exclusions were made from partial profiles, again based on the differences observed between the reference and questioned results. Partial inclusions were made in three of the sample comparisons. For all partial inclusions, to determine the likelihood that questioned hair originating from someone other than the buccal swab donor, statistical calculations were performed, resulting in likelihood ratios as low as 1 in 140 individuals (profile with 4 alleles detected), and as discriminating as 1 in 9 million individuals (profile with ten alleles detected). Depending on the circumstances surrounding a

particular case, these numbers could serve as corroborating evidence, but would rarely provide an absolute answer as to the source of the evidence.

Often when analyzing forensic samples with limited amount of nuclear DNA, such as hairs, no STR typing results are obtained. This is the least favorable outcome—when a hair sample does not produce a nuclear DNA type, no information has been gained and a portion of the hair has been consumed. This was the case for 28, or 56%, of hair samples analyzed in this study.

As previous research has shown (Linch *et al.*, 1998) the success of nuclear DNA typing can be directly correlated to the stage of root growth. Only telogen hairs with follicular tags and anagen/catagen hairs absent sheath material are considered good candidates for DNA typing. In this study, the screening analysts performing macroscopic comparisons and the analyst collecting hair samples for DNA analysis did not characterize the root morphology of the hairs, perhaps because these individuals lack knowledge in this area, and because it is not a common practice for them. The root morphology was reported for eighteen of the hairs examined by qualified microscopic hair analysts. Thirteen of these hairs were characterized as good candidates for nuclear DNA analysis, and five were not. All five hairs described as lacking sufficient root material did not produce nuclear DNA typing results, as did three of the good candidates. Full nuclear DNA profiles were obtained from the remaining ten good candidates. These results indicate that proper characterization of root morphology can serve to direct subsequent analyses, but that it cannot always be relied upon as a predictor of success—even those hairs with sufficient root material may not produce nuclear DNA typing results.

MtDNA Analysis

MtDNA typing utilizing the Linear Array™ kit was successful in correctly characterizing the source of 96% of the sample sets. Twenty-three exclusions from non-matching sets and twenty-five inclusions from matching sets were made based on the results of this assay.

Two inclusions were made for non-matching sample sets. One inclusion was made between a reference and hair sample from a non-matching set, both having the Linear Array™ type '1111112111' and a second inclusion was made between samples from a non-matching set having the type '1131121111'. While the results from the assay are accurate in these two examples, they are not unique. Among the individuals in the study, these particular Linear Array™ types occurred most frequently. '1111112111' was observed four times, or 8%, and '1131121111' observed five times, or 10%. In the FBI's mtDNA Database, these particular sequences were observed in 24% ('1111112111') and 16.95% ('1131121111') of individuals in the database, showing they are very common types. The results from these two non-matching sample sets illustrate the limitation of the assay in terms of inclusions—several people, related or not, will share the same Linear Array™ type. Therefore, all inclusions should be further characterized using mtDNA sequencing, nuclear DNA typing, or microscopic comparison.

However, during this study, the Linear Array™ kit proved its usefulness for making exclusions. Exclusions were made for all 25 non-matching sets—when the Linear Array™ types from reference and questioned samples differed they did not originate from the same individual. On the other hand, when Linear Array™ types are the same, the samples could have originated from the same person, or from two different

individuals. The results from this assay can also be obtained quickly—results from 24 hair samples could be obtained in less than two days. Nuclear DNA analysis from 24 hair samples would take twice as long, and may not provide results at all, depending on the amount of root material present. The ability to obtain mtDNA results in a short amount of time could be of importance in cases where a suspect is being held under tenuous circumstances, or when a victim's accusations are being questioned. Due to the ability to get quick results from multiple samples at a time, this assay could also be efficient when faced with large numbers of questioned samples or large numbers of exemplars—exclusions and inclusions can often direct the analysis towards the most probative evidence.

When two Linear Array™ types are not identical, it is important to consider the weight of particular differences. For example, an individual who has the Linear Array™ type '1131112111' can certainly be excluded as the source of a questioned sample with the type '1111113522' due to the number of differences occurring. However, in a situation where an individual having the type '11111111w11' is compared to a questioned sample '1111111101', it would be unreasonable, given the potential causes of such a difference (e.g. non-specific binding, low input DNA), to exclude him/her as being the source of the questioned sample. Also, considering that heteroplasmy can manifest in one tissue but not another (Calloway *et al.*, 2000), a reference buccal swab having the haplotype 'w1/w2 111111111' could not be excluded as coming from a hair having the type '1111111111'. Therefore, care must be taken when interpreting results when using this system and laboratories will need to define their criteria for exclusion based on validation studies.

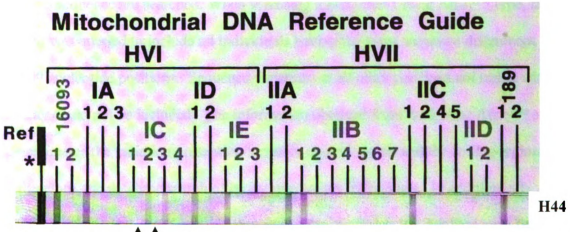
Four types of banding patterns were detected on the strips—a single band, a weak band, no band, or two bands. Most commonly a single band was detected and the sequence determination could be made as in Figure 3. Both 'w' and '0' calls were also observed in the Linear Array™ types from several individuals. Seventeen individuals, or 34%, had a '0' in their Linear Array™ type, representing the absence of a band, and thirteen individuals, or 26%, had a 'w', representing the presence of a weak band in their Linear Array™ type. The high instance (34%), of blank, or '0', calls on the strips is consistent with the 52% observed in the NIST study of the Linear Array™ typing strips (Butler, 2004). In the NIST study, 21% of samples had a weak signal, which is comparable to the 26% observed in this study. In this study, when a 'w' or '0' signal was observed in an individual's hair sample, it was also seen in that individual's buccal swab, indicating that they were accurate calls. The Linear Array™ types from three individuals, or 6%, had regions with two bands, representing a mixture of two sequences. Again, the same Linear Array™ types were seen in both the buccal and hair samples from these individuals. However, while a sequence determination could not be made for the regions in which a weak, no, or more than one signal is present, it did not hinder comparison to the other sample in the set.

Multiple bands in a region could indicate contamination, heteroplasmy, or some other polymorphism not targeted by the assay. For the samples with two-banded patterns, the possibility of contamination was unlikely since the results could be duplicated using a new cutting from the samples. The buccal and hair samples from two individuals had a w2/w3 signal in the IC region, which targets polymorphisms at 16304 and 16311. Examples of w2/w3 signals can be seen in Figures 7 and 8. One possibility

for this two-banded pattern is the presence of a sequence variant known to occur in <1% of individuals (Calloway *et al.*, 2000). This variant has been researched and contains base changes at both 16304 and 16311. These changes cause weak hybridization to two non-complementary probes (IC2 and IC3), resulting in a weak two-banded signal. It is also possible, yet less likely, that the banding pattern in caused by heteroplasmy at both 16304 and 16311, both in equal ratios. A second set of samples from one individual had multiple probe signals in two different regions, 1/w2 at position 16093, and w1/w2 at position 73. Figure 7 includes the results from these samples. No other regions showed a two-banded pattern for these samples. Again, one potential cause for the two-banded patterns seen in this sample is heteroplasmy occurring in two sites.

Heteroplasmy has been reported to occur in 2–11.6% of individuals (Holland and Parsons, 1999; Calloway *et al.*, 2000). The NIST study of the Linear Array™ kit observed heteroplasmy in 7 out of 666 individuals, or 1%, at various sites including 16093, 16363, 146, 152, and 189. Calloway *et al.* (2000) found that heteroplasmy has occurred at some positions more frequently than others, including 16093 and 73. Although a possibility for this set of samples, heteroplasmy should not be assumed when using the Linear Array™ kit, since other polymorphisms could cause altered hybridization of the mtDNA to the probes, resulting in a two-banded pattern.

Thirty-five different Linear Array™ types were observed among the fifty participants, and 28 of these were unique. However, there were maternally related individuals participating in this study (one mother and her son, one mother and her two daughters, and one set of fraternal twins), the number of unique Linear Array™ types among unrelated individuals is likely to have been underestimated.



Sample Description	16093	IA	IC	ID	IE	IIA	IIB	IIC	IID	189
Hair sample 44	1	1	w2/w3	1	1	2	1	1	0	1

	16304	**16311**
IC1	G T A C A T A G T A	
IC2	G C A C A T A G T A	
IC3	G T A C A T A G C A	
IC4	G T A C A T G G T A	
ICw2/w3	G C A C A T A G C A	

Figure 8. Example of multiple probe signals at IIC. Arrows indicate the two-banded pattern at IC. The table shows the Linear Array™ type for this sample. One known variant sequence hybridizes to two non-complementary probes, resulting in two weak signals within IC. This variant sequence, which contains base changes at 16304 and 16311, hybridizes weakly to both the IC2 and IC3 probes, which detect a single variant at 16304 and 16311 respectively. The probe sequence designations for IC, including the IC w2/w3 variant sequence are also included.

The frequency of each Linear Array™ type was calculated and then compared to the FBI's mtDNA Database. When using this database, sequences differing from the Anderson sequence are searched. When searching a particular Linear Array™ type, the database was queried to include all individuals having the same sequence differences at up to 19 nucleotide positions. Sequence variations at all other positions not tested with the assay could not be included. The information obtained from the Linear Array™ strips underestimates the variation present in the entire control region and the coding region, and therefore, the database search often included many individuals. In addition, all positions having a 'w', a '0', or multiple bands were queried in the database such that any nucleotide could be present at those locations, a drawback to these kind of results. For example, the Linear Array™ type '1w101120111' would be entered such that any polymorphism could be present at the 2nd, 3rd, and 7th positions. The only search criteria different from the Anderson sequence for this sample was that a G had to be present at position 73, which 81.53% of individuals in the database have. The database was found to be useful only for samples that have a single band in each region on the strip, and the frequencies estimated for Linear Array™ types with a 'w', '0', and multiple signal results were of little value since a specific polymorphism could not be entered for these positions—they may include many more individuals than if the polymorphism causing the signal was searched.

Proposed Strategies

As previously noted, current protocols for hair examination vary among laboratories, and there is no consensus as to the most appropriate strategy. Some laboratories perform a macroscopic examination followed by nuclear DNA analysis, and

although this DNA test is sometimes not successful on hairs, it is often the only one available in the lab. Other laboratories require that a microscopic examination precede nuclear DNA or mtDNA analysis. Typically, laboratories that are unable to perform mtDNA sequencing in-house will outsource hair evidence for mtDNA sequencing only if money is available, and only if the hair is the best evidence in the case. The ability to characterize hairs ultimately depends on the examinations included in a laboratory's protocol, and the order in which those analyses are performed.

When a laboratory decides to develop a set strategy for hair examination, it must consider the abilities, techniques, and expertise available to it, the time and cost of each procedure, and the accuracy of results obtained. The most important goal is to achieve positive identification or a high degree of discrimination. One strategy would be to begin with the quickest and least expensive method possible (macroscopic examination), followed by progressively more demanding procedures. Of course, if initial method used (e.g. macroscopic examination) has a high error rate, it may be more detrimental than informative. In contrast, perhaps time could be saved in the long run if the most exacting technique was used from the start. However, if the success rate of this method (such as STR analysis of hairs) is very low, a large amount of effort and expense can be used without gaining results. Sample conservation is a second factor to consider when developing strategies. Logically, all non-destructive examinations should be performed prior to DNA-based analyses, which will consume portions of the hair. A third consideration when establishing strategies is cost and time needed for analyses. The analyses, in increasing order of cost and time needed for examination are as follows: macroscopic examination, microscopic examination, mtDNA typing using a SSO

hybridization assay, nuclear DNA typing using STRs, and finally mtDNA sequencing. Considering all of these factors, three strategies will be proposed.

The first strategy proposed will assume that unlimited resources are available to the laboratory. The second will be designed around the many laboratories with limited resources. Finally, a strategy will be proposed for instances when the hair evidence is limited. An example of this would be the presence of a hair shaft less than one centimeter, or the presence of a hair root with little to no shaft.

The first strategy, with open funds, could be implemented if time and money are not limited. The analysis would begin with sample collection and a general macroscopic examination to collect all hairs and document their visual characteristics. All hairs collected would be sent to a hair examiner for complete microscopic analysis. The root material of all hairs would then be cut for DNA extraction. If the root was described as a good candidate for nuclear DNA typing, a portion of this extract could be used for STR analysis. The examination would end if an exclusion or identification was made using nuclear DNA analysis. If a partial profile resulted in an inclusion, the value of the information obtained would be evaluated. If the statistical values calculated were above the identification criteria (e.g. 1 in 260 billion), the analysis would end. If the partial result was not highly discriminatory, or if no nuclear DNA typing results were obtained, another volume of the DNA extract could then be used for mtDNA SSO typing. An exclusion at this level would end the examination. However, if an inclusion resulted, the remaining portion of the extract, or another sample from the hair, would be submitted for mtDNA sequencing. The purpose of performing the mtDNA SSO assay prior to sequencing would be to eliminate the time needed for sequencing samples excluded by

the assay. This strategy would use all available methods of analysis, and would therefore be the most informative and exhaustive. Figure 9 is a flow chart illustrating the progression of this analysis.

The second strategy could be implemented if there were budgetary restraints and time and labor were limiting factors. As in the first strategy, the analysis would begin with sample collection and a general macroscopic examination for documentation purposes. All hairs collected would be sent to a hair examiner for general analysis, regardless of the macroscopic characterization. At this point, the hair examiner would perform a truncated microscopic examination. During this examination, the analyst would photograph the entire hair, making sure that detailed images at the microscopic level were captured of the proximal and distal ends. The root end or distal end may be consumed for DNA analysis, so it is imperative that the features of these areas are well documented for future comparison purposes. Other general characteristics of the hair would be recorded, and finally the stage of root growth would be assessed for nuclear DNA typing. The root end of the hair would then be cut and DNA would be extracted. If the root end was considered a good candidate for nuclear DNA typing, this analysis would proceed. If the root was not suitable for this nuclear DNA analysis, or if nuclear DNA analysis did not yield discriminating results, a volume of the DNA extract would be taken for mtDNA typing using the hybridization assay. If the results of the hybridization assay did not offer an exclusion, the remaining portion of the extract, or an additional portion of the hair, would be submitted for mtDNA sequencing. Finally, if additional information were needed, the hair would be returned to the hair examiner for a complete comparison utilizing the documentation initially generated. Using this strategy would

potentially allow for highly discriminating results, while conserving sample and labor. By using the hybridization assay as a screening tool prior to sequencing, money will only be spent on the most probative evidence. Figure 10 depicts this strategy.

A final strategy would be implemented if the evidence was limited. Occasionally analysts only have a small portion of the hair shaft (less than one centimeter) or just a root of a hair with little shaft material to perform comparisons. In these situations, all non-destructive examinations must be performed prior to all destructive examinations. Therefore, a general macroscopic examination would be performed, and the hairs collected would be submitted to the hair examiner for microscopic comparison. The hair fragment would then be extracted for nuclear and/or mtDNA typing. If the hair fragment does not have a root, the DNA extract would be submitted for mtDNA SSO typing. If an inclusion results, the remaining extract would be submitted for mtDNA sequencing. If the hair fragment has a root, the DNA extract would be submitted for nuclear DNA analysis. If successful, the analysis would end, and if unsuccessful, the remaining portion of this extract would be submitted for mtDNA typing using the hybridization assay, and finally mtDNA sequencing if necessary. This strategy would provide the most information possible considering the evidence available, and is illustrated in Figure 11.

These three strategies should serve to provide the most information available from hair evidence. However, due to the variation in laboratory structure, personnel, and protocols, these strategies cannot be applied in all situations.

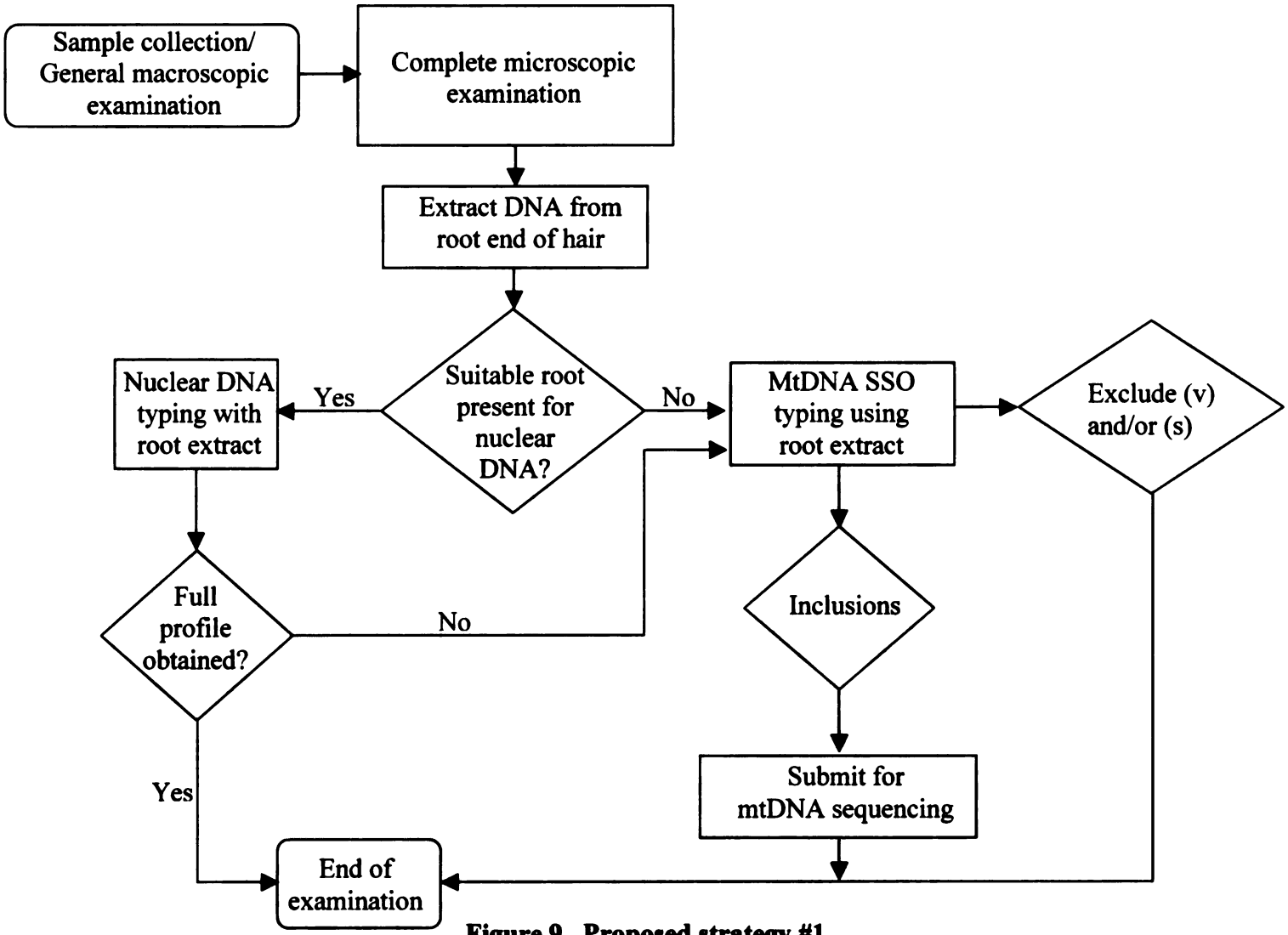


Figure 9. Proposed strategy #1

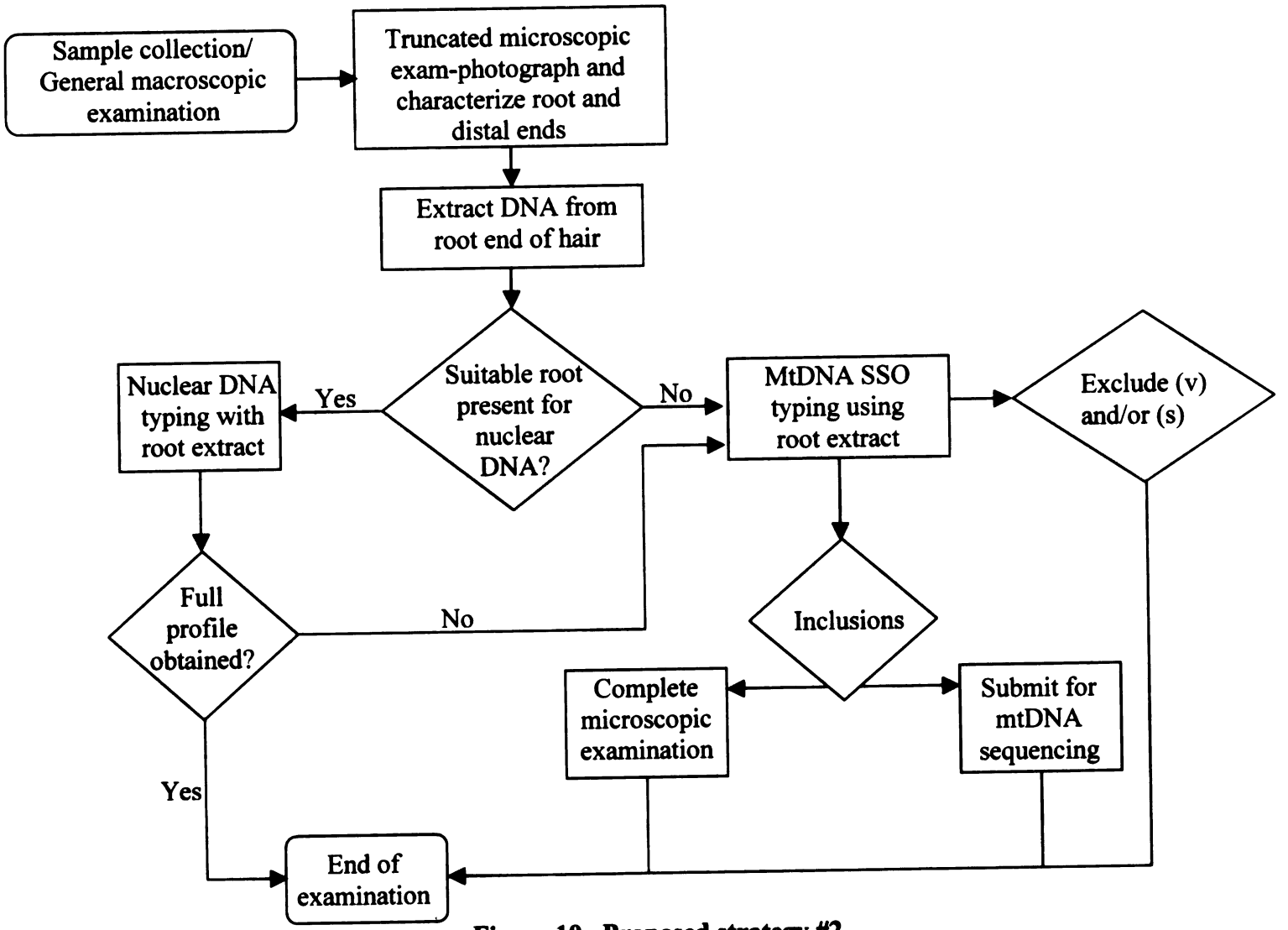


Figure 10. Proposed strategy #2

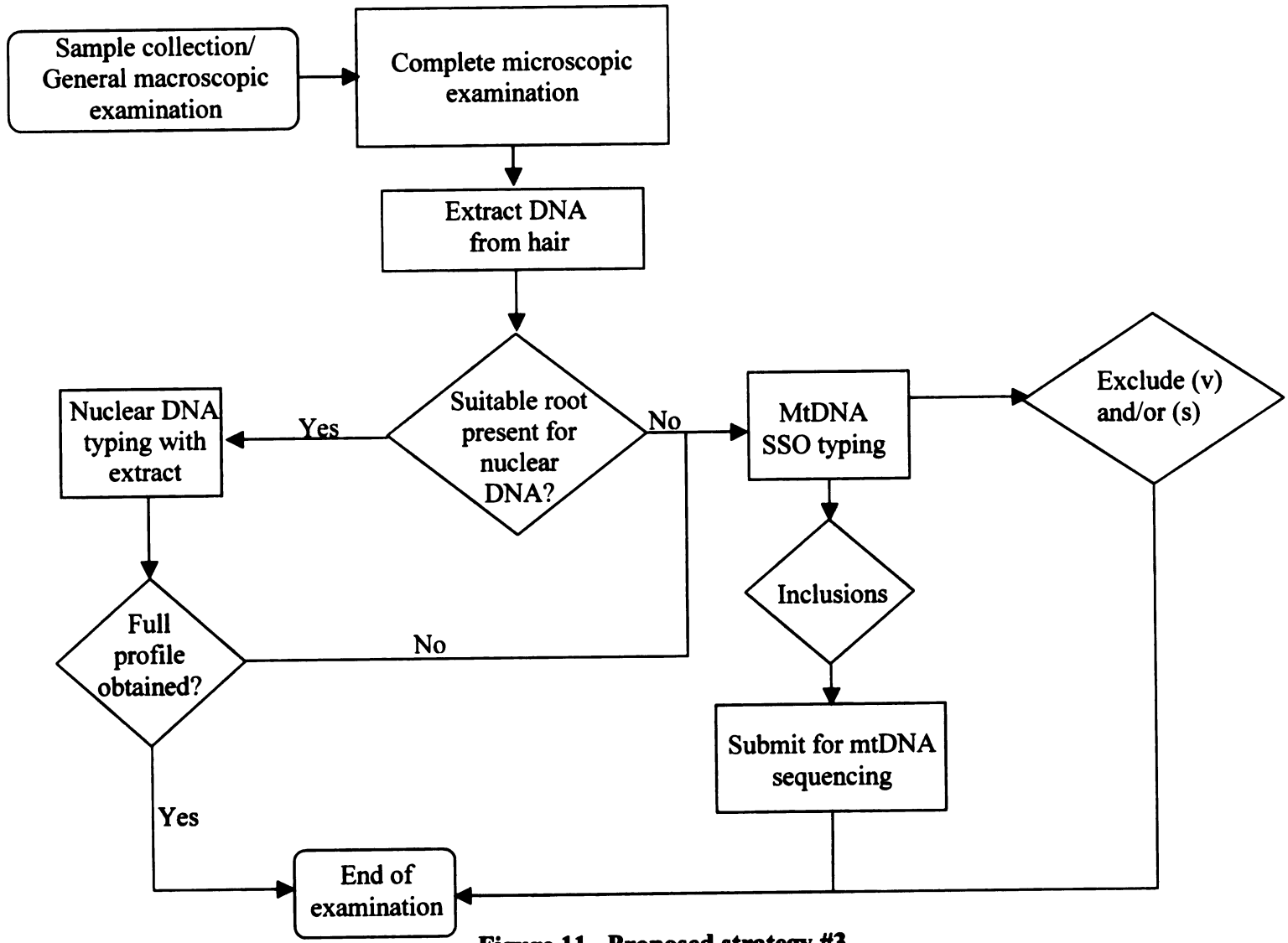


Figure 11. Proposed strategy #3

Conclusions

The results of this study illustrate the advantages and disadvantages of the different methods used for hair comparison in a forensic setting. Although very quick and inexpensive, the macroscopic evaluation of hairs was proven to be highly subjective and erroneous. For this reason, the results of a macroscopic examination should not determine whether or not additional testing should be performed on a questioned hair. In the absence of better evidence in a case, all potentially probative hairs should be examined further. The microscopic examinations were relatively accurate; however, incorrect inclusions, incorrect exclusions, and inconclusive results were reported, regardless of analyst experience and length of examination.

Ideally, the forensic scientist should employ whatever methods necessary to identify all probative hairs, and to avoid overlooking any discriminating evidence. The findings in this study show that if possible, all hairs examined microscopically should also be analyzed at the molecular level. Microscopic examinations, while potentially time-consuming, are important for the collection of trace evidence, sample screening, and also for determining the stage of root growth, an important factor in nuclear DNA analysis. When nuclear DNA and mtDNA analyses do not yield discriminating result, the analyst must rely on the microscopic results.

MtDNA analysis using a SSO hybridization assay, such as the Linear Array™ kit, can be extremely successful in establishing exclusions and inclusions, as shown in this study. All inclusions reported using this assay should probably be further characterized using nuclear and/or mtDNA sequencing because the Linear Array™ types are not typically unique. MtDNA sequencing was not performed in this study, however, it may

serve to better discriminate among matching Linear Array™ types, and potentially identify heteroplasmy and other sequence variations that could not be resolved. In addition, individual laboratories would need to assess the value of implementing the SSO assay. The information gained using mtDNA sequencing is always more discriminating than the information gained using the SSO assay. However, if a laboratory already conducts mtDNA sequencing, the SSO assay could serve to save time and money. Finally, the SSO assay may only be cost-effective when analyzing a large number of samples, thus a laboratory would have to evaluate its use depending on the caseload encountered.

Considering the advantages and disadvantages of each method explored in this study, three strategies were proposed. When developing these strategies, the ultimate goal was the ability to identify all probative hairs, while also considering constraints faced by examiners in the field. Some of these constraints include sample conservation, cost, time, and workflow. Although these strategies cannot be implemented by all laboratories due to personnel or budgetary limitations, they can offer insight on how to best modify current practices to better identify probative hairs from sexual assault cases

References

Anderson, S., A.T. Bankier, B.G. Barrell, M.H.L deBruijn, A.R. Coulson, J. Drouin, I.C. Eperon, D.P. Nierlich, B.A. Rose, F. Sanger, R.H. Schreier, A.J.H. Smith, R. Staden, I.G.Young. (1981) Sequence and organiztion of the human mitochondrial genome. *Nature* 290: 457-465.

Applied Biosystems (1999) AmpF*l*STR® COfiler™ Amplification Kit User's Manual. Foster City, CA.

Applied Biosystems (2000) AmpF*l*STR® Profiler Plus™ Amplification Kit User's Manual. Foster City, CA.

Applied Biosystems (2001) AmpF*l*STR® Identifiler™ Amplification Kit User's Manual. Foster City, CA.

Brandstätter, A., Parsons, T.J., Parson, W. (2004) mtDNA coding region SNPs for rapid screening and haplogroup identification of forensic samples. *International Congress Series* 1261: 422-424.

Branicki, W., Kupiec, T., Wolańska-Nowak, P., Parys-Proszek, A., Ba, G. (2004) Beyond HV1 and HV2-identification of valuable mitochondrial DNA single nucleotide polymorphisms. *International Congress Series* 1261: 100-102.

Budowle, B., Wilson, M.R., DiZinno, J.A., Stauffer, C., Fasano, M.A., Holland, M.M., Monson, K.L. (1999) Mitochondrial DNA regions HVI and HVII population data. *Forensic Sci. Int.* 103: 23-35.

Budowle, B., Allard, M.W., Wilson, M.R. (2002) Critique of interpretation of high levels of heteroplasmy in the human mitochondrial DNA hypervariable region I from hair. *Forensic Sci Int.* 126(1): 30-33.

Budowle, B., Carmody, G., Chakraborty, R., Monson, K.L. (2000) Source Attribution of a Forensic DNA Profile. *Forensic Science Communications* 2(3): http://www.fbi.gov/hq/lab/fsc/backissu/july2000/index.htm (25 Oct 2004).

Butler, J.M. (2004) SNPs and Strips: Approaches to Rapid Screening of mtDNA types. *American Academy of Forensic Sciences 56th Annual Meeting, mtDNA Workshop.*

Calloway, C.D., Reynolds, R.L. *et al.* (2000) The frequency of heteroplasmy in the HVII region of mtDNA differs across tissue types and increases with age. *Am. J. Hum. Genet.* 66(4): 1384-1397.

Calloway, C.D. (2004) Applications and Validation of the Linear Array™ Mitochondrial DNA HVI/HVII Region-Sequence Typing Kit. *California Association of Criminalists Spring Seminar, DNA Workshop.*

Coble, M.D. Just, R.S., O'Callaghan, J.E., Letmanyi, I.H., Peterson, C.T., Irwin, J.A., Parsons, T.J. (2004) Single nucleotide polymorphisms over the entire mtDNA genome that increase the power of forensic testing in Caucasians. *Int. J. Legal Med.* 118(3): 137-146.

Coene, A., Vanderheyden, N., Gilissen, A., Cassiman, J.J., Decorte, R. (2004) Analysis of SNP-variation in the coding region of mitochondrial DNA. *International Congress Series* 1261: 97-99.

Deedrick, D.W. (2000) Hairs, Fibers, Crime, and Evidence: Part 1: Hair Evidence. *Forensic Science Communications* 2(3): http://www.fbi.gov/hq/lab/fsc/backissu/july2000/index.htm (25 Oct 2004).

Exline, D.L., Smith, F.P., Drexler, S.G. (1998) Frequency of Pubic Hair Transfer During Sexual Intercourse. *J. Forensic Sci.* 43(3): 505-508.

Forens-DNA User's Survey, posted 27 May 2004. http://groups.yahoo.com/group/forens-dna/messages

Gabriel, M.N., Calloway, C.D. *et al.* (2001) Population variation of human mitochondrial DNA hypervariable regions I and II in 105 Croatian individuals characterized by immobilized sequence-specific oligonucleotide probe analysis. *Croat. Med. J.* 42(3): 328-335.

Gabriel, M.N., Calloway, C.D. *et al.* (2003) Identification of human remains by immobilized sequence-specific oligonucleotide probe analysis of mtDNA hypervariable regions I and II. *Croat. Med. J.* 44(3): 293-298.

Gaudette, B.D. (1976) Probabilities and Human Pubic Hair Comparisons. *J. Forensic Sci.* 21(3): 514-517.

Giannelli, P. (2001) Impact of Post-Conviction DNA Testing on Forensic Science. *New England Law Review* 35(3): 627-638.

Grzybowski, T. (2000) Extremely high levels of human mitochondrial DNA heteroplasmy in single hair roots. *Electrophoresis* 21:548-553.

Hicks, J.W. (1977) *Microscopy of Hairs: A Practical Guide and Manual,* Federal Bureau of Investigation, U.S. Government Printing Office, Washington, DC

Higuchi, R. von Beroldingen, C., Sensabaugh, G.F., Erlich, H.A (1988) DNA typing from single hairs. *Nature* 332(7): 543-546.

Holland, M.M., Parsons, T.J. (1999) Mitochondrial DNA Sequence Analysis-Validation and Use for Forensic Casework. *Forensic Sci. Rev.* 11: 21-49.

Houck, M.M., Budowle, B. (2002) Correlation of Microscopic and Mitochondrial DNA Hair Comparisons. *J. Forensic Sci.* 47(5): 964-967.

Inman, K., Rudin, N. (2001) *Principles and Practice of Criminalistics: The Profession of Forensic Science,* CRC Press, Boca Raton, FL.

Keating, S.M. (1982) The Cross Transference of Pubic Hairs During Sexual Intercourse. A Preliminary Study. *Metropolitan Police Forensic Science Laboratory Report* 23: 1-6.

Keating, S.M. (1988). The laboratory's approach to sexual assault cases. Part 2: Demonstration of the possible offender. *J. Forensic Sci.* 28(2): 99-110.

Kline, M.C., *et al.* (2004) Semi-automation of mtDNA arrays: results from 666 population samples and comparisons. *Proceedings of the 14th International Symposium on Human Identification.*

Linch, C.A., Smith, S.L., Prahlow, J.A. (1998) Evaluation of the Human Hair Root for DNA Typing Subsequent to Microscopic Comparison. *J. Forensic Sci.* 43(2): 305-314.

Linch, C.A., Whiting, D.A., Holland, M.M. (2001) Human Hair Histogenesis for the Mitochondrial DNA Forensci Scientist. *J. Forensic Sci.* 46(4): 844-853.

Mann, M. (1990) Hair Transfers in Sexual Assault: A Six-Year Case Study. *J. Forensic Sci.* 35(4): 951-955.

Melton, T., Clifford, S., Kayser, M., Nasidze, I., Batzer, M., Stoneking, M. (2001) Diversity and Heterogeneity in Mitochondrial DNA of North American Populations. *J. Forensic Sci.* 46(1): 46-52.

Monson, K.L., Miller, K.W.P. Wilson, M.R., DiZinno, J.A., Budowle, B. The mtDNA Population Database: An Integrated Software and Database Resource for Forensic Comparison. *Forensic Science Communications* 4(2): http://www.fbi.gov/hq/lab/fsc/backissu/april2002/miller1.htm (10 Nov 2004).

Moretti T.R., Baumstark A.L., Defenbaugh D.A., Keys K.M., Smerick J.B., Budowle B. (2001) Validation of short tandem repeats (STRs) for forensic usage: performance testing of fluorescent multiplex STR systems and analysis of authentic and simulated forensic samples. *J. Forensic Sci.* 46(3): 647-660.

Moretti T.R., Baumstark A.L., Defenbaugh D.A., Keys K.M., Brown A.L., Budowle B. (2001) Validation of STR typing by capillary electrophoresis. *J. Forensic Sci.* 46(3): 661-676.

National Institute of Standards and Technology Certificate of Analysis (2003) Standard Reference Material® 2392, Mitochondrial DNA Sequencing (Human). http://srmors.nist.gov/certificates/2392.pdf (4 Nov 2004).

Ogle, R.R., Fox, M.J. (1999) *Atlas of Human Hair Microscopic Characteristics.* CRC Press, Boca Raton, FL.

Petraco, N., Fraas, C., Callery, F.X., DeForest, P.R. (1988) The Morphology and Evidentiary Significance of Human Hair Roots. *J. Forensic Sci.* 33(1): 68-76.

Reynolds, R., Walker, K. *et al.* (2000) Detection of sequence variation in the HVII region of the human mitochondrial genome in 689 individuals using immobilized sequence-specific oligonucleotide probes. *J. Forensic Sci.* 45(6): 1210-1231.

Roche Applied Sciences (2003) Linear Array™ Mitochondrial DNA HVI/HVII Region-Sequence Typing Kit Package Insert. Indianapolis, IN.

Saferstein, R. (2004). *Criminalistics: An Introduction to Forensic Science.* Pearson Education Inc., Upper Saddle River, NJ.

Sekiguchi, K., Sato, H., Kasai, K. (2004) Mitochondrial DNA Heteroplasmy Among Hairs from Single Individuals. *J. Forensic Sci.* 49(5): 986-991.

Serological Research Institute (2004) http://www.serological.com (20 Nov 2004).

Soules, M.R., Pollard, A.A., Brown, K.M., Verma, M. (1978) The forensic laboratory evaluation of evidence in alleged rape. *Am. J. Obstet. Gynecol.* 130: 142-147.

Smith, S.L., Linch, C.A. (1999) A review of major factors contributing to errors in human hair association by microscopy. *Am. J. Forensic Med. Pathol.* 20(3); 269-273.

Stoneking, M., Hedgecock, D. *et al.* (1991) Population variation of human mtDNA control region sequences detected by enzymatic amplification and sequence-specific oligonucleotide probes. *Am. J. Hum. Genet.* 48(2): 370-382.

Taupin, J.M. (2004) Forensic hair morphology comparison—a dying art or junk science? *Sci. Justice.* 44(2); 95-100.

Vallone, P.M., Just, R.S., Coble, M.D., Butler, J.M., Parsons, T.J. (2004) A multiplex allele-specific primer extension assay for forensically informative SNPs distributed throughout the mitochondrial genome. *Int. J. Legal Med.* 118(3): 147-157.

U.S. Census Bureau (2004). www.census.gov (19 Nov 2004).

Wilson, M.R., Stoneking, M., Holland, M.M., DiZinno, J.A. (1993) Guidelines for the Use of Mitochondrial DNA Sequencing in Forensic Science. *Crime Laboratory Digest* 20(4): 68-77.

Wilson, M.R., Polanskey, D., Butler, J., DiZinno, J.A., Replogle, Budowle, B. (1995) Extraction, PCR Amplification and Sequencing of Mitochondrial DNA from Human Hair Shafts. *Biotechniques* 18(4): 662-669.

Wraxall, B., Fedor, T., Watkins, J., Greco, T. (rev. 2002, 1999) Population Database for 9 STRs In Three Populations. Serological Research Instititute, Richmond, California.