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rec'd 10-7-03

SUPERIOR COURT OF THE STATE OF CALIFORNIA

FOR THE COUNTY OF STANISLAUS

THE PEOPLE OF THE STATE OF CALIFORNIA, Plaintiff, vs. SCOTT LEE PETERSON, Defendant.	Case No. 1056770 NOTICE OF MOTION AND MOTION IN LIMINE TO EXCLUDE MITOCHONDRIAL DNA EVIDENCE (Evidence Code Section 402) DATE: October 20, 2003 TIME: 8:30 a.m. PLACE: Dept 2

TO: STANISLAUS COUNTY DISTRICT ATTORNEY; and

TO: CLERK OF THE ABOVE-ENTITLED COURT:

PLEASE TAKE NOTICE that on October 20, 2003 at the hour of 8:30 a.m., or as soon thereafter as counsel can be heard, Defendant Scott Lee Peterson ("Mr. Peterson"), through counsel Mark J. Geragos, will move this Court for an order excluding all evidence regarding mitochondrial deoxyribonucleic acid ("mtDNA") testing and analysis. Mr. Peterson hereby also requests a hearing to determine the reliability of mtDNA sequence analysis pursuant to *People v. Kelly* (1976) 17 Cal.3d 24.

The motion is made on the grounds that [1] mtDNA testing and analysis is a novel scientific technique, which is not generally accepted in the scientific community, [2] the procedures used to analyze the mtDNA are not generally accepted in the scientific community, and [3] the statistical probability of the mtDNA analysis in the instant case was not only ambiguous, it was insignificant and therefore incapable of helping the fact finder determine a fact in dispute. Alternatively, if the Court finds that mtDNA testing meets the requirements for admissibility under the *Kelly/Frye* standards, Mr. Peterson respectfully moves the Court to exclude the mtDNA evidence on the grounds that there was a complete break in the chain of custody based on the handling of the evidence items and the search conducted by the Modesto Police Department.

The motion is based on this notice of motion, the memorandum of points and authorities served and filed herewith, the attached declaration, on all the papers and documents on file in this action, and on such oral and documentary evidence as may be presented at the hearing on the motion.

Dated: October 6, 2003

Respectfully submitted,
GERAGOS & GERAGOS

By: _____
MARK J. GERAGOS
Attorney for Defendant
SCOTT LEE PETERSON

MEMORANDUM OF POINTS AND AUTHORITIES

I.

INTRODUCTION

The issue presented is one of first impression in this state – whether mtDNA testing meets the legal requirements for admissibility of novel scientific evidence and, if so, whether the basis for the calculation of statistical probability employed by the testing laboratory satisfied the foundation requirements of *People v. Kelly, supra.*

Mr. Peterson hereby moves this Court to exclude evidence of mtDNA testing and analysis pursuant to the standard set forth in *People v. Kelly, supra*, which standard requires, *inter alia*, that the reliability of a new technique has gained general acceptance in the relevant scientific community, and that the methods used to calculate the statistical probability of a match be reliable and scientifically valid. As set forth below, mtDNA testing is a novel scientific technique, which has not yet acquired general acceptance by experts in the relevant community. MtDNA is greeted by experts with great skepticism because it presently lacks the reliability and exactitude that is required before evidence of mtDNA analysis should be admitted in criminal cases.

Furthermore, the mtDNA evidence in this case must be excluded based on yet another reason: the negligent handling of the evidence items and the way in which the search was conducted by the Modesto Police Department. In fact, the evidence sought to be introduced at the preliminary hearing came about after a complete break in the chain of custody during which two Modesto Police Department detectives unilaterally decided to check out of property what was alleged to be a single hair (Evidence Item #144a). Miraculously, this single hair then somehow spontaneously multiplied into several hairs. This observation and spontaneous generation of "hairs" occurred without anyone present from the Department of Justice laboratory observing and while the Modesto Police officers were reviewing the evidence alone and without any independent supervision.

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II.

STATEMENT OF FACTS

On or about December 27, 2002, the Modesto Police Department ("MPD") executed a search warrant at the Peterson residence located at 523 Covena Avenue, Modesto, California. As indicated in the reports prepared by the officers, the search warrant team conducted an in depth examination of the entire residence. Thereafter, on the same day, the same officers who searched the Peterson residence served a search warrant and searched Mr. Peterson's business warehouse located at 1027 North Emerald Avenue, Suite B-1, in Modesto, California. During the search of the warehouse, numerous items of evidence were collected by the officers. Among the various items collected was a pair of needle nose pliers found at the bottom of a boat, identified as Evidence Item #144. Supposedly, there was a **single** "black colored hair" attached to the pliers, which, according to police reports, appeared to be approximately 5-6 inches in length. The hair itself was collected as Evidence Item #144a. The plier was photographed with one hair and a specific numbered placard in the picture. Indeed in the multiple reports prepared by the officers present during the search of the warehouse over the next two months it was indicated that only a **single** hair was collected. The picture taken at the search shows only a single hair.

Nearly three months later, on February 12, 2003, officers Al Brocchini and Dodge Hendee apparently decided to do their own forensic examination of the heretofore single hair. At that time, the officers claim to now have observed two hairs. The officers, apparently believing their own forensic skills had been exhausted, then decided to forward a hairbrush belonging to Laci Peterson to be examined for hairs and compared with that recovered from the pliers to the Department of Justice.

On February 13, 2003, the hair from the pliers, along with the supposed "new" second strand of hair, and two hair brushes used by Laci Peterson were submitted to the Department of Justice ("DOJ") crime laboratory by an L. Conner of the Modesto Police Department. On February 26, 2003, Criminalist Rod Oswalt performed an examination

and opined that the two strands of hair and at least a portion of the head hairs recovered from the hair brushes "could have been donated by the same individual".

The hairs were apparently analysed four months later and because they did not contain nuclear DNA, the testing and analysis was accomplished by the use of a more novel scientific technique involving the analysis of mitochondrial DNA ("mtDNA"), or DNA obtained from the mitochondria of the cell rather than from the nucleus. This technique is specifically what the defense challenges by way of this motion.

III.

THE COURT SHOULD EXCLUDE EVIDENCE OF MTDNA TESTING BECAUSE IT FAILS TO MEET THE STANDARDS ARTICULATED IN *PEOPLE VS. KELLY.*

A. Applicable Legal Standard - *People v. Kelly.*

The party attempting to introduce evidence that is based on a new or novel scientific technique bears the burden of establishing the reliability of that evidence before it is admitted under the rule of *People v. Kelly*,17 Cal.3d 24, 30 (1976) and *Frye v. U.S.*, 293 F. 1013, 1014 (1923). The *Frye* rule was expressly adopted in California in *Huntington v. Crowley* (1966) 64 C2d 647, 653, and reaffirmed in *Kelly*. The *Kelly* rule is based on the notion that juries may give undue weight to experimental techniques presented by credentialed experts whose testimony may convey an unjustified aura of scientific certainty. The rule only tests the fundamental validity of the new scientific technique. The degree of professionalism with which the methodology is applied is relegated to the weight of the evidence. *See People v. Cooper* (1991) 53 C3d 771, 812; *People v. Farmer* (1989) 47 C3d 888.

Under the *Kelly/Frye* rule, a proponent of evidence which is derived from a new scientific methodology must satisfy three prongs, by showing, first, that the reliability of the new technique has gained general acceptance in the relevant scientific community, second, that the expert testifying to that effect is qualified to do so, and, third, that correct scientific procedures were used in the particular case. *People v. Leahy* (1994) 8 Cal.4th

587, 612; *People v. Jackson* (1996) 13 Cal.4th 1164, 1212; *People v. Venegas* (1998) 18 Cal.4th 47, 81.

As explained below, although the scientific validity and reliability of *nuclear* DNA testing has previously been upheld by courts in California and courts in other jurisdictions, the scientific validity of the significantly different mtDNA testing has never been subject to a *Kelly/Frye* analysis and has never been upheld in a California court.

In fact, there is only one California case which involves mtDNA testing, and in that case the court does not even address the admissibility of mtDNA and the case itself is unpublished. *See People v. Gomez*, 2003 WL 21675518 (Cal.App.6 Dist.) (2003). In *Gomez*, the defendant sought to compel a third party to submit a sample for mtDNA analysis to determine if the third party could be connected to a burgundy shirt that had a single hair attached to it. In denying the defendant's request, the court stated, *inter alia*, that "the fact that counsel sought only mitochondrial DNA testing is significant because mitochondrial testing "**is not a unique identifier because it is shared by individuals with a given maternal line.**" *People v. Gomez*, 2003 WL 21675518 (Cal.App.6 Dist.) (2003), *citing State v. Pappas*, 256 Conn. 854, 882 (2001) (emphasis added).

Furthermore, the analysis set forth below clearly establishes that mtDNA evidence lacks the reliability and exactitude required to be admissible in court.

B. *Kelly's* First Prong - MtDNA Is Not Generally Accepted in the Scientific Community.

The admissibility of expert testimony based on "a new scientific technique" requires proof of its reliability–i.e., that the technique is "'sufficiently established to have gained general acceptance in the particular field to which it belongs'" *People v. Frye*, 293 F. at 1014. Moreover, a witness testifying to such reliability "must be properly qualified as an expert to give an opinion on the subject." *Kelly*, F. at 30. As discussed below, mtDNA fails to meet the first prong of *Kelly/Frye* as it lacks reliability, has many disadvantages, is greeted with skepticism, and is not generally accepted in the scientific

community.

1. Overview of mtDNA Testing and Analysis.

Since the admissibility of mtDNA evidence is an issue of first impression in this state, it is helpful to review the process of mtDNA sequence analysis. Mitochondrial DNA analysis was first implemented for forensic purposes by the Federal Bureau of Investigation in June of 1996. MtDNA is used in cases where the source typically does not contain sufficient DNA for nuclear DNA analysis, such as bones, teeth, and hair.

DNA is the genetic material carried by each living organism. DNA molecules are replicated in the cell and copies are transmitted from generation to generation. The vast majority of the DNA in a cell is stored in cell centers called the "nucleus," and the DNA found there is termed "nuclear DNA." Its length and sequence are the result of the combination of two different sets of DNA, a set inherited from the mother, and a set inherited from the father. With the exception of identical twins, no two human beings have exactly the same DNA. *See* Exhs.1A-D, containing several articles about mtDNA.

Mitochondria, however, are much smaller molecules that significantly differ from nuclear DNA not only in location but also in sequence and mode of inheritance. A mitochondrion is a compartment in the cell known as the "powerhouse" because it is responsible for providing the cell with energy. The DNA located within mitochondria is called mitochondria DNA or mtDNA. *See* Exhs.1A-D.

MtDNA differs from nuclear DNA with respect to its location within a cell, and more importantly, its uniqueness among individuals, sequence length and its mode of inheritance. First, mtDNA is found within mitochondria, which are circular structures surrounding the cellular nucleus that provide a cell with energy. Second, mtDNA, unlike nuclear DNA, **cannot be used to establish positive identification because mtDNA consists of but a single "marker" that is approximately 16,569 base pairs in length.** A matching sequence offers only probabilistic evidence of identity or non-identity. By comparison, nuclear DNA consists of approximately three billion base pairs and many discrete markers, or loci, that may be compared to establish a positive match between

DNA samples. Because mtDNA has only one marker, **the probability of a random match is much higher between mtDNA samples than between nuclear DNA samples.** As a result, mtDNA is significantly less probative of identity than is nuclear DNA. Finally, whereas nuclear DNA is inherited from both parents, mtDNA is inherited maternally. Consequently, mtDNA cannot discriminate between two individuals who are maternally related, as nuclear DNA analysis is able to do. *See* Exhs.1A-D.

2. Advantages/Disadvantages of mtDNA Analysis:

As discussed mtDNA has advantages and disadvantages as a forensic typing locus, especially compared to the more traditional *nuclear* DNA markers that are typically used. As mentioned above, mtDNA is maternally inherited, so that any maternally related individuals might be expected to share the same mtDNA sequence. However, because of meiotic recombination and the diploid (bi-parental) inheritance of nuclear DNA, the reconstruction of a nuclear profile from even first degree relatives of a missing individual is rarely this straightforward. The maternal inheritance pattern of mtDNA is therefore also considered problematic. Because all individuals in a maternal lineage share the same mtDNA sequence, mtDNA cannot be considered a unique identifier. In fact, apparently unrelated individuals might share an unknown maternal relative at some distant point in the past. *See* Exh. 1A.

Furthermore, the substitution or change rate for mtDNA is significantly high. This means that a higher number of cases, than originally expected, have been found and will be found where mother and child do not match. MtDNA is often employed to compare questioned samples to presumed maternal references. However, because the mtDNA substitution rate is sufficiently high, the differences between true maternal relatives will be encountered frequently, thus providing the grounds for false inclusions. *See* Exhibits.1C-D.

Additionally, at the present time the available database of human mitochondrial DNA sequences is in its early days of existence, with around 5000 sequences available for a search of a casework sequence. Because of the relatively small size of this database

compared to nuclear DNA databases, the current convention in the event of an inclusion (a match between questioned and reference sample sequences) is for the analyst to report the number of times the observed sequence is present in the database to provide some idea of its relative frequency in the database. *See* Exh.1A.

Therefore, due to the various disadvantages and inadequacies of mtDNA testing and analysis described above, the first prong of *Kelly* is not met.

C. *Kelly's* Third Prong - The mtDNA Procedures Used in this Case are Not Generally Accepted in the Scientific Community.

The third prong of the *Kelly* foundational test of admissibility of evidence based on new scientific technique, inquires into whether procedures actually utilized in the case were in compliance with methodology and technique generally accepted by the scientific community. Cal. Evid. Code section 402; *see also People v. Barney*, 8 Cal.App.4th 798, 825 (1992). The third prong is case specific and cannot be satisfied by relying on a published appellate decision. *People v. Venegas*, 18 Cal.4th 47 (1998). A hearing is necessary to determine whether proper scientific procedures were used and whether the statistical data derived from the mtDNA test is correct.

1. Methods Utilized by FBI to Conduct mtDNA Analysis.

In the case at hand, the mtDNA examination was conducted by using a population database, identified as CODISmt version 1.2, containing 5071 individuals,[1] and the published Cambridge Reference Sequence (rCRS)[2]. As discussed above, it is critical in a *Kelly/Frye* analysis to determine whether the proponent of the evidence used the correct scientific procedures in its mtDNA calculations.

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[1]The CODIS was developed by the FBI as a national database containing DNA profiles of convicted felons. CODIS allows law enforcement at all levels to compare DNA profiles electronically.

[2]CRS is the mtDNA sequence against which the mtDNA sequences generated are compared. It was determined by a group of researchers as being the most common sequence found in native Europeans.

2. Statistical Significance of mtDNA Sequence Match.

Assuming *arguendo* that the prosecution can meet the requirements of *Kelly/Frye*, the statistical probability of the mtDNA analysis in the instant case is so insignificant and ambiguous that it is not capable of helping the fact finder determine a fact in dispute.

To assess the probability in question, one needs to calculate how frequently each mtDNA sequence is found in a target population. Thereafter, one must calculate the statistical probability that the DNA sequence of one person, selected at random from the relevant population, would likewise have a DNA sequence matching that of the evidentiary sample. That probability is usually expressed as a fraction–i.e., the probability that one out of a stated number of persons in the population (e.g., 1 out of 100,000) would match the DNA profile of the evidentiary sample in question. A greater probability, that is to say, a fraction with a smaller denominator (e.g., 1 out of 10,000), would tend to favor the suspect by increasing the probability that one or more other persons has a DNA profile matching the evidentiary sample. *See* Exhs. 1A-D; *People v. Soto*, 21 Cal.4th 512 (1999).

In order to calculate the statistical significance of the match within a particular racial or ethnic population, tests are performed to determine the frequency of appearance of the different bands within the target population. Thus, a database would be created by selecting a number of people from the relevant population which would be, theoretically, the same population to which the suspect belonged. Therefore, if the suspect was Hispanic then the Hispanic database would be employed to establish a frequency of occurrence of a given sequence pattern within the Hispanic population. The underlying theory behind all of this is that the ratio of sequence patterns will vary among different racial and ethnic groups. In other words, while a DNA sequence pattern may not be distinct to particular racial or ethnic groups, it may occur with different frequency within different racial or ethnic groups.

As set forth in *People v. Axel*, 235 Cal.App.3d 836 (1991), once a match has been declared, the next step is to determine its statistical significance. "To make a statistical

evaluation of the data obtained from a DNA typing, it is necessary to know how frequently in the population a band of a certain size will be found, a question answered according to the principles of population genetics. *Axell*, 235 Cal.App.3d at 846. *Axel* utilized an ethnic database to reach a statistical probability. *Axel* concluded that the "calculation of statistical probability is an integral part of the process and the underlying method of arriving at that calculation must pass muster under *Kelly/Frye*." *Axell*, 235 Cal.App.3d at 866-867. Where DNA results are so unreliable or completely lack evidentiary foundation, they are inadmissible as a matter of law.

Furthermore, as the Delaware Supreme Court noted in *Nelson v. State*, 628 A.2d 69 (Del.1993), involving comparison of nuclear DNA samples, "'[t]o say that two patterns match, without providing any scientifically valid estimate ... of the frequency with which such matches might occur by chance, is meaningless.'" *Nelson*, 628 A.2d at 76. Indeed, courts have even considered the statistical calculation step as the more important of the two pieces of information which constitute DNA evidence. *U.S. v. Porter*, 618 A.2d 629, 640 (D.C.1992). The Court in *Nelson* held that it was error for the trial court to admit evidence of a match after finding the corresponding statistical calculation to be inadmissible as scientifically unreliable. *Nelson*, 628 A.2d at 76.

In conclusion, although mtDNA testing may be accepted as a reliable technique in research laboratories, the use of mtDNA technology for criminal identification of forensic samples is not necessarily accepted as reliable in the scientific community. There is simply not a sufficient body of research or literature to determine the likelihood or unlikelihood of false positives under these forensic conditions.

IV.

THE COURT SHOULD EXCLUDE THE EVIDENCE BASED ON THE BREAK IN THE CHAIN OF CUSTODY.

An improper chain of custody precludes testimony or evidence on the issue involved. The chain of custody is established when the party offering a particular item in

evidence shows that it is reasonably certain the evidence has not been altered. *See People v. Lucas* (1995) 12 C4th 415, 444; *People v. Diaz* (1992) 3 C4th 495. The requirement of reasonable certainty is not met when some vital link in the chain of possession is not accounted for, because then it is as likely as not that the evidence analyzed was not the evidence originally received. Left to such speculation the court must exclude the evidence. *People v. Catlin*, 109 Cal.4th 81 (2001).

In the case *sub judice* it is reasonably certain the evidence has been altered in some way. The numerous reports prepared immediately after the search of the warehouse detailing the items seized and the observations made by the detectives, all indicate that only a **single** black hair was recovered. A vital link in the chain of custody is that the evidence was originally one hair and later is submitted for testing as two hairs. The break in the chain of custody begins when two untrained officers spontaneously decide to review what was originally characterized as a single black hair (Evidence Item #144a). Upon their review, these two Modesto Police officers supposedly found a second strand of hair while reviewing the evidence alone and without any supervision by a criminalist or lab technician.

V.

CONCLUSION

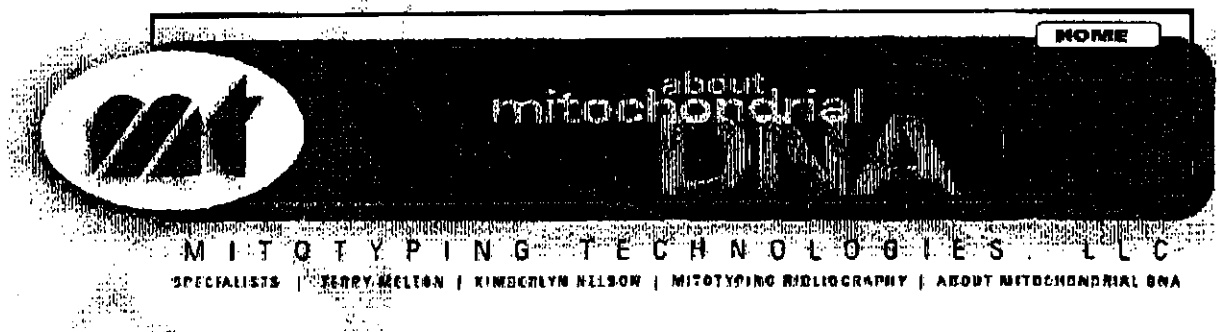
WHEREFORE, in light of the foregoing, Mr. Peterson respectfully moves this Court for an order excluding all evidence regarding mtDNA testing and analysis.

Dated: October 6, 2003

Respectfully submitted,
GERAGOS & GERAGOS

By: _____
MARK J. GERAGOS
Attorney for Defendant
SCOTT LEE PETERSON

EXHIBITS 1A-D



Mitochondrial DNA (mtDNA) provides a valuable locus for forensic DNA typing in certain circumstances. The high number of nucleotide polymorphisms or sequence variants in the two hypervariable portions of the non-coding control region can allow discrimination among individuals and/or biological samples. The likelihood of recovering mtDNA in small or degraded biological samples is greater than for nuclear DNA because mtDNA molecules are present in hundreds to thousands of copies per cell compared to the nuclear complement of two copies per cell. Therefore, muscle, bone, hair, skin, blood and other body fluids, even if degraded by environmental insult or time, may provide enough material for typing the mtDNA locus. In addition, mtDNA is inherited from the mother only, so that in situations where an individual is not available for a direct comparison with a biological sample, any maternally related individual may provide a reference sample.

A mtDNA analysis begins when total genomic DNA is extracted from biological material, such as a tooth, blood sample, or hair. The polymerase chain reaction (PCR) is then used to amplify, or create many copies of, the two hypervariable portions of the non-coding region of the mtDNA molecule, using flanking primers. Primers are small bits of DNA that identify and hybridize to or adhere to the ends of the region one wishes to PCR amplify, therefore targeting a region for amplification and subsequent analysis. Care is taken to eliminate the introduction of exogenous DNA during both the extraction and amplification steps via methods such as the use of pre-packaged sterile equipment and reagents, aerosol-resistant barrier pipette tips, gloves, masks, and lab coats, separation of pre- and post-amplification areas in the lab using dedicated reagents for each, ultraviolet irradiation of equipment, and autoclaving of tubes and reagent stocks. In casework, questioned samples are always processed before known samples and they are processed in different laboratory rooms. When adequate amounts of PCR product are amplified to provide all the necessary information about the two hypervariable regions, sequencing reactions are performed. These chemical reactions use each PCR product as a template to create a new complementary strand of DNA in which some of the As, Ts, Cs, and Gs (nucleotide bases) that make up the DNA sequence are labeled with dye. The strands created in this stage are then separated according to size by an automated sequencing machine that uses a laser to "read" the sequence, or order, of the nucleotide bases. Where possible, the sequences of both hypervariable regions are determined on both strands of the double-stranded DNA molecule, with sufficient redundancy to confirm the nucleotide substitutions that characterize that particular sample. At least two forensic analysts independently assemble the sequence and then compare it to a standard, commonly used, reference sequence. The entire process is then repeated with a known sample, such as blood or saliva collected from a known individual. The sequences from both samples, about 780 bases long each, are compared to determine if they match. The analysts assess the results of the analysis and determine if any portions of it need to be repeated. Finally, in the event of an inclusion or match, the SWGDAM mtDNA database, which is maintained by the FBI, is searched for the mitochondrial sequence that has been observed for the samples. The analysts can then report the number of observations of this type based on the nucleotide positions that have been read. A written report is provided to the submitting agency.

While mtDNA is useful for forensic examinations, it has also been used extensively in two other major scientific realms. First, there are a number of serious human diseases caused by deleterious mutations in gene-coding regions of the mtDNA molecule, which have been studied by the medical profession to understand their mode of inheritance. In addition, molecular

anthropologists have been using mtDNA for almost a decade to examine both the extent of genetic variation in humans and the relatedness of populations all over the world. Because of its unique mode of maternal inheritance it can reveal ancient population histories, which might include migration patterns, expansion dates, and geographic homelands. Recently mtDNA was extracted and sequenced from a Neanderthal skeleton. These results allowed anthropologists to say with some conviction that modern humans do not share a close relationship with Neanderthals in the human evolutionary tree. While all the applications of mtDNA, including forensic, are relatively recent, the general methods for performing a mtDNA analysis are identical to those used in molecular biology laboratories all over the world for studying DNA from any living organism. There have been over a thousand published articles regarding mtDNA.

MtDNA has advantages and disadvantages as a forensic typing locus, especially compared to the more traditional nuclear DNA markers that are typically used. As mentioned above, mtDNA is maternally inherited, so that any maternally related individuals would be expected to share the same mtDNA sequence. This fact is useful in cases where a long deceased or missing individual is not available to provide a reference sample but any living maternal relative might do so. Because of meiotic recombination and the diploid (bi-parental) inheritance of nuclear DNA, the reconstruction of a nuclear profile from even first degree relatives of a missing individual is rarely this straightforward. However, the maternal inheritance pattern of mtDNA might also be considered problematic. Because all individuals in a maternal lineage share the same mtDNA sequence, mtDNA cannot be considered a unique identifier. In fact, apparently unrelated individuals might share an unknown maternal relative at some distant point in the past.

At the present time the available forensic database of human mitochondrial DNA sequences has around 4800 sequences available for a search of a casework sequence. The current convention in the event of an inclusion (a match between questioned and reference sample sequences) is for the analyst to report the number of times the observed sequence is present in the database to provide some idea of its relative frequency in the database. A frequency statistic may also be used, and a 95% or 99% confidence interval is placed around the calculated frequency to account for the inherent uncertainty in the frequency calculation. While most types appear to be rare or at least infrequent in each of the racial databases (African or African-origin, Asian or Asian-origin, Caucasian or European-origin, and Hispanic), there is one type which is seen in around 7% of Caucasians. However, almost two thirds of newly-typed samples have novel sequences, so we have not yet uncovered all the variation present in the general human population. For novel types, a 95% or 99% upper bound frequency calculation may be performed. In general, the pattern observed in most populations around the world, with the exception of a few populations of anthropological interest, is that the vast majority of sequences is uncommon, and relatively few types present at frequencies greater than 1% in the databases. Because of this fact, it will be possible to exclude greater than 99% of the population as potential contributors of a sample in most cases, except where one is dealing with a more "common" type.

In contrast, a multilocus nuclear DNA typing profile provides vastly superior discriminatory power, such that we can now approach the possibility that a typed individual has a unique profile with respect to any other person in the world. Therefore, mtDNA can never provide the resolution of individuality that nuclear typing can. For this primary reason, it should be reserved for cases or samples for which nuclear typing is simply not possible. Candidates for mtDNA typing analyses would most likely be: 1) shed hairs with no follicle, tissue, or root bulb attached, 2) hair shaft fragments, 3) bones or teeth which have been subjected to long periods of high acidity, high temperature, or high humidity, 4) stain or swab material that has been previously unsuccessfully typed for nuclear markers, and 5) tissue (skin, muscle, organ) that has been previously unsuccessfully typed for nuclear markers. Hair roots, when available, should be removed from the shaft and processed separately for nuclear DNA markers prior to attempting mtDNA analysis on the hair shaft. Hair shafts or fragments are only suitable for mtDNA analysis as they can contain fewer than 100 copies of the mtDNA molecule and virtually no nuclear DNA. The same is generally true for older skeletal remains. While mtDNA typing of blood stains is possible, it is more likely that mixtures will be obtained, due to the extreme sensitivity of this form of typing in samples that unlike hairs and bones are difficult to clean

before DNA extraction.

Finally, it must be noted that mtDNA analyses are the most rigorous and time-consuming of DNA forensic analyses. Based on informal statistics available from all laboratories performing these typings, the rate of throughput is approximately 1-2 cases/analyst/month. The reasons for this include: 1) small/degraded samples requiring numerous PCR reactions to obtain sufficient DNA template for sequencing, 2) exhaustive procedures to control for contamination, and 3) sequencing analyses of both strands of DNA in both hypervariable regions. In addition, for some types of samples, especially hairs, mtDNA analysis is more likely to consume the whole sample than nuclear DNA typing. For example, a single mtDNA analysis could be performed on a 0.5-2 cm hair fragment. A 4 cm fragment could have duplicate testing for confirmation of the sequence. In both cases the fragment would be totally consumed. However, a root ball, follicle, or skin tissue attached to a hair would also be consumed in a nuclear typing effort. For both mtDNA and nuclear DNA testing there is a possibility that sufficient extracted DNA might remain for duplicate testing in another lab. Swatch, swab, stain, bone, and tooth analyses are less likely to consume all material, as these samples can often be divided, although the difficulties of obtaining enough DNA for analysis could result in consumption of these materials as well. For the reasons above, pre-analysis documentation (microscopy, photography) is desirable.

Most importantly, mitochondrial DNA testing should only be performed by laboratories with considerable experience in handling the unusually difficult samples that require this form of testing. The primary reason for this is that experienced labs can extract minimal amounts of mtDNA from difficult samples. In the event of a sample failure, an inexperienced lab would never know whether their extractions and PCRs were simply not sensitive enough, or whether the sample lacked non-degraded DNA altogether. In addition, contamination controls are heightened in a mitochondrial DNA laboratory, where working at the limits of sensitivity is standard operating procedure.

Because of the advantages and in spite of the limitations mentioned above, mtDNA analysis has found a place in the forensics arena. Several dozen cases have been tried in US courtrooms using mtDNA evidence to augment more traditional forms of evidence, and several post-conviction exonerations have been obtained in cases where microscopically examined hairs have recently been analyzed for mtDNA. All appellate decisions handed down to date have upheld mtDNA testing and written decisions may be viewed at www.denverda.org. MtDNA forensic testing should be utilized primarily in situations where nuclear DNA typing is not an option, or in the event that nuclear typing has been attempted and is unsuccessful. In these cases, mtDNA typing can provide additional information about the relationship of an individual to a biological sample heretofore unavailable.

The Wellcome Trust

Fred Sanger: Fridge magnate

1/7/02. By DJS

Professors Robert Lightowlers and Doug Turnbull of the University of Newcastle's Mitochondrial Research Group owe a special debt to Fred Sanger. Not just because of his two Nobel Prizes, the second of which (for his work on DNA sequencing) laid the foundations for their field. But also for a remarkable feat of memory...

"Can you imagine? After 20 years, finding a test-tube the size of the smallest part of your little finger, somewhere in the Sanger labs, which must cover half of Cambridge!" Professor Turnbull laughs: "I suppose it fits: only a sequencer would have the right kind of mind!"

That test-tube contained the original DNA sample which, in the late 1970s, Dr Sanger's group used to sequence the first human genome - the 16 500 base pair human mitochondrial DNA. Now known as the 'Cambridge Reference Sequence' (CRS), it has been an indispensable reference for studies of human evolution, population genetics and mitochondrial disease since its publication in 1981.

Fred Sanger's group sequenced the first human genome – the mitochondrial genome – in the late 1970s.

But what did the Newcastle Group want with the original sample? The story begins in Texas, where Professors Turnbull and Lightowlers, funded by a Biomedical Research Collaboration Grant from the Wellcome Trust, were visiting Professor Neil Howell. The main aim of the study was to combine the Texas group's fundamental knowledge of mitochondrial genetics with their own more clinically related studies. They found Professor Howell increasingly frustrated by apparent differences between the CRS sequence and what was known from other research.

Mitochondrial DNA (mtDNA) has two unusual characteristics. First, it is

B

extremely variable: mutations are common. And second, it is inherited only through the maternal line: mutations can be clearly followed through generations. They act as 'markers' that help to track different human populations and ethnic groups.

But in the CRS some of these common markers 'didn't fit' - in particular, there was a non-European mutation bang in the middle of the reference sequence for European groups. Errors in the original sequencing were one possible explanation, but the differences could simply have reflected individual variation. Not knowing what lay behind the differences was a frustrating stumbling block.

After the visit, Professor Lightowlers decided to phone Professor Alan Coulson in Cambridge with a bizarre request - did any of the original material still exist? Professor Coulson enthusiastically offered to contact Dr Sanger, who had been retired for some time. "And," Professor Lightowlers continues, "He knew where it might be - not only which freezer, but whereabouts in the freezer - and he came in and found it!"

ACCURACY OF NEW DNA TEST IS CALLED INTO QUESTION

by Laurie P. Cohen

The FBI has a powerful new weapon that gets its man nearly every time it is deployed. One catch: In some cases, the weapon may demolish the innocent along with the guilty. Another catch: Defendants can use it, too.

The weapon, called mitochondrial DNA testing, is a new way to analyze crime-scene evidence. An important step beyond standard DNA testing, it has been used by the Federal Bureau of Investigation only since August 1996 and has resulted in six convictions in six attempts.

It will be tried by a defendant for the first time in the long-running saga of Jeffrey MacDonald, the former Army surgeon who was convicted in 1979 of killing his wife and two daughters but who has always insisted a marauding band of drug-crazed hippies did the deed instead. If he is right, mitochondrial DNA testing of strands of hair found under his daughters' fingernails, and preserved all these years, might yet show that he was not the killer.

Though most people outside of scientific circles have never heard of mitochondrial DNA, it has been used to identify Czar Nicholas II's bones and to prove that the body in Jesse James's grave is truly that of the outlaw. Since 1991, the military has also employed it to identify soldiers' remains.

Realm of the Living

The FBI crime lab is responsible for moving the technique into the realm of the living, despite scientific concerns about the accuracy of the method. Thanks to the FBI, mitochondrial DNA testing has already occurred in about 70 cases that haven't yet reached trial.

These days, viewers of Court TV know just about as much as the average biology student about DNA, or deoxyribonucleic acid, which contains a person's unique genetic code within the nucleus of each body cell. With the exception of identical twins, no two individuals share the same genetic code-making ordinary "nuclear" DNA testing a popular means of identification in court cases.

Since 1988, when nuclear DNA testing was first used by the FBI in criminal trials, it has played a part in more than 30,000 cases nationwide. Next year alone, the FBI predicts, it will employ the method in as many as 2,500 cases.

Nonetheless, nuclear DNA testing suffers from a major limitation: It can't

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be used unless the evidence is fresh and in good shape; crucially, there have to be cell nuclei present, and hair that has been pulled away from its roots doesn't contain any nuclei.

Whole New Worlds

In contrast, mitochondrial DNA is located outside of a cell's nucleus and is much more plentiful than nuclear DNA. Indeed, a single cell will have hundreds, even thousands, of mitochondria, tiny particles that are responsible for converting food to usable energy. That means more DNA can be extracted from smaller, older and less-well-preserved fragments of evidence.

The distinction opens up whole new worlds for prosecutors, who sometimes have nothing more to work with than a strand or two of hair or old skeletal remains. The FBI lab's small mitochondrial DNA unit in Washington, which employs just two examiners and focuses on hair analysis, is now gearing up to meet rising demand. "We have calls coming in daily from prosecutors," says Joseph DiZinno, chief of the unit.

In the scientific community, though, the much-sought-after forensic tool is being greeted with skepticism. While juries may assume one type of DNA is the same as another, the truth is that mitochondrial DNA—which is inherited from the mother's side only—doesn't provide the same kind of unique fingerprint as nuclear DNA. The same mitochondrial DNA sequence is shared by siblings and their mother and all of a person's maternal relatives for many generations. And a 1993 British study found that even among unrelated people, four out of 100 who were tested shared the same mitochondrial DNA sequence.

Reliability Concerns

So even if a defendant is linked to crime-scene evidence through mitochondrial DNA, there is a small but realistic possibility that he or she had nothing to do with the crime. "The FBI is bringing mitochondrial DNA into the courtroom and painting it with the same reliability as other DNA typing," says one critic, William Shields, a biologist at the State University of New York in Syracuse. But, he adds, "It isn't as unique to an individual as nuclear DNA."

Says Edward Blake, a DNA expert with Forensic Science Associates in Richmond, Calif.: "We don't know enough about mitochondrial DNA in hair to be giving scientific testimony about it." That's also the view of the nation's largest private DNA lab, Cellmark Diagnostics Inc., in Germantown, Md. A company spokesman says further study is needed before the lab will begin doing mitochondrial DNA analysis on hair in criminal cases.

Such resistance doesn't faze the FBI's Dr. DiZinno. "We wouldn't have gone on-line if were weren't confident this was a reliable technique," he

says. Dr. DiZinno and Mark Wilson, both former FBI hair examiners, began researching mitochondrial DNA in 1992. By August 1996, they were ready to unveil it in a Chattanooga, Tenn., courtroom. There, the technique helped win the conviction of rape-and-murder defendant Paul Ware in a tangled case in which much of the other prosecution evidence was weak.

The government was fortunate to be able to use Tennessee as its proving ground for mitochondrial DNA testing. Tennessee is one of only six states in which defendants aren't entitled to pretrial hearings before DNA evidence is admitted. In most states, such a hearing would have subjected the FBI to tough questioning by defense lawyers and the judge regarding scientific procedures and validity. Only then would the judge have ruled on whether the evidence could be heard by a jury. But in Tennessee, a state statute passed in 1991, before anyone involved had heard of mitochondrial DNA testing, decreed that DNA test results are always admissible in court.

FBI Is Prevailing

In a legal system built on precedent, the successful application of the technique in Tennessee was later used by the government as a wedge to help it get mitochondrial DNA testing approved in states in which pretrial hearings are required. "The FBI was happy to legitimize mitochondrial DNA analysis in this state," says Barry Steelman, a state prosecutor who worked on the Ware case, though Dr. DiZinno says the FBI didn't have any control over where the first case would be tried.

Since the Ware case, even when the defense has presented experts to oppose mitochondrial DNA evidence, the FBI has prevailed.
Early this year, the FBI's mitochondrial tests linked a former police officer to the 1993 murder of a man in Boone, N.C. After years of delay, defense lawyer Bruce Kaplan says that mitochondrial testing produced "the only physical evidence" linking the defendant to the crime.

Mr. Kaplan says use of the test wasn't a close call for the judge, even though the defense argued that the method wasn't scientifically sound. "The FBI comes in and testifies that mitochondrial DNA has been previously admitted elsewhere and is accepted in scientific circles, and that was that," Mr. Kaplan says.

Prosecutors have also been helped by the fact that not every defense lawyer has attempted to challenge the method. "I didn't think I was going to win an admissibility hearing, so I didn't ask for one," says Fred Brown, the defense lawyer in a Waco, Texas, case in which a defendant was accused of mailing a bomb to his estranged wife. Postconviction appeals in these cases, objecting to the use of mitochondrial DNA testing, haven't yet been heard by appellate courts.

In explaining their new forensic weapon to juries, FBI agents DiZinno and Wilson won't say that mitochondrial DNA can be used to make a positive

identification. Instead, they speak of the frequency that a particular DNA sequence appears in its current database of 1,043 individuals. (Sandy Zabell, a Northwestern University math professor, has argued in court, so far unsuccessfully, that the FBI's database is too small and too narrowly drawn to lead to any conclusion at all.)

Jurors Are Confused

By contrast, in standard DNA cases, FBI agents give jurors statistics indicating the likelihood that a defendant's DNA could have come from another person. That likelihood is usually very small, on the order of one in 200 billion.

Nonetheless, the distinctions between nuclear and mitochondrial DNA appear to be lost on many jurors. Indeed, the six jurors in mitochondrial DNA cases who were interviewed for this article spoke—incorrectly— of mitochondrial DNA's powerful capacity to identify suspects.

"Is there a difference between kinds of DNA?" asks Linda Hicks, a juror in the North Carolina case. "All I can say is the DNA showed it pretty well matched" the defendant. Says Phillip Summerlin, a hospital chaplain who was a juror in the Ware case, "I thought mitochondrial DNA was a good way of identifying people." Hank Hill, the lawyer for Mr. Ware, says jurors in DNA cases have been heavily influenced by the O.J. Simpson case, which involved standard nuclear DNA testing rather than mitochondrial testing. He says they now tend to be uncritical of all DNA evidence because they believe Mr. Simpson was wrongly acquitted. "After O.J., most of middle-class America, which is where juries come from, figure, 'If you've got DNA, you have to convict,' " Mr. Hill says. "They don't distinguish between this DNA and that. It's all DNA to them."

'Fatal Vision' Case

What hurts most criminal defendants, though, may be the only thing left that can help Dr. MacDonald, who was the subject of the Joe McGinniss book "Fatal Vision" and the made-for-TV movie that followed. He has a chance to prove his innocence, after being shut down in one appeal after another, because the government itself threw open the door to a technique that courts otherwise might not have approved.

Since his conviction, Dr. MacDonald's lawyers had repeatedly tried and failed to get courts to give Dr. MacDonald a new trial. The odds of getting any court to listen were growing more difficult with time; U.S. Supreme Court rulings in recent years had made it all but impossible for prisoners who have been through the appeals process once to have their cases reopened. But last April, the much-publicized troubles at the FBI Laboratory gave Dr. MacDonald a fresh opening to try again.

Dr. MacDonald had always claimed that the intruders who had killed his family were led by a woman wearing dark clothing, a floppy hat and a long,

blond wig. His lawyers tried to reopen the case in 1990 based on the discovery of blond synthetic fibers they claimed must have come from the woman's wig. But the theory was shot down by Special Agent Michael P. Malone, at that time the top hair-and-fiber examiner in the FBI crime lab. In his affidavit in the MacDonald case, Mr. Malone said the fibers he examined came from dolls and couldn't have come from a woman's wig.

In the course of his evaluation of the fibers in the MacDonald case, Mr. Malone also examined a human hair from the crime scene that he said was "forcibly removed and appears to have a piece of skin tissue attached" to it. At the time, Mr. Malone said the hair couldn't be microscopically identified and was too old for standard DNA testing, which was the only kind then available.

Suddenly, in 1997, the new mitochondrial DNA technique—which could be used on such a piece of evidence even if no cell nuclei were present—offered Dr. MacDonald a "last-gasp claim," says Andrew Good, one of his defense lawyers. Meanwhile, in the wake of the Justice Department's April report on the crime lab, Mr. Malone's credibility was now in question.

Back to Court

The Justice Department report, which criticized 13 FBI crime-lab analysts, was particularly tough on Mr. Malone for giving inaccurate testimony in an unrelated case. Meanwhile, a front-page article in The Wall Street Journal also raised questions about the credibility of Mr. Malone's testimony in a number of cases, including Dr. MacDonald's. The Journal article reported, among other things, that Mr. Malone's conclusion about the origins of the blond fibers found at the MacDonald crime scene wasn't supported by other experts whom Mr. Malone had interviewed or by textbooks available in the FBI's own library.

Believing these revelations might influence a judicial panel, Dr. MacDonald's lawyers again asked a federal appeals court to reopen the case, this time seeking the right to conduct mitochondrial DNA testing on crime-scene evidence. To help with this new approach, they brought in DNA specialist Barry Scheck, best known for his role in cross-examining the government's DNA expert in the O.J. Simpson case.

In October, the court, without comment, granted the request. It was Dr. MacDonald's first court victory of any kind in almost two decades. "It is poetic justice that the same mitochondrial DNA testing that the FBI is using as a sledgehammer to prosecute people is the way I can now get back into court in my murder case," says Dr. MacDonald, who is serving a life sentence in Sheridan, Ore.

The fateful test in his case is expected to take place early next year.

Results are due about one month later

Mitochondrial DNA (mtDNA) - What you really need to know.

1. An mtDNA match is not conclusive in proving identity. A matching sequence offers only probabilistic evidence of identity or non-identity.

2. A difference in mtDNA sequences is not conclusive in proving that two samples come from different individuals.

3. Four unrelated individuals in the original British Caucasian Database of 100 have the same mtDNA sequences in the control region and other pairs of unrelated individuals are also identical.

4. At least 178 individuals in the total database of about 1000 individuals match at least one other unrelated person in the database.

5. The substitution or change rate for mtDNA is much higher than anticipated. This means that a higher number of cases, than originally expected, have been found and will be found where mother and child DO NOT match.

The use of mtDNA testing as the sole means of identification, must be stopped! At this stage, it's use as the primary evidence for identification is probably dangerous.

Here is some of what the scientific community has to say:

From Nature Genetics Volume 15 April 1997 ' "A high observed substitution rate in the human mitochondrial DNA Control Region" by Holland, Parsons et al. - "Our results have implications for the use of CR sequences in forensic identity testing, **mtDNA is often employed to compare questioned samples to presumed maternal references. It is now clear that the mtDNA substitution rate is sufficiently high that differences between true maternal relatives will be encountered not infrequently, providing the grounds for false exclusion."**

From Science Vol. 279 2 January 1998 - "...Parsons and Holland, in their work identifying 220 soldiers' remains from World War II to the present, now have new guidelines - adopted by the FBI as well - to

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account for a faster mutation rate. When a missing soldier's or criminal suspect's mtDNA comes up with a single difference from that of a relative or evidence at a crime scene, the scientists no longer call it a "mis-match." Instead the results are considered "inconclusive."

From New Scientist February 28th, 1998 - **"Bearing False Witness Chance Matches Are Much More Likely with mtDNA Tests" - "A type of genetic fingerprinting with a high chance of producing a false match** has helped convict six people in the US. The increasing use of this type of DNA evidence, based on DNA from the mitochondria (mt) of a cell, **has sparked heated debate about whether it should be admissible in courts at all...."**

"Because of these limitations, **Britain's Forensic Science Service in Birmingham uses mt DNA only to eliminate suspects or to back up other pieces of evidence,** Dave Werrett, director of research and DNA services at the FSS says it is vital that expert witnesses explain to the jury that a random match is far more likely with mt DNA. "What we do is put all the caveats up front."

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DOCUMENT THREE

MOTION IN LIMINE TO EXCLUDE GPS TRACKING EVIDENCE