Indexed as:

R. v. Murrin

Between
Her Majesty the Queen, and
Shannon Leonard Murrin

[1999] B.C.J. No. 2715
Vancouver Registry No. CC971114

British Columbia Supreme Court
Vancouver, British Columbia
Henderson J.

Heard: April 1, 6 - 9, 12, May 10 - 14, 17 - 20, 31,
June 1 - 3 and 7 - 11, 1999.
Judgment: November 17, 1999.
(134 paras.)

Counsel:

J. Wood, Q.C., P. Johnstone and D. Neave, for the Crown.
P. McMurray and P. Wilson, for the accused.

RULING

RE: ADMISSIBILITY OF MITOCHONDRIAL DNA EVIDENCE

¶ 1 **HENDERSON J.**:— The prosecution is tendering, for the first time in a Canadian courtroom, evidence of the results of a mitochondrial DNA ("mtDNA") analysis. This type of analysis is to be distinguished from nuclear DNA analysis, from which it differs in important respects. Since mtDNA analysis is a novel scientific theory or technique, this voir dire has been conducted to determine whether the evidence meets the criteria of admissibility set out in R. v. Mohan (1994), 89 C.C.C. (3d) 402, [1994] 2 S.C.R. 9, 114 D.L.R. (4th) 419 (S.C.C.).

¶ 2 Shannon Murrin is charged with the first degree murder of Mindy Tran, a young friend of a girl living in the same residence as Murrin in Kelowna. She disappeared on the night of August 17, 1994 after last being seen in the area of Murrin's residence. Her body was found two months later in a shallow grave in a nearby park.

¶ 3 There is no issue that Mindy Tran was murdered. Mr. Murrin has denied having had any contact with her on the night in question and has provided details of his alibi to

the police. Thus, the sole issue is whether the Crown can prove beyond a reasonable doubt that Murrin killed Mindy Tran. The Crown alleges that three hairs were left by the killer at the crime scene, and that they contain the same mtDNA sequence as blood samples taken from Mr. Murrin.

The Issues

¶ 4 The accused concedes that mtDNA analysis will at some point become a viable scientific technique. He argues that, in its present state of development, the difficulties and uncertainties render the technique insufficiently reliable for consideration by the trier of fact.

¶ 5 The accused's first point is that the danger of contamination is so great, and its potential to produce erroneous results so high, that mtDNA results are inherently unreliable.

¶ 6 Second, he argues that the relatively recent discovery of a condition known as "heteroplasmy" within the regions of the mtDNA chain which are examined provides further uncertainty. An individual is heteroplasmic if his mtDNA sequence differs slightly (usually at just one base position) from cell to cell in his body. The accused says the existence of heteroplasmy has not been provided for adequately in the data bases in which mtDNA sequences are collected. The incidence of heteroplasmy is unknown.

¶ 7 Third, he argues that fundamental aspects of the science of mtDNA are still unknown: the possibility exists that recombination and paternal inheritance contribute to mtDNA sequences, although the present technology was designed at a time when the assumption was that they do not.

¶ 8 The combination of these uncertainties, says the accused, demonstrates that mtDNA analysis is not yet ready for the courtroom.

The Crown's Circumstantial Evidence of Identity

¶ 9 When Mindy Tran's body was discovered, police investigators found three hairs in her underpants. A known sample of Mr. Murrin's blood was obtained in January 1995 from bloodied clothing seized from him at the time of his arrest. Later, a sample of his blood was taken.

¶ 10 The three hairs were unsuitable for analysis by the traditional techniques of nuclear DNA typing. By 1995, laboratories in other countries were beginning to perform mtDNA analysis, although there is still no laboratory in Canada capable of carrying out this procedure. The hairs (referred to as hairs 11, 12 and 13) were sent to the Forensic Science Services ("FSS") Laboratory in Birmingham, England, together with the known blood sample from the stained clothing, in February, 1998. The second known blood sample was sent to the FSS Laboratory for mtDNA analysis in June, 1998.

¶ 11 The analysis was performed by Mr. John Bark, the leader of the mtDNA team at the FSS Laboratory. Mr. Bark has concluded that all three hairs have the same mtDNA sequence and that it matches the sequence in the known blood samples taken from Mr. Murrin. He has also concluded (from an analysis of blood samples taken from Mindy Tran's parents) that the sequence could not be that of Mindy Tran.

¶ 12 By convention, FSS scientists use a set of descriptive phrases to describe the strength of the opinion being asserted. The descriptors used by the FSS are, in ascending order of strength: inconclusive, weak support, moderate support, moderately strong support, strong support, very strong support, and conclusive. It is not the custom of FSS scientists to provide statistical estimates of the frequency with which a particular mtDNA sequence is found in the general population.

¶ 13 Mr. Bark will testify that his analysis provides "strong support" for the hypothesis that the three hairs originated from Shannon Murrin or, in the alternative, from any person related to him in the maternal line. He will also testify that the sequence found in the hairs is an uncommon one. Mr. Bark compared the sequence to several data bases containing collections of sequences obtained at random. He observed the questioned sequence once in a collection of 163 sequences from unrelated British caucasians and once in a collection of 233 unrelated North American caucasians. He also observed the sequence twice in a larger collection, the "Miller Concordance", which consists of 4,774 sequences.

¶ 14 Forensic scientists at the Federal Bureau of Investigation ("FBI") are less reticent about statistical estimates. The Crown intends to call evidence from FBI scientists who have compared the sequence from the three hairs to the sequences in an American mtDNA data base. These experts will say that 99.76% of the general population of caucasians have sequences which differ from that found in the three hairs. The statistical analysis leading to this opinion provides a 95% confidence level.

¶ 15 The defence opposes the admission of all of this evidence, arguing that the technique of mtDNA typing is so new, and so fraught with as-yet-unresolved problems, that it does not meet the threshold level of reliability contemplated in Mohan.

¶ 16 This voir dire, which lasted 24 days, has examined the development and present state of the science of mtDNA typing in considerable detail. The Crown called Dr. Gillian Tully and Mr. John Bark from the FSS and Dr. Bruce Budowle and Mr. Mark Wilson of the FBI. The defence called three experts: Dr. Donald Riley , Dr. William Shields, and Dr. Peter D'Eustachio. All are well qualified scientists, although they have varying levels of experience with mitochondrial (as opposed to nuclear) DNA analysis.

Mitochondrial DNA Sequences

¶ 17 Many of the mechanical steps in mtDNA analysis are similar to those in nuclear DNA analysis. There are, however, important differences, and it was these differences which provided the focus of this voir dire.

¶ 18 The "backbone" of a DNA molecule contains pairs of chemicals called nucleotides, or "bases". There are four different bases, known as guanine ("G"), cytosine ("C"), adenine ("A"), and thymine ("T"). The bases pair up to form "base pairs". G always pairs with C and A always pairs with T. A particular order, or sequence, in which these base pairs appear along the backbone of the molecule provides the property which makes DNA a useful tool in the identification of humans.

¶ 19 Most cells in the body contain DNA. The vast majority of DNA in a cell is stored in the nucleus. This nuclear DNA is the result of the combination of two different sets of DNA inherited from the subject's mother and father.

¶ 20 Another type of DNA is found in a different part of the cell called the mitochondria. It is a much smaller molecule and differs from nuclear DNA in its location, sequence, and mode of inheritance.

¶ 21 Cells may contain thousands of mitochondria, each of which may contain many copies of mtDNA. Thus, there are far more copies of mtDNA present in a cell, although nuclear DNA is much larger than mtDNA. This has important consequences. The greater prevalence of mtDNA makes it possible to extract useful information in circumstances where nuclear DNA analysis is not possible. On the other hand, the smaller size presents problems and dangers which will be discussed below.

¶ 22 Mitochondrial DNA is inherited only from the mother. Although no two people (with the exception of identical twins) have the same nuclear DNA sequence, all maternally related individuals will (in the absence of a mutation) have the same mtDNA sequence. This fact can be both an advantage and a limitation. On the one hand, it is possible to infer the mtDNA sequence of a subject by obtaining and analyzing a blood sample from, for example, her brother or father. On the other hand, mtDNA analysis is capable only of suggesting that the suspect, or any other person related to her in the maternal line, could have left the identifying material at the crime scene.

¶ 23 Base pairs are arranged along the DNA backbone in a sequence which differs between individuals. The sequence can be expressed as a string of letters, each identifying a base, such as this: GCATTGCGTACAGTC. There are approximately 16569 bases in a complete human mtDNA sequence.

¶ 24 A complete sequence, known as the "Anderson sequence", has been published and is used as a reference. In any human sequence, most of the bases will be identical to the bases in the Anderson sequence at the same position. Each base pair in the Anderson sequence is assigned a position number. In forensic testing, a sequence is described by listing only the position numbers of bases which differ from those in the Anderson sequence. The following hypothetical sequence

Position	Base
78	A

265	C
16129	C
16342	T

shows that the subject differs from the Anderson sequence at only four base positions and specifies the bases found in the test sequence at those positions.

¶ 25 There are two regions in the mtDNA sequence, called "hypervariable" regions 1 and 2 ("HV1" and "HV2") or, collectively, the "control" regions, which show the greatest degree of variability between unrelated individuals. As a consequence, mtDNA analysis focuses on these regions. The FBI analyzes approximately 600 base pairs in the two regions while the FSS, although focusing on the same two regions, extends its analysis to about 780 base pairs (this is one of several differences in the way in which the two labs operate). On average, a caucasian differs from the Anderson sequence in about eight base positions. The known and questioned samples in the present case contain a sequence with 16 deviations from the Anderson sequence.

How the Process Works

¶ 26 The first step in the analysis of hair is the washing of the questioned sample to remove any contaminating materials found on it. Inadequate washing could cause the resulting sequence to represent the contaminating material rather than the sample itself. The FBI gives hair a detergent treatment at high temperature in a sonicator. The FSS agitates the hair in a solution in a vortexer. The defence argues that the FSS method is inferior and can lead to errors.

¶ 27 To extract DNA from the hair after washing, both labs follow a process of organic extraction with filtration and precipitation. These techniques are well known and have been used for years in nuclear DNA analysis.

¶ 28 After extraction comes amplification by a Polymerase Chain Reaction ("PCR") . The two strands of the DNA double helix are separated from each other by heating. Certain primers and an enzyme are used to copy the existing DNA in cycles. Each cycle doubles the amount of DNA. The FBI uses 36 cycles. The FSS uses a "nested" process which results in the use of 50 cycles. The number of cycles is important - the higher the number, the greater the risk that contamination will cause erroneous results. While the PCR process is also used in nuclear DNA analysis, mtDNA testing uses more cycles and thereby poses an increased risk of error.

¶ 29 After amplification, the FBI uses a capillary electrophoresis machine to quantify the amount of DNA which has been produced. Amplification may have to be repeated if the PCR was unsuccessful. The result of the PCR may contain a "mixture", ie. two or more DNA sequences. If so, the additional sequences will be the result of contamination. As long as the quantity of DNA from the test sample is at least ten times as great as the quantity from the contaminating material, the FBI will proceed to the next step.

¶ 30 The FSS does not use capillary electrophoresis to quantify the DNA produced during amplification. It uses an agarose gel electrophoresis to permit the examiner to inspect the material visually and apply her experience and judgement to determine whether the contamination level is high enough to invalidate the results. The FBI's 10:1 rule is not used.

¶ 31 The next step is sequencing. The FSS use dye primer chemistry to prepare the DNA for sequencing. The FBI technique, which is very similar, is called dye terminator chemistry. The resulting solution is sequenced automatically by a computer which labels the bases and produces a "chromatogram", a graph with four horizontal lines showing which of the four nucleotides is found at each position.

¶ 32 A trained examiner can label the bases more accurately than the software currently in use. The base calls by the computer are always checked independently by an examiner. Both the FSS and the FBI require that a second examiner, acting independently and without knowledge of the first examiner's decisions, check the bases assigned by the computer and amend them as needed. (In the instant case, the FSS had a third examiner check the bases as well.) In the five years that Mr. Bark has been doing mtDNA analysis, he has never seen an instance of examiners disagreeing on a base call.

¶ 33 For a variety of reasons, some bases may appear ambiguous; these are assigned an "N". Where a sequence is heteroplasmic at a certain base, that is recorded as an "N" in the database. The FSS uses an internal guideline of a maximum of ten N's in 100 bases. Sequences exceeding that level are not "reportable" and cannot be relied upon.

¶ 34 After the known and questioned samples have been sequenced, the two sequences are compared. If there is a match at every base position, sequence "concordance" has been established.

¶ 35 In the present case, there were no caucasians in the FBI data base with an "N", or inconclusive, value at a base position who would otherwise have matched Mr. Murrin's sequence. Aside from the one sequence which did match, the closest differed by five base pairs.

¶ 36 The experts do not purport to be offering an opinion on identification of the person who left the questioned sample. This distinguishes mtDNA typing from fingerprinting. An inculpatory opinion is to the effect that the accused, because an mtDNA sequence matching his was found at the crime scene, "cannot be excluded" from the group of people who could have deposited the questioned sample there. Any other person with the same mtDNA sequence could also have done so. Since mtDNA is inherited through the maternal line, any person maternally related to the accused is included in the group of possible donors.

¶ 37 An exculpatory opinion is called an "exclusion"; the suspect is excluded from the group of possible donors of the questioned sample. Because of heteroplasmy, a difference between the known and questioned samples of a single base pair is not

sufficient to result in an exclusion. The FBI deems such results "inconclusive". A difference of two base pairs or more is required by the FBI for an exclusion.

¶ 38 The FSS approaches the heteroplasmy question in a different and somewhat more flexible manner. A single base pair difference is scrutinized by the FSS examiner, together with all other information derived from the analysis, and a judgement is made as to whether the difference is the result of heteroplasmy. If it is, the donor of the sample is not excluded. In fact, heteroplasmy could account for a two-base or even a three-base difference, and the FSS recognizes the remote possibility that an examiner will decide there is no exclusion even with a difference of this magnitude.

¶ 39 Once it is determined that the known and questioned samples show a sequence concordance, the ultimate question is: how often does this sequence appear in the population as a whole? Opinions in this area fall in the realm of population genetics.

¶ 40 The FBI, the FSS, and a number of other organizations maintain data bases of mtDNA sequences observed in the population. By searching these data bases, one can determine how frequently the sequence of interest appears. For example, Mr. Wilson of the FBI will say that Mr. Murrin's sequence has been observed once in the TWGDAM data base of 1,219 caucasians. The Technical Working Group on DNA Analysis and Methods ("TWGDAM") was a group of forensic scientists, largely American, who met regularly for the purpose of establishing quality assurance guidelines for DNA analysis. The organization is now known as the Scientific Working Group on DNA Analysis Methods, or "SWGDAM". In the TWGDAM data bases maintained for other racial groups, this sequence did not appear at all.

¶ 41 Expressing the frequency in this manner is referred to as the "counting method" and is the preferred method of the FBI for expressing their opinion in court. The FSS does not, as a matter of practice, use the counting method but prefers to confine its evidence to the use of the descriptive phrases given above.

¶ 42 In searching the data bases, the experts make an allowance for heteroplasmy. For example, in the case of an mtDNA sequence from a questioned sample which is heteroplasmic ("C/T") at position 16129, any sequence in the data base which shows a C (alone) or a T (alone) at that position and otherwise matches would be treated as an "inconclusive". It would be excluded entirely from any statistical analysis. It would not count as a sequence that matches the subject sequence or as one which differs. Dr. Shields, a defence expert, argues that such a sequence should be counted as a match.

¶ 43 Given that the data bases represent a random selection of DNA sequences from the general population, standard statistical methods can be used to extrapolate a percentage of the general population who are excluded from the group that could have deposited the sample. Statistics also provides a confidence level pertaining to such an estimate. Although the FSS declines to testify to such estimates, the FBI does so from time to time. In the present case, the FBI experts will say that 99.76% of the general

caucasian population are excluded as possible sources of the three hairs and that a 95% confidence level attaches to their opinion.

Applicable Case Law

¶ 44 In R. v. Mohan, supra, the Supreme Court of Canada identified four criteria upon which the admission of expert evidence depends: relevance, necessity in assisting the trier of fact, the absence of any exclusionary rule, and a properly qualified expert.

¶ 45 The latter three criteria present little difficulty.

¶ 46 The expert evidence must be "necessary" in the sense that it provides information likely to be outside the experience and knowledge of a judge or jury. In other words, the subject matter must be such that ordinary people are unlikely to form a correct judgement about it if unassisted by persons with special knowledge. This criterion is easily met in the present case. The potential of the expert evidence to overwhelm the jury and thereby distort the fact finding process may also be considered as an aspect of necessity, although I prefer to address this below under the heading of relevance.

¶ 47 The third criterion, the absence of any exclusionary rule, is satisfied here.

¶ 48 The fourth criterion, a properly qualified expert, has also been established. All of the Crown and defence experts have acquired special or peculiar knowledge of DNA analysis through study and hands-on experience.

¶ 49 The issue of substance is what the Supreme Court of Canada referred to as "relevance".

¶ 50 In its narrow sense, relevance means that the evidence is so related to a fact in issue that it tends to establish it. The evidence proffered here is circumstantial evidence tending to establish identity and is clearly relevant in the narrow sense.

¶ 51 The broader meaning given to relevance in Mohan requires what the court described as a "cost benefit analysis". Logically relevant evidence may be excluded if its probative value is overborne by its prejudicial effect. The evidence may prove to be misleading, in the sense that its effect on the jury will be out of proportion to its reliability.

¶ 52 Expert evidence is usually dressed up in scientific language which is, on the one hand, difficult to comprehend and, on the other, suggestive of a degree of certainty and infallibility that the evidence may not deserve. The fact that the experts usually have impressive academic credentials and extensive experience may also serve to lend an air of "mystic infallibility" to the evidence. These dangers become more pronounced as the nature of the opinion approaches the ultimate issue more closely.

¶ 53 The danger of a misuse of expert evidence by the jury is off-set by the standard instruction as to the limits of expert evidence and the uses to which it can be put. In the case of DNA evidence, the jury should also be instructed not to be overwhelmed by the aura of scientific infallibility associated with it: R. v. Terceira (1998), 38 O.R. (3d) 175, 123 C.C.C. (3d) 1, 15 C.R. (5th) 359 (Ont.C.A.). The jury should be told to use their own common sense in weighing the DNA evidence and should be reminded that it is only one piece of circumstantial evidence offered to prove identity: Terceira, supra, at 123 C.C.C., p. 28.

¶ 54 In addition, the cost benefit analysis requires some consideration of whether the evidence requires an amount of trial time which is out of proportion to its probative value. I am satisfied that this is not a concern in the present case. While it will require several days of trial time to adduce the mtDNA evidence, the opinions go to the heart of the only issue of substance in the trial - the identity of the killer of Mindy Tran. The time the evidence will require is not inconsistent with its probative value.

¶ 55 I am also satisfied that the evidence will not distort the fact-finding process in the manner contemplated in Mohan. The limitations of the science can be made clear to the jury. The primary cause for concern with nuclear DNA evidence - that the statistical probability of someone other than the accused having the same DNA sequence and having deposited the questioned evidence is extremely small - does not arise in mtDNA analysis. There is a much higher probability of two people possessing the same mtDNA sequence, so the danger that the jury will accept the opinion of the expert as, in effect, deciding the ultimate issue largely disappears. Mitochondrial DNA evidence is just another link in the chain of evidence tending to prove identity. It is not a "genetic fingerprint".

¶ 56 The primary issue on this cost benefit analysis is the question of reliability. Before admitting the evidence, I must be satisfied on the balance of probabilities (see Terceira, supra) that the evidence achieves a threshold level of reliability which qualifies it as appropriate for consideration by the trier of fact. It is the science itself that is on trial. Since mtDNA analysis is a novel scientific theory or technique, I must be satisfied that it is derived from a body of scientific knowledge of reasonable reliability and that the methods and procedures involved are likely to provide scientifically valid results.

¶ 57 There is an important distinction between assessing the threshold reliability of the science as a whole and determining the accuracy of a single application of it in particular factual circumstances (such as the case at bar): Terceira, supra, pp. 15-16. If I am satisfied that mtDNA analysis attains a threshold level of scientific reliability which makes it fit for consideration by a trier of fact, the question of whether the particular tests in the present case were flawed is for the jury to determine. Otherwise, my ruling would usurp the role of the jury.

¶ 58 The issue in Mohan was the admissibility of expert evidence to show that character traits of the accused person did not fit the psychological profile of the

perpetrator of the offences. The analysis of that issue required extensive reference to the general rule excluding evidence of disposition and character. This complicating factor is not present in the case of mtDNA evidence. In addition, DNA analysis is much closer to traditional or "hard" science than the psychiatric opinions under consideration in Mohan. One result of these differences is that the court did not attempt to provide in Mohan any extensive analysis of what must be established to demonstrate a threshold level of reliability for a novel scientific theory or technique.

¶ 59 An appropriate starting point is to ask whether the principles and conclusions upon which mtDNA analysis is based bear the traditional hallmarks of scientific knowledge. These hallmarks are: falsifiability, peer review and publication, general acceptance within the relevant academic community, a known rate of error and the existence and maintenance of standards. These criteria are identified and discussed by the United States Supreme Court in Daubert et al v. Merreli Dow Pharmaceuticals, Inc. 113 S.C.T. 2786, (1993), 509 U.S. 579.

¶ 60 Scientific method involves the generation of hypotheses and the testing of them to see if they can be falsified or refuted. An hypothesis which is inherently incapable of falsification is also incapable of scientific verification; it may amount to a belief or opinion, but it can never be shown to be fact.

¶ 61 Peer review is the process of submitting one's hypothesis, methods and conclusions to the scrutiny of other, independent experts in the field. Publication in a refereed journal of wide circulation in the field is a major, although not the only, method of inviting peer review. When a scientific conclusion has been published and a reasonable time has elapsed without any meritorious criticism of it, the publication and peer review process provides a circumstantial guarantee of trustworthiness.

¶ 62 Many scientific methods and procedures will be associated with a known or potential rate of error. Where the error rate is entirely unknown, that will militate against a finding of reliability. The existence and maintenance of standards and proficiency testing tend to compensate for the absence of a known error rate.

¶ 63 Where widespread or general acceptance of the novel scientific technique can be shown to exist in the relevant academic community, that fact will weigh heavily in favour of a threshold finding of reliability. Widespread or general acceptance is not, however, a precondition: R. v. Blanchari (1996), 146 Nfld. & P.E.I. R. 316 (Nfld. C.A.); R. v. Beamish (1996), 144 Nfld. & P.E.I. R. 310 (P.E.I.S.C.) . The "Frye Test" has not been adopted in Canada: see Frye v. U.S. 293 F. 1013, 54 App. D.C. 46 (1923)

¶ 64 In R. v. Johnston (1992), 69 C.C.C. (3d) 395, 12 C.R. (4th) 99, the Ontario Court (General Division) has provided (at p. 415) a list of some additional factors for assessing novel scientific evidence which are worth considering. These include:

> The care with which the scientific technique has been employed and whether it is susceptible to abuse; The presence of fail-safe characteristics;

> Whether there are analogous relationships with other types of scientific techniques that are routinely admitted into evidence;
>
> The novelty of the technique in its relationship to more established areas of scientific analysis;
>
> The nature and breadth of the inference adduced;
>
> The clarity with which the technique may be explained;
>
> The extent to which basic data may be verified by the court and jury; and
>
> The availability of other experts to evaluate the technique.

¶ 65 No one criterion is conclusive, either for or against admissibility. By a consideration of all of the factors mentioned above, an accurate view of the present degree of reliability of the novel scientific theory or technique can be arrived at. It is useful to remember that science is an ongoing process - a new theory or technique which fails to attain the requisite degree of reliability today may, at some future time, be proven sufficiently reliable.

¶ 66 The criteria mentioned so far relate to the reliability of the evidence. When, in the course of the cost benefit analysis, the prejudicial effect of the evidence falls to be considered, other factors become germane. A useful list of these is found in R. v. Melaragni (1992), 73 C.C.C. (3d) 348 (Ont.Ct. (Gen.Div.)), which was mentioned with approval in Mohan. That list includes:

> Is the evidence likely to assist the jury in its fact finding mission, or is it likely to confuse and confound them?
>
> Is the jury likely to be overwhelmed by the "mystic infallibility" of the evidence, or will the jury be able to keep an open mind and objectively assess its worth?
>
> Will the evidence, if accepted, conclusively prove an essential element of the crime which the defence is contesting, or is it simply a piece of evidence to be incorporated into a larger puzzle?
>
> Are there a sufficient number of experts available so that the defence can retain its own expert if desired?
>
> Can the procedures or tests be performed independently by the defence experts?
>
> Has the process destroyed the evidence upon which the conclusions have been based, or has some of the evidence been preserved for defence analysis?

Are the Hallmarks of Scientific Knowledge Present?

¶ 67 Mitochondrial DNA analysis is best viewed as an off-shoot of nuclear DNA analysis. Although there are important differences, many of the basic principles and procedures are identical. It is fair to say that mtDNA analysis represents a further step in the march of science in this area but is not a quantum leap. Time and again, the experts

referred to nuclear DNA analysis techniques for the purpose of illustrating and explaining points in their evidence. Indeed, the defence experts had relatively little experience with mitochondrial, as opposed to nuclear, DNA analysis. The accused argued, and I accept this as correct, that the procedures and problems in the two types of analysis are sufficiently similar that experience and observations from nuclear DNA analysis have considerable relevance to mtDNA testing.

¶ 68 The hypothesis that a DNA sequence can be extracted and identified from human mitochondria, and the further hypothesis that its relative frequency in the general population can be described and can serve as cogent evidence supporting an identification, both meet the requirement of falsifiability (also referred to as refutability or testability) . Thus, the first hallmark of scientific knowledge identified in Daubert is present.

¶ 69 Much of the scientific literature on mtDNA testing and analysis has been entered in evidence. It consists, for the most part, of published articles in refereed journals. The journals include BioTechniques, The International Journal of Legal Medicine, Human Genetics, The American Journal of Human Genetics, Nature, Nature Genetics, Forensic Science International, Crime Laboratory Digest, Proceedings of the Royal Society of London, Electrophoresis, Journal of Forensic Sciences, etc. In general, there has been little, if any, published criticism of the important articles. While the science is very new, enough time has passed for a conclusion that, in the absence of such published criticism, the methodology and conclusions in these articles are accepted by the scientific community. I find that the second Dauhert criterion, publication and peer review, is present here.

¶ 70 Has mtDNA testing attained widespread or general acceptance in the scientific community? The evidence demonstrates that a substantial majority of scientists in the field have accepted it. A minority of scientists, exemplified by the three defence experts, question its current viability. In essence, these scientists say that the process has promise but is too uncertain to be safe for use at the present time. There is a distinction between widespread or general acceptance and unanimity which is relevant here. Although the scientific community is not unanimous, there is a widespread and general acceptance of the reliability of mtDNA analysis in forensic casework.

¶ 71 Is there a published rate of known or potential error associated with mtDNA testing? The evidence does not reveal that an error rate, in the traditional sense, has been identified. However, the potential sources of error are known and understood and certain fail-safe procedures have been adopted by the laboratories, including duplication of testing and proficiency testing. The FSS always duplicates each test if there is enough sample material available to permit that. (In addition to duplicating the test itself, the FSS always has a second examiner "call" the bases as a check on the accuracy of the judgement of the first examiner). The FBI prefers to leave the duplication of testing to the defence and makes an effort to save enough sample material to facilitate that.

Proficiency Testing

¶ 72 Laboratory personnel are required to take periodic proficiency tests to confirm the accuracy of their procedures. TWGDAM and SWGDAM guidelines require laboratory personnel to pass a proficiency test twice per year. The FBI adheres to this guideline. The proficiency tests are devised and graded by an outside agency.

¶ 73 The FSS does not adhere to the proficiency testing standards established by TWGDAM. The FSS has been accredited by the United Kingdom Accreditation Services. The accreditation process requires an independent audit of the laboratory operations. The FSS mtDNA laboratory has completed successfully two proficiency tests, in 1995 and 1998. Both of these tests were created and graded internally. Mr. Bark was assisted by two laboratory technicians in his work on this case. Mr. Bark and one of those technicians have each completed the two proficiency tests successfully; the other technician joined the laboratory in 1997 and has passed just one proficiency test.

¶ 74 There is a crucial element of subjectivity in the process by which FSS scientists read "mixed" sequences when contamination or heteroplasmy or both are present. The need for difficult judgement calls is not unique to this sort of testing - such diverse fields as fingerprint comparison and tool mark examination require similar judgement calls. Dr. D'Eustachio said that mixtures of DNA sequences cannot be typed reliably. In his view, it is not possible for an examiner to repeatedly and reliably identify bases in the face of contamination problems and the complicating factor of heteroplasmy. The Crown experts said that their extensive experience in reading mixed sequences has developed their ability to do so reliably.

¶ 75 On this issue, I prefer the evidence of the Crown's experts. Dr. D'Eustachio does not have the advantage of having performed mtDNA sequencing himself, although his academic qualifications are admirable. Mr. Bark has had several years of experience in sequencing mtDNA, reading chromatographs, and dealing with problems caused by mixtures arising from contamination and heteroplasmy. He has developed his ability to read sequences resulting from mixtures to a high level of sophistication. His evidence (given in re-examination) concerning the need to read the chromatogram as a whole, and not focus on a small portion of it, was convincing. Scientists (like the defence experts) engaged in pure research do most of their work with pristine blood samples and do not face the difficulties posed by mixtures on a regular basis.

¶ 76 The occasional proficiency testing done internally at the FSS Laboratory provides some reassurance about the accuracy of the examiners there. I have ordered that the defence is entitled to disclosure of these proficiency tests and the results. In addition, the chromatographs showing the actual sequences obtained from the various samples in this case have been disclosed to the defence and examined by its experts. Thus, the individual values assigned to the bases can be explored in cross-examination of the Crown experts. (I should also repeat that no evidence of heteroplasmy was observed in the questioned samples or known samples taken from Mr. Murrin in this case.)

Validation Studies

¶ 77 From the beginning, the need for validation studies of mtDNA analysis has been recognized: see, for example, Wilson and others, "Guidelines for the Use of Mitochondrial DNA Sequencing in Forensic Science", Crime Laboratory Digest (1993) 20(4): 68 at p. 72.

¶ 78 In a study published in a peer-reviewed journal in 1995, FBI scientists concluded that "correct typing results can be achieved when the ratio of sample to contaminant is greater than or equal to 10 to 1": Wilson and others, "Extraction, PCR Amplification and Sequencing of Mitochondrial DNA from Human Hair Shafts", Biotechniques (1995) 18(4): 662 at p. 667. This study was conducted on hair and blood samples from approximately 30 people. The study (at page 668) recommended further validation studies on hairs contaminated with such substances as semen or blood "to confirm that the cleaning procedure effectively removes adhering contaminants prior to DNA extraction".

¶ 79 In a subsequent study, a total of 224 tests were performed on hairs that had been contaminated in a variety of ways: Wilson and others, "Validation of Mitochondrial DNA sequencing for Forensic Casework Analysis", International Journal of Legal Medicine (1995) 108:68-74. The conclusion reached (at p. 72) was that "sequencing amplified mtDNA is a reliable method of DNA typing from human hairs, blood and semen". Analysis using a polymerase chain reaction was described as "robust and reliable".

¶ 80 The hairs used in this latter study were taken from only six people. Dr. Shields, a defence expert, criticized the sample size in this and other validation studies as being too small to demonstrate the reliability of the science. It must be observed, however, that this validation study was published in a peer-reviewed journal in 1995 and there has been no published criticism of it since then. Dr. Shields' own criticism has been submitted for publication but not yet published in a refereed journal.

¶ 81 The European DNA Profiling Group ("EDNAP") is an organization comprising a number of mtDNA laboratories in various European countries. A variety of PCR strategies are in use in the different laboratories. Two of the laboratories use nested PCR amplification while the others do not.

¶ 82 In a study published in 1998, each of the EDNAP laboratories analyzed three blood stains and all reported the same results. The conclusion of the study was that mtDNA typing is a "valid, robust and reliable means of forensic identification": Carracedo and others, "Reproducibility of mtDNA Analysis Between Laboratories", Forensic Science International (1998) 97:165-170. The FSS Laboratory was one of the participants. The study was concerned only with pristine blood stains and does not purport to address the more problematic aspects of analyzing contaminated hairs.

¶ 83 In another study, shed hairs gathered during investigation of three robberies in Sweden were analyzed together with other types of samples from the four suspects. The results confirmed the accuracy of the methodology, although the authors warned (at page

463) of the need for further study in several areas to avoid false exclusions: Allen and others, "Mitochondrial DNA Sequencing of Shed Hairs and Saliva on Robbery Caps", Journal of Forensic Science (1998) 43(3): 453-464.

¶ 84 In July, 1995, the Defense Science Board Task Force in the United States on "The Use of DNA Technology for Identification of Ancient Remains" published its report. After a thorough investigation, the Task Force concluded (at page 55) that "identification of so-called ancient skeletal remains by a program of mtDNA testing is possible, particularly in association with other information". It went on to say that:

> The Task Force finds that the present probability of coincidental matches between mtDNA control region sequences is no more than a few percent. Once sequences from 500 members of a population have been determined, precise statements about the chance of a false association of a set of remains with a family will be able to be made.

¶ 85 On the subject of contamination, the Task Force said:

> The Task Force finds that control of contamination is essential to PCR based laboratory testing. Some contamination is unavoidable, particularly in mtDNA testing of ancient remains, but it does not preclude reliable case work testing where redundancy, good laboratory practices, and appropriate cautionary language are used and constant oversight is maintained.

The authors of the Task Force report were aware of the existence of heteroplasmy within the control regions, although they considered its incidence to be "generally low": ibid., page 19.

¶ 86 Dr. Shields testified that he has examined unpublished data from a validation study by LabCor, a division of Hoffman LaRoche. He said that, despite extensive efforts, the LabCor scientists were unable to validate the hypothesis that mtDNA typing of hair can be done reliably. Dr. Shields was handicapped by having had no personal involvement in this study, a difficulty which led to his erroneous assumption (at one point in his testimony) about the type of machine LabCor was using to wash hair. The LabCor study remains unpublished; it has not been subjected to peer review.

¶ 87 I am somewhat troubled by the small sample sizes in the validation studies. However, these validation studies have been placed in the public domain in peer-reviewed journals and have survived without criticism for a considerable period of time. This approbation by silence represents the judgement of the scientific community. If the studies were inadequate to support their conclusions, the process of peer review would have called attention to their deficiencies by now. That has not happened.

Conclusion on Daubert Criteria

¶ 88 On balance, I find that the hallmarks of scientific knowledge described in Daubert are present in the body of knowledge underpinning mtDNA analysis. The criteria of falsifiability, publication and peer review, and general acceptance within the relevant academic community have been established. Quality assurance standards are in place and adhered to. A particular rate of error has not been identified, but the published validation studies and proficiency testing provide comfort in this area.

¶ 89 I turn now to a consideration of the particular problems identified by the defence: contamination, heteroplasmy, and uncertainties about recombination and paternal inheritance.

Contamination

¶ 90 The expert witnesses were in general agreement that the prospect of contamination presents the greatest risk of a false conclusion in mtDNA analysis. It is also true that the difficulties posed by contamination are somewhat greater in the extraction of DNA from hair than from, for example, blood or semen. The Crown experts said that this risk, although always present, can be contained and managed by the application of proper fail-safe techniques. The defence disagreed. It argues that the risk of contamination is so pervasive and overwhelming that the science of mtDNA analysis does not achieve the necessary threshold level of reliability.

¶ 91 At the outset, it is necessary to distinguish between the impact of the risk of contamination upon the novel scientific theory or technique as a whole and the particular problems with contamination in the analyses done in the present case. The former is an issue to be considered and decided on this voir dire, as it relates to the admissibility of expert evidence on mtDNA analysis and testing in general. If, however, the science as a whole is found to meet the threshold level of reliability, then particular dangers posed by contamination in the individual analyses done in this case are a matter for the jury. Any sort of scientific test or analysis can be suspect because of contamination of the evidence. The presence of contamination in the tests done in the present case is one of several things the jury will have to assess in determining how much weight, if any, to place upon the test results.

¶ 92 Nuclear DNA testing also presents a risk of contamination, but the risk is much less. In mtDNA testing, the extraction process produces a much smaller amount of DNA than is the case with nuclear DNA. This requires the use of a greater number of cycles in mtDNA amplification to produce a legible sequence. Each cycle doubles the amount of DNA, but this is true for any DNA which may have found its way into the sample. In the process of amplifying the target DNA sequence, any other DNA which is present as a contaminant will be amplified also. Since the absolute amount of mtDNA at the start of amplification is much less than would be the case in a nuclear DNA test, the relative concentration of any contaminant in the mtDNA solution will be correspondingly greater. The use of a large number of amplification cycles (50 in the present case) will have the effect of amplifying the contaminant to a much greater degree than is the case in

conventional nuclear DNA testing. For that reason, the risk of an erroneous result is greater also.

¶ 93 Both the FSS and the FBI Laboratories make strenuous efforts to reduce contamination. The precautions adopted by the two labs differ somewhat.

¶ 94 At both laboratories, rooms and work surfaces are cleaned on a regular basis. Containers are cleaned and sterilized with heat and ultraviolet light. FSS examiners wear gloves, disposable lab coats, caps, face masks, and dedicated shoes. All of this clothing is donned in a "clean room" which has its own air supply at the FSS lab. Dr. Shields, one of the defence experts, said the use of a clean room was the best method he had seen for avoiding contamination. Amplification is always carried out in a different room from extraction. The various solutions and reagents are created in the clean room and brought to the amplification room. Any products of the amplification remain in that room.

¶ 95 A number of controls are used by both laboratories. A "reagent blank" consisting of all of the components (except the DNA sample) used in the extraction process is subjected to the same procedures as the DNA sample itself and tested for the presence of contaminating DNA. A "negative control" is used in a similar fashion at the amplification stage to check for the presence of contaminating DNA. A "positive control", consisting of material containing a known DNA sequence, is used during testing to provide yet another check.

¶ 96 In addition to spatial separation of the processes, the two labs use temporal separation - the questioned sample is always tested before the known sample, and a period of days is allowed to intervene.

¶ 97 When the DNA is to be obtained from hair, the first step is to wash the hair to remove any contaminating substance. The FSS uses a vortexer to wash hair in a detergent solution; the FBI submits hair to a sonic bath by transmitting sound waves through a liquid containing the hair. The defence expended some effort in an attempt to demonstrate that the FBI method is more effective. I am not satisfied that it is. I accept the assertion of Dr. Tully of the FSS that the methods are different but equivalent. I note that the vortexing procedure is carried out three separate times by the FSS.

¶ 98 During sequencing, the amount of contamination present is assessed and a decision is made as to whether to discard the sample or accept the result as meaningful. The level at which contamination will cause a result to be discarded at the FSS lab is somewhat subjective and dependent upon the judgement of the analyst.

¶ 99 The FBI approach is somewhat different. Rather than leaving the operator to judge subjectively the level of contamination and its affect on the validity of the test, the FBI uses the 10:1 rule mentioned above. A test result will be accepted whenever the ratio of sample to contaminant is at least 10:1. The 10:1 rule was arrived at after testing to determine a level of contamination at which ambiguities and errors were not observed:

Wilson and others, "Extraction, PCR Amplification and Sequencing of Mitochondrial DNA from Human Hair Shafts", Biotechniques (1995) 18(4): 662. The FBI does not use as many as 50 cycles, contenting itself with a maximum of 36 cycles.

¶ 100 The use of a lower number of cycles and the imposition of the 10:1 rule may suggest a more conservative approach by the FBI Laboratory. However, these safeguards are counter-balanced by the FSS's insistence upon duplication of testing where possible. All FSS tests are carried out in duplicate whenever there is enough sample material to permit this. Mr. Bark said he could not remember a case where the same contaminant was observed in both halves of a duplicated test. The FBI does not duplicate its tests, although it does attempt to save some sample material to enable a defence expert to duplicate the test.

¶ 101 Having considered these differences between the protocols of the two laboratories, Dr. Budowle of the FBI Laboratory said that the FSS protocol is a valid one. A defence expert, Dr. D'Eustachio, described the FSS controls and checks as "impressive" (although he is of the view that they failed in the instant case).

Results in the Present Case

¶ 102 In the instant case, DNA was extracted by the FSS from hair 11 (once) and from hair 13 (in duplicate) on the same day, April 28, 1998. Not enough of hair 11 was available to permit duplicate testing of it.

¶ 103 Both reagent blanks used in the extraction from hair 13 contained evidence of contamination and, in each case, it exceeded the 10:1 ratio adopted by the FBI. In each case, however, the DNA sequence in the reagent blank did not match the sequence in the questioned sample, which tends to confirm that the sequence from the questioned sample was not the result of contamination. Mr. Bark considered that the result was valid.

¶ 104 With respect to the single extract from hair 11, Mr. Bark found no evidence the result was affected by contamination although, because of the lack of duplication, the result is deemed "unconfirmed" in the lexicon of the FSS.

¶ 105 Hair 12 was tested in duplicate and the two extractions were done five days apart in September, 1998. One of the reagent blanks contained a "trace" of contamination but the contaminating sequence was different from the one obtained from the questioned material.

¶ 106 There was a sequence concordance between each of the five hair extracts, and sequence concordance between them and the known samples obtained from Mr. Murrin.

¶ 107 It will be noted that, if the FSS had felt itself bound by the 10:1 rule formulated and used by the FBI Laboratory, the results of both analyses of hair 13 would have been discarded. Mr. Bark's acceptance and use of the hair 13 results is buttressed, however, by one "unconfirmed" result from hair 11 and two results from hair 12 derived from

extractions on separate days. The hair 12 results, having been duplicated, provide a greater degree of reliability than a single FBI test would have provided. In my view, the duplication of testing by the FSS is a safeguard which, at the least, counterbalances the absence of an absolute upper limit for the contamination level.

Conclusion on Contamination

¶ 108 I do not find that the risk of contamination presents a reason for rejecting the evidence of this novel scientific theory or technique. The safeguards described by the Crown experts and in use at the FSS and FBI laboratories are impressive. The FSS requirement for duplication (where possible) provides a measure of confidence which justifies their more flexible approach to contamination levels. A consideration of all of the evidence, including the validation studies described below, demonstrates that the Crown experts are correct when they assert that contamination is no more than an ever-present complicating factor which must be managed carefully to avoid invalidating the results.

Heteroplasmy

¶ 109 The initial assumption of scientists was that every person has one, but only one, sequence in their mtDNA. On this assumption, a sample of any bodily substance from any part of a particular individual would necessarily yield the same mtDNA sequence. The original decisions by law enforcement agencies, including the FSS and the FBI, to use mtDNA analysis for forensic purposes were made while this assumption was still current. For example, Mr. Wilson, one of the Crown's experts from the FBI, wrote in 1993 that:

> An important feature of mtDNA which simplifies DNA sequencing is its monoclonal nature. With the exception of certain disease conditions where tissue specific deletions of large segments of the mitochondrial genome have been detected, for practical purposes, all copies of an individual's mtDNA sequence are identical.
>
> (Wilson and others, "Guidelines for the Use of Mitochondrial DNA Sequencing in Forensic Science", Crime Laboratory Digest (1993) 20(4): 68.)

¶ 110 This assumption is now recognized as false. Many observations have now been made of mtDNA sequences taken from the same person which differ at one or more base positions. This condition, known as heteroplasmy, is now seen as common and may be universal (although not, as yet, universally detectable). The prevalence of heteroplasmy is being revealed as research continues: see Wilson and others, "A Family Exhibiting Heteroplasmy in the Human Mitochondrial DNA Control Region", Human Genetics (1997) 100: 167-171; Bendall and others, "Variable Levels of a Heteroplasmic Point Mutation in Individual Hair Roots", American Journal of Human Genetics (1997) 61: 1303-1308; Gill and others, "Identification of the Remains of the Romanov Family by DNA Analysis", Nature Genetics (1994) 6: 130; Ivanov and others, "Mitochondrial DNA

Sequence Heteroplasmy in the Grand Duke of Russia", Nature Genetics (1996) 12: 417; Bendall and others, "Heteroplasmic Point Mutations in the Human mtDNA Control Region", American Journal of Human Genetics (1996) 59: 1276-1287; Sullivan and others, "A Single Difference in mtDNA Control Region Sequence Observed between Hair Shaft and Reference Samples from a Single Donor", (1997) Proceedings of the 7th International Symposium on Forensic Identification; Bendall and Sykes, "Length Heteroplasmy in the First Hypervariable Segment of the Human mtDNA Control Region", American Journal of Human Genetics (1995) 57: 248-256.

¶ 111 Two types of heteroplasmy have been observed: "length" heteroplasmy and "sequence" heteroplasmy. There are areas in an mtDNA sequence which consist of long, uninterrupted stretches of a particular base. Length heteroplasmy occurs when the number of bases in this string differs between mtDNA sequences taken from the same individual. Sequence heteroplasmy is found when a particular base position has differing values in two mtDNA samples from the same person. Length heteroplasmy is much more common.

¶ 112 Most sequence heteroplasmy observed so far has manifested itself as a variation at a single base position. Mr. Bark, for example, has never seen a case of sequence heteroplasmy at more than one position in a single person. The possibility of a person being heteroplasmic at 2, 3, or even 4 base positions is recognized by the experts. (The only heteroplasmy observed in the present case was found in the mtDNA sequence taken from the victim's mother.)

¶ 113 Before the existence of heteroplasmy was recognized and taken into account, a different value at a single base position was enough to exclude a subject. The existence of heteroplasmy has forced a re-evaluation of what is needed for an exclusion.

¶ 114 Differing values at a single base position may be due to heteroplasmy. Whether heteroplasmy provides the explanation of a single base difference is assessed by FSS scientists in what is, in effect, a judgement call. The location of the base in question is important to this assessment - some positions are known "hot spots" likely to produce differing values in the same individual. Additional tests of known samples may be done to confirm heteroplasmy. When, in the judgement of an FSS scientist analyzing a sequence, the one base pair difference is not due to heteroplasmy, the result is an exclusion. If the difference appears to be the result of heteroplasmy, the result is a "failure to exclude". This approach may produce a false exclusion if heteroplasmy goes unrecognized.

¶ 115 The FBI approach to heteroplasmy is somewhat different from that of the FSS. It also recognizes the possibility that a single base pair difference may be due to heteroplasmy. Any comparison of sequences which produces a one base pair difference is termed "inconclusive". Where two or more base pair differences exist, the result is an exclusion. When there is complete sequence concordance, the FBI expert will say that he "cannot exclude" the possibility that the questioned material was deposited by the suspect or any other person with the same maternal lineage.

¶ 116 Defence experts spoke of the danger of "false inclusions" because of heteroplasmy. An example would be the hypothetical case of a questioned sample found at a crime scene which yields an mtDNA sequence which is heteroplasmic at position 16129 - it shows values of both C and T (C/T) at that position. An individual whose sequence showed complete concordance with the questioned sequence, including C/T heteroplasmy at position 16129, would not be excluded. Also not excluded would be individuals showing sequence concordance with a C (only) or a T (only) at position 16129, provided the examiner felt the subject was heteroplasmic at this position. (I note that when a group of researchers compared a heteroplasmic mtDNA sequence to sequences in British and American data bases, they simply ignored the value found at the heteroplasmic base: Ivanov and others, op. cit., page 419.)

¶ 117 If more was known about the DNA sequence of the second and third subjects in this example, either or both of them might be excluded. If one could demonstrate that either person was not heteroplasmic at position 16129, i.e., that every mtDNA sequence which could be extracted from that person's body contained one, and only one, base at position 16129, then the individual could be excluded. Failing exclusion, the opinion of the analyst would be that "the crime material could have come from that person or anyone else with the same sequence". The subject has not been excluded from the group of possible donors of the questioned sample although, if more was known about her genetic makeup, her exclusion might be possible. This is what the defence experts call a false inclusion. The false inclusion of someone who differs from the questioned sample at only one base position is a result of the manifest impossibility of examining every mtDNA sequence in a person's body. It is a necessary result of the mechanical limitations of this forensic technique. The Crown experts give implicit recognition to this, as they do not speak of "inclusions" but of a "failure to exclude".

¶ 118 Dr. Shields, in his unpublished paper entitled "Analysis of the FBI's Validation Studies of PCR Amplification and Automated Sequencing of Mitochondrial DNA for Forensic Use", says (at page 29) that, because of heteroplasmy, any sequence found in a database which differs from the questioned sequence at only one base position should be treated as a match. That approach would increase the number of reported matches considerably. He says this is not the current practice of the FBI.

¶ 119 The evidence is unclear as to how the FSS and the FBI treat a sequence found in a database which differs from the questioned sequence at just one base position. The issue does not arise in the present case with respect to the TWGDAM database because there were no such sequences found there. The evidence on the voir dire has not revealed how many such sequences were found in the other databases which have been examined.

¶ 120 This issue is, of course, fertile ground for cross-examination. It does not amount to a reason for finding that the science as a whole has failed to attain a threshold level of reliability.

Conclusion re Heteroplasmy

¶ 121 In my view, neither the discovery of heteroplasmy within the control region nor the alleged danger of "false inclusions" provide a reason for rejecting evidence of this novel scientific theory or technique. Like the ever present danger of contamination, the existence of heteroplasmy is a complicating factor which requires substantial exercise of judgement by mtDNA analysts. The effect of heteroplasmy and its treatment in the data bases will have to be explained carefully to the jury to enable them to understand the true frequency of the questioned mtDNA sequence in the general population.

How Much Do We Know?

¶ 122 Mr. Wilson of the FBI conceded in cross-examination that fundamental tenets of the science are being questioned. The assumption that there is no recombination between mitochondrial lineages and that the inheritance of mitochondria is clonal is now being questioned. Several studies have suggested recombination and some degree of "paternal leakage" as possibilities worthy of further consideration and investigation: see Eyre-Walker and others, "How Clonal are Human Mitochondria?", Proc. R. Lond. B. (1999) 266: 477-483; Hagelberg and others, "Evidence for Mitochondrial DNA Recombination in a Human Population of Island Melanesia", Proc. R. Soc. Lond. B. (1999) 266: 485-492; Parsons and others, "A High Observed Substitution Rate in the Human Mitochondrial DNA Control Region", Nature Genetics (1997) 15: 363.

¶ 123 This does not mean, however, that the science lacks utility from a forensic viewpoint. If it can be established that an mtDNA sequence can be extracted accurately and reliably from biological material, and if the relative frequency of that particular sequence in the general population can be estimated, then the evidence has some value as circumstantial evidence of identity. These propositions can be advanced and weighed independently of any need to consider the biological puzzles underlying them.

¶ 124 Whether or not recombination occurs, whether there is paternal leakage, and the rate at which mutations occur are, no doubt, fascinating questions for scientists in the field but their resolution is not necessary to render mtDNA analysis valuable in the court room. If a suspect observed leaving a crime scene has red hair, that fact could be put before a jury routinely as some evidence tending to establish identity. The usefulness of the evidence is in no way dependent upon the genetic and biological explanations for hair redness. All that matters is that the suspect has red hair but most people in the general population (in Vancouver, at any rate) do not. It is a piece of identifying circumstantial evidence which, considered with other such evidence, may or may not serve to identify the accused as the perpetrator.

Conclusion on Admissability

¶ 125 The hallmarks of scientific method are, for the most part, present. Neither the difficulties posed by contamination and heteroplasmy, nor our lack of knowledge about some of the fundamental aspects of the science, are reasons for rejecting the evidence.

¶ 126 Many of the factors mentioned in R. v. Johnston, supra, are also present. The techniques in use for mtDNA analyses are employed with care and incorporate fail-safe characteristics. There is an analogous relationship with nuclear DNA evidence, a subject which is now routinely introduced in Canadian courtrooms. The relevant proficiency tests and chromatograms may, if the defence chooses, be placed before the jury. It is clear that independent experts exist who may be retained to evaluate the work of the Crown experts.

¶ 127 As for the possible prejudicial effect of the evidence, it is important to note that the probability of two individuals having the same mtDNA sequence is far higher than is the case with nuclear DNA. That goes a long way to avoid the possibility of the jury being overwhelmed by the "mystic infallibility" of the evidence. In the words of Melaragni, supra, the evidence, if accepted, will not prove identity conclusively but will simply stand as a piece of evidence to be incorporated into a larger puzzle.

¶ 128 The three hairs here have been used up during the Crown testing, so it is not possible for the defence experts to perform their own independent tests. However, the effect of this is ameliorated considerably by the production to the defence of the chromatographs showing the base calls made by the Crown examiner, Mr. Bark.

¶ 129 Overall, I consider that any prejudicial effect the evidence might otherwise have can be removed by a proper caution to the jury.

¶ 130 After weighing all of the evidence and measuring it against these criteria, I am satisfied on a balance of probabilities that the evidence has a threshold level of reliability. It may be placed before the jury.

¶ 131 The question of whether the samples in the present case were contaminated to such a degree as to invalidate the results is for the jury to consider. The significance of the evidence concerning the 3 hairs on this voir dire is to illustrate the application of this new science to a particular forensic investigation and nothing more. If I am wrong in that conclusion, I am nevertheless of the view that the specific analyses in the present case attain a threshold level of reliability of the sort contemplated in Mohan.

Statistical Estimate

¶ 132 It has become routine in nuclear DNA evidence for the courts to admit an estimate, derived through standard statistical methodology, of the frequency with which the questioned sequence is found in the general population: R. v.Singh (unreported) March 5, 1993 (B.C.S.C.), affirmed without reference to this point at (1996), 77 B.C.A.C. 185, 108 C.C.C. (3d) 244 (B.C.C.A.); R. v. Beamish, supra; R. v. Henson, [1997] O.J. No. 5585 (Ont. C.J.) (Gen. Div.)); R. v. Lafferty, [1993] N.W.T.R. 218, 80 C.C.C. (3d) 150, [1993] 4 W.W.R. 74, (N.W.T. S.C.); R. v. Legere (1994), 156 N.B.R. (2d) 321, 95 C.C.C. (3d) 139, 35 C.R. (4th) 1 (N.B.C.A.); R. v. Terceira (1998), 123 C.C.C. (3d) 1 (Ont. C.A.).

¶ 133 The Crown proposes to lead the opinion of Mr. Wilson or Dr. Budowle, or both, that a standard statistical analysis of the one observation in 1,219 caucasians from the TWGDAM data base, employing a 95% confidence level, suggests that 99.76% of the general caucasian population are excluded from the group that could have deposited the questioned sample. Approaching the estimate another way, this means that 24 people in a population of 10,000, or 240 in a population of 100,000, could have left the three hairs at the crime scene.

¶ 134 I see no reason to exclude this statistical estimate. The reasons given in Singh and the other authorities cited above admitting similar evidence in nuclear DNA cases apply with equal force to an mtDNA analysis. The statistical estimate is admissable.