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Nuclear and Mitochondrial DNA in the Courtroom

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NUCLEAR AND MITOCHONDRIAL DNA IN THE COURTROOM

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INTRODUCTION

The overwhelming value of DNA evidence is now well recognized and accepted. During the last fifteen years, the use of DNA information has significantly changed the legal landscape in three different areas. First, in the area of criminal litigation, particularly in the prosecution of violent crimes, the introduction of DNA evidence has convinced the courts of the guilt of many defendants and has persuaded countless others to plead guilty or to plea bargain. At the same time, the introduction of DNA evidence in criminal appeals has, as of October 2004, led to the exoneration of 154 individuals, many of whom faced the death penalty.[1] Second, the value of DNA evidence has also been recognized in the resolution of numerous parentage and even grandparentage disputes. Prior to the availability of DNA information, U.S. courts recognized the limited evidentiary value of blood group data and correspondingly restricted the use of such data to the identification of paternity exclusions,[2] though several European countries have

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[1] For information on the use of DNA evidence to exonerate wrongfully convicted individuals, see http://www.innocenceproject.org (last visited Dec. 23, 2004).

[2] K.S. Broun & H.D. Krause, *Paternity Blood Tests and the Courts*, *in*

traditionally admitted such data to support positive claims of paternity.[3] Today, DNA information is frequently admitted to establish inclusion probabilities of paternity.[4]

Finally, DNA evidence has entered the courtroom in cases involving the identification of human remains recovered from the sites of mass disasters and from the battlefield. For example, in recent years, DNA evidence has been invaluable in the identification of bodies exhumed from mass graves in Bosnia and Haiti[5] and recovered from airplane crashes, and of fragments of bodies recovered from the ruins of the World Trade Center.[6]

Although the principles involved in the interpretation of DNA evidence are the same in all of these contexts, their application often is complicated by the nature of the biological samples. In many cases, the human remains are in an advanced state of decomposition, and it can be difficult to extract DNA that has not itself decomposed.[7] Furthermore, multiple fragments may be recovered in different stages of decomposition and thus possess varying qualities of DNA information. In the World Trade Center attack, for example, about 15,000 separate body parts were recovered, although there were fewer than 3,000 victims.[8] In airplane crashes, it is common for several members of the same family to perish.[9] Distinguishing between the remains of related individuals, whose DNA is necessarily related, presents special

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INCLUSION PROBABILITIES IN PARENTAGE TESTING 171-207 (R.H. Walker ed., American Association of Blood Banks 1983).

[3] W. F. Bias et al., *Theoretical Underpinning of Paternity Testing*, *in* INCLUSION PROBABILITIES IN PARENTAGE TESTING, *supra* note 2, at 51-61.

[4] HOWARD C. COLEMAN & ERIC D. SWENSON, DNA IN THE COURTROOM: A TRIAL WATCHER'S GUIDE 62 (GeneLex Press, 1994).

[5] Eliot Marshall, *International Experts Help Probe Haiti's Bloody Past*, 269 SCI. 1812, 1812-13 (Sept. 29, 1995).

[6] C.H. Brenner & B.S. Weir, *Issues and Strategies in the DNA Identification of World Trade Center Victims*, 63 THEORETICAL POPULATION BIOLOGY 173, 173 (May 2003).

[7] Brian Vastag, *Out of Tragedy, Identification Innovation*, 288 JAMA 1221, 1221-23 (Sept. 11, 2002).

[8] Brenner & Weir, *supra* note 6, at 174.

[9] *Id.* at 177.

challenges.[10]

In the early 1990s vigorous challenges to the admissibility of DNA evidence were mounted in both the legal[11] and scientific arenas.[12] More recently, however, DNA evidence has become widely accepted, and challenges based on *Daubert v. Merrell Dow Pharmaceuticals*[13] or *Frye v. United States*[14] are now few and far between. Nevertheless, interpreting DNA evidence often is not simple and requires a sound understanding of the theory involved and the underlying assumptions. The current use of two different categories of DNA evidence—mitochondrial DNA and nuclear DNA—adds an additional layer of complexity.

I. THE TWO DNA GENOMES

The extensive publicity surrounding the Human Genome Project and the announcement in 2001 of the publication[15] of a proof of the sequence have perhaps overshadowed the fact that the

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[10] *Id.*

[11] *See* United States v. Yee, 134 F.R.D. 161 (N.D. Ohio 1991) (finding that the government met its burden of showing that the general scientific community accepted the FBI's protocol and procedures for determining a match of DNA fragments in estimating the likelihood of encountering a similar pattern).

[12] *See* Leslie Roberts, *Fight Erupts Over DNA Fingerprinting*, 254 SCI. 1721, 1721-23 (Dec. 20, 1991); R.C. Lewontin & Daniel L. Hartl, *Population Genetics in Forensic DNA Typing*, 254 SCI. 1745, 1745-50 (Dec. 20, 1991); B.S. Weir, *Population Genetics in the Forensic DNA Debate*, 89 PROC. NAT'L ACAD. SCI. U.S.A. 11654 (Dec. 1992).

[13] Daubert v. Merrell Dow Pharm. Inc., 509 U.S. 579, 585-97 (1993) The Court held that:

> 'General acceptance' is not a necessary precondition to the admissibility of scientific evidence under the Federal Rules of Evidence, but the Rules of Evidence . . . do assign to the trial judge the task of ensuring that an expert's testimony both rests on a reliable foundation and is relevant to the task at hand.

Id. at 597.

[14] Frye v. United States, 293 F. 1013 (D.C. Cir. 1923) (requiring the proponent of testimony based on scientific procedures to show that the procedures were generally accepted in their field).

[15] *Initial Sequencing and Analysis of the Human Genome*, 409 NATURE 860, 860-921 (Feb. 15, 2001).

human cell—and, in general, all animal cells—possess two genomes with very different characteristics. While the term "human genome" commonly refers to the DNA contained within the nucleus, the largest and most prominent organelle within the cell, the "other" genome is contained within a second, much smaller organelle within the cell, the so-called mitochondrion. The mitochondrial genome is many orders smaller than the human genome and is comprised of a single circular DNA strand of only 16,569 bases, or "letters." In contrast, the human genome consists of some 300 million bases distributed among 46 linear strands, or chromosomes. The differences between the two genomes are summarized in Table 1.

While DNA information from both genomes has been held admissible in both criminal and civil litigation, the two genomes possess radically different properties and each has unique advantages, disadvantages, and probative values specific to the context in which it is used. This article will examine the different characteristics of the two genomes and the means by which information obtained from them is most appropriately presented in the courtroom.

A. Characteristics of the Two Genomes

A striking feature of the nuclear genome appreciated by early geneticists is that the number of copies per cell is constant. Each cell contains one nucleus possessing 23 pairs of chromosomes. Each gene, or unit of inheritance, is present in two copies. By contrast, the mitochondrial genome consists of one circular DNA strand, but is present in varying numbers of copies within the cell. A cell can contain up to 1,000 mitochondria and each mitochondrion can contain between 2 and 10 genome copies.[16] Thus, a cell can contain as many as 10,000 copies of the mitochondrial genome—far more copies than the nuclear genome contains. This difference acquires significance when it is

[16] Bruce Budowle et al., *Forensics and Mitochondrial DNA: Applications, Debates and Foundations*, 4 ANN. REV. GENOMICS & HUMAN GENETICS 119, 121 (Sept. 2003).

considered that forensic samples, such as a single hair follicle, may contain extremely small quantities of DNA or may have been recovered in an advanced state of decomposition.[17] In such cases, it may only be possible to obtain information from the mitochondrial DNA genome by virtue of the large number of copies within the cell.

B. Patterns of Inheritance

The human and mitochondrial genomes differ fundamentally in their patterns of inheritance. Whereas a child inherits one set of 23 chromosomes (and, by extension, one copy of each gene) from the mother and one set from the father (biparental inheritance), the mitochondrial genome is maternally inherited. In other words, a child will inherit the mitochondrial genome of the mother. This pattern of inheritance follows from the nature of the fertilization process of the egg cell by a spermatozöon. Fusion of an egg cell with a spermatozöon, which is several orders of magnitude smaller than the egg, involves the injection of the nucleus of the spermatozöon into the egg. All other cellular components, including mitochondria carried by the spermatozoa, remain outside of the egg and eventually are degraded or broken down. This maternal pattern of inheritance has several implications for the evidentiary value of mitochondrial DNA. Because a father contributes no mitochondrial DNA to his offspring, mitochondrial DNA will obviously have no evidentiary value in paternity cases. However, mitochondrial DNA can be extracted from both blood and semen samples, and so can have evidentiary value in identifying male perpetrators of crimes of violence.

It follows from the maternal inheritance of mitochondrial DNA that the offspring of a single mother, as well as all maternal relatives, should have the same mitochondrial genome. Consequently, mitochondrial DNA may be of limited value in the identification of tissue recovered from airplane crashes and similar disasters in which several family members may have perished together. On the other hand, the mitochondrial DNA of maternal

[17] Vastag, *supra* note 7, at 1221-23.

relatives can assist in the identification of individuals separated by more than one generation. Although litigation involving these situations is relatively rare, several such cases have been heard in Argentina during the last decade.[18] These cases were initiated by the *Abuelas de Plaza de Mayo* (Grandmothers of the Plaza de Mayo) in their efforts to gain custody of their grandchildren, whose parents were assassinated during the military junta led by General Leopoldo Galtieri in the late 1970s and early 1980s.

C. "Reading the genomes"

The great probative value of DNA in identifying and distinguishing between individuals lies in hypervariable regions in both the nuclear and mitochondrial genomes. Outside of the hypervariable regions, the genomes of two randomly chosen individuals exhibit few differences. In contrast, within the hypervariable regions, two randomly chosen individuals will exhibit a number of differences. The mitochondrial genome possesses two hypervariable regions, which are characterized by random permutations of the bases, or "letters," in the DNA sequence. Currently, it is necessary to determine the sequence of the letters in these two regions—a procedure that can be expensive and often time consuming.

The pattern of variation in the hypervariable regions of the nuclear genome is quite different. Rather than random permutations of bases, the nuclear hypervariable regions possess differing numbers of tandem repeats of a fixed signature base sequence termed a "motif." Nuclear DNA possesses two types of hypervariable regions with this pattern of variation, defined by the length of the motif. The first hypervariable regions to be identified in the 1980s had motifs ranging in length from 15 to 35 bases and were known as "Variable Number Tandem Repeats" (VNTR) loci.[19] The use of these VNTR loci was eclipsed in the 1990s for

[18] *See, e.g.*, Carmen Aguiar de Lapaco v. Argentina, Case 12.059, Report 21/00, OEA/SER.L/V/II.106 Doc. 3 rev. at 340 (1999), *available at* http://www1.umn.edu/humanrts/cases/21-00.html.

[19] It is customary in genetics to refer to a delineated region of DNA, such as a region containing VNTRs or STRs, as a gene or a locus (plural: loci). In the

technical reasons[20] by the so-called "Short Tandem Repeats" (STR) loci—hypervariable regions with the same structure as VNTR's but with motifs of only three or four bases in length. Figure 1 shows a diagram of an STR locus. Biotechnological advances in the last ten years have allowed the rapid and relatively inexpensive determination of the variants in the VNTR and STR regions. Technical advances in the future may allow the determination of these variants at the scene of a crime in less than an hour.

II. DETERMINATION OF A MATCH BETWEEN THE FORENSIC SAMPLE AND THE DNA OF A DEFENDANT

The first step necessary in the analysis of DNA information is to determine whether there is a match between the forensic DNA sample and one or more reference DNA samples (in criminal litigation, these are taken from one or more defendants). The procedure for determining a match differs for nuclear DNA and mitochondrial DNA.

A. Nuclear DNA

Determination of a match between a forensic sample and the DNA of a defendant is conceptually easy to understand. DNA fragments carry an electric charge, so small pieces of DNA will move when placed in an electric field. The size of the DNA fragment will influence how rapidly it moves. Thus, DNA

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context of the legal application of DNA information, the terms gene and locus are equivalent and are used interchangeably.

[20] Forensic samples typically contain extremely small quantities of DNA. It is therefore often necessary to amplify the quantity of DNA recovered using a chemical procedure known as Polymerase Chain Reaction (PCR) to obtain enough DNA for analysis. PCR can only amplify fragments up to a certain size. Fragments containing STR loci are much shorter than VNTR loci, as the length of the repeat motif is much shorter. Consequently, all fragments containing STR loci, but only a few containing VNTR loci, may be amplified by PCR. PCR is a procedure that was first described only twenty years ago and did not become widely used in DNA forensic analysis until the 1990s. The application of PCR in DNA forensic analysis thus stimulated the shift from VNTR to STR loci.

fragments can be separated by size if they are placed in a gel (to control their movement) and an electric current is applied across the gel. This process is known as electrophoresis. For any one STR or VNTR locus, a number of different forms, or alleles, may exist. Since each allele possesses a different number of motif repeats, different alleles will have different sizes. Figure 2 shows the DNA profile for one VNTR locus from an actual case.[21] For each individual, two bands of DNA are seen, corresponding to two alleles of different sizes. Although a visual comparison of the DNA fragments from a forensic sample and the defendant may show a striking similarity in their mobility, a statistical test is required to evaluate whether they are in fact the same. The mobility of the DNA in the gel is subject to random variation or experimental error caused, for example, by small changes in temperature across the gel. Technological improvements during the last ten years have substantially reduced this experimental error. This period has also seen extensive development in the statistical procedures involved, and there is now little controversy regarding

[21] Successful identification of the alleles of a VNTR or STR locus involves a two-step process. First, long lengths of DNA must be cut into short fragments of DNA containing the VNTR or STR locus, and each DNA molecule must be cut at exactly the same place. Second, the fragments containing the VNTR or STR locus must be identified from the mixture of millions of random DNA fragments derived from other parts of the genome that do not possess the copies of the VNTR or STR locus. The first step is accomplished by the use of a particular category of DNA degrading enzymes, known as restriction enzymes, which cleave the DNA chains into fragments at specific points, defined by a sequence of (typically) four to six bases. For this reason, variation at a VNTR or STR locus is sometimes called a Restriction Fragment Length Polymorphism (RFLP). To distinguish VNTR or STR containing fragments from all others, the DNA (usually transferred to a nylon membrane for ease of manipulation) is allowed to hybridize with a radioactive "probe" containing copies of the motif sequences of the VNTR or STR locus. This "probe" hybridizes only with DNA fragments containing VNTR or STR alleles. A photographic film is then overlaid on the nylon membrane, or gel. The film is exposed at the positions of the now-radioactively labeled VNTR or STR alleles, resulting in the autoradiograph shown in Figure 2. A more complete description of these methods and procedures can be found in COLEMAN & SWENSON, *supra* note 4, at 29-59; NATIONAL RESEARCH COUNCIL, THE EVALUATION OF FORENSIC DNA EVIDENCE (National Academy Press, 1996).

the criteria for determining a match.

B. Mitochondrial DNA

In principle, the determination of a match for mitochondrial DNA is even simpler to understand. Because the two hypervariable regions of mitochondrial DNA must be sequenced, or "read," base by base, theoretically all that is required is a comparison of the two base sequences and a determination of whether they are the same. However, one feature of mitochondrial DNA complicates interpretation. In comparison to nuclear DNA, the replication machinery of mitochondria is less precise. As a consequence, changes in the DNA sequence are sometimes observed. Such changes are thought to be responsible for the observation in some individuals of more than one type of mitochondrial DNA—a phenomenon known as heteroplasmy.[22] Typically, the different types of mitochondrial DNA found in a single individual will differ by only one base, rarely by two, and, even more rarely, by three or more bases. Furthermore, the pattern of heteroplasmy may vary. Thus, individuals may be observed who are homoplasmic, that is, they possess only one mitochondrial DNA type in one tissue, but are heteroplasmic in another. Alternatively, individuals may be homoplasmic in two tissues, but for a different mitochondrial DNA type. For reasons related to the developmental origins of hair, hair samples are more likely to be heteroplasmic than other tissues. This is significant, as human hair forensic samples are frequently used as sources of mitochondrial DNA. Such samples typically do not contain sufficient amounts of nuclear DNA for analysis.

Heteroplasmy necessarily complicates the determination of a match between mitochondrial DNA extracted from a forensic sample and mitochondrial DNA extracted from an individual. It is perhaps not surprising, therefore, that this phenomenon has often been cited (albeit unsuccessfully) in admissibility challenges to the introduction of mitochondrial DNA evidence.[23] At the present time, the following guidelines exist for the interpretation of

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[22] *See* Budowle et al., *supra* note 16, at 127.

[23] *Id.* at 128-30.

mitochondrial DNA evidence:[24]

> 1. If both samples are homoplasmic and possess the same sequences in the two hypervariable regions, then there is a match.
>
> 2. If both samples are heteroplasmic and both samples possess the same mitochondrial DNA sequences, then there is a match.
>
> 3. If one sample is homoplasmic and the other is heteroplasmic, but the mitochondrial DNA sequence in the homoplasmic sequence is the same as one of those in the heteroplasmic samples, then there is a match.
>
> 4. If both samples are homoplasmic and differ at one base, then analysis of further samples is recommended. If this is not possible or if further analysis does not resolve the issue, then it is considered that there is insufficient evidence to reach a conclusion of exclusion or inclusion.

This last possibility merits some discussion. While the two samples may only differ by one base, samples originating from two random individuals would be expected to differ by more than one base.[25] In this respect, mitochondrial DNA analysis is fundamentally different from nuclear DNA forensic analysis. In nuclear DNA analysis, a comparison of the two samples can lead to one of two conclusions: either there is a match, resulting in inclusion, or there is no match. In mitochondrial DNA analysis, the lack of conclusive evidence for a match is a third possible option.

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[24] Bruce Budowle et al., *Interpretation Guidelines for Mitochondrial DNA Sequencing*, presented at the Tenth International Symposium on Human Identification (1999), *available at* http://www.promega.com/geneticidproc/ussymp10proc/content/37Budowle.pdf; *see* Budowle et al., *supra* note 16, at 128.

[25] *See* Budowle et al., *supra* note 16, at 128.

III. FREQUENCY OF DNA PROFILES IN THE POPULATION

The probative value of DNA information is directly related to the frequency of a given DNA profile in the population. We are now accustomed to seeing frequencies of nuclear DNA profiles that are vanishingly small. For example, the frequency of the DNA profile obtained from the stain on Monica Lewinsky's dress was reported to be 1 in 7.9 trillion.[26] This number is truly impressive considering that the world's population at the end of the twentieth century has been estimated to be around 6 billion. It is perhaps difficult to resist the temptation to conclude that the individual responsible for the stain could not have existed. In contrast, frequencies for mitochondrial DNA profiles typically are several orders of magnitude higher, on the order of 1 in 5,000, or even higher. An understanding of how these frequencies are determined provides some insight into their evidentiary value today and in the recent past.

A. Determination of the Frequency of a Nuclear DNA Profile

The value of VNTR and STR genes to discriminate between individuals lies in the large number of different forms, or *alleles*, they may take. Although alleles may vary in their frequency in the population, few are very rare and none are exceptionally common. Like other genes in the nuclear genome, two copies of each VNTR and STR locus are present in every cell, one copy being inherited from the father and the other from the mother. For any VNTR or STR gene, the alleles may be denoted by the number of copies of the motif that the allele contains. For example, an individual may possess one allele with 7 copies of the motif and another allele with 19 copies of the motif. The genetic constitution, or *genotype*, of that individual may then be designated (7,19). Alleles may have as few as 7 copies of the motif and as many as 44, so that the total

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[26] *See* University of Michigan, DNA Fingerprinting, Genetics and Crime: DNA Testing and the Courtroom, Determining the Frequency of the Genetic Profile in the Population, *at* http://www.fathom.com/course/21701758/session3.html (last visited Feb. 27, 2004).

number of different alleles would be 38. If there are 38 different alleles, there will be many different genotypes: (7,7), (7,8), (7,9), and so on. In fact, the total number of different genotypes can be calculated using a simple equation—$n\times(n+1)/2$—where n is the number of different alleles. In this example, the number of different alleles is 38, so the number of different genotypes will be 741.

One approach to estimating the frequency of each genotype in the population would be to determine the genotype for each individual in the population sample. With 741 different genotypes, however, the task would be daunting. For example, with a sample size of 1,000, even if every one of the 741 genotypes had the same frequency, it is quite unlikely, simply by chance, that all genotypes would be found in the sample. Even with a sample size of 10,000 individuals, all 741 genotypes may not be seen, even though they all are present in the population. Thus, impractically large sample sizes would be required to obtain accurate estimates of genotype frequencies. To circumvent this problem, the frequencies of the different alleles are first estimated and then used to estimate the genotype frequencies. The task of estimating allele frequencies in the population is considerably easier: there are only 38 alleles, whereas there are 741 different genotypes. Moreover, each individual possesses two alleles for each VNTR or STR gene, so that a sample of 500 individuals contains 1,000 alleles. To estimate the frequency of each allele, the genotype of each of the 500 individuals is determined, and the number of each allele type is counted. The frequency of each genotype can then be estimated by multiplying the frequencies of the alleles together.

To understand how these frequencies are determined, it is instructive to invoke the analogy of a roulette wheel. For example, the likelihood that the numbers 14 and 35 will come up in two spins of the wheel will be $2 \times 1/38 \times 1/38 = 1/722$. Note that there are two ways in which the numbers 14 and 35 may come up in two spins of the wheel: 14 may come up first and then 35, or 35 may come up first and then 14. Thus, $1/38 \times 1/38$ is multiplied by two. Genotype frequencies are calculated from allele frequencies using the same logic, but also take into consideration that individual allele frequencies will differ. To extend the analogy, it is helpful

now to imagine a roulette wheel that has different sized slots for each number on the wheel. With such a roulette wheel, the frequency with which the roulette ball will end up in each slot will vary, with the ball landing in larger slots more frequently on average than in smaller slots. Thus, the different sizes of the slots may be estimated by spinning the wheel 1,000 times and counting the number of times the ball ends up in the different slots. These sizes are analogous to allele frequencies.

These simple calculations permit the estimation of the frequencies of the DNA genotypes or profiles at one VNTR or STR locus. The frequencies of the genotype will vary somewhat, but most of the frequencies will range from 1 in 500 to 1 in 2,000. A number of VNTR loci have been identified and are located on all of the 23 pairs of chromosomes. To determine the frequency of a genotype, considering two different VNTR or STR loci, the frequencies of the genotype at each locus are multiplied together. This act of multiplication invokes some basic laws of genetics, among them that population frequencies are stable, that is, at equilibrium.[27] For example, if the frequency of a genotype (DNA profile) at one locus is 1 in 1,000, and the frequency of a genotype (DNA profile) at a second locus is also 1 in 1,000, then the combined frequency of the DNA profile for both loci will be 1 in 1,000,000. With the use of more loci, it is easily possible to arrive at extremely small frequencies on the order of 1 in 7.9 trillion.

Although the first case[28] in which a U.S. court was asked to consider DNA evidence was heard in 1987, it was not until the 1990s that the legal community fully appreciated the evidentiary power of DNA. Challenges to the reliability of DNA evidence under *Frye*[29] intensified as an increasing number of cases invoked this type of evidence. Abstruse scientific terms, such as "Hardy-Weinberg Law" and "Linkage Disequilibrium"—well known to population geneticists but to few others—began appearing more frequently in court transcripts.[30]

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[27] In technical terms, the population is at linkage equilibrium.

[28] Andrews v. State, 533 So.2d 841 (Fla. Dist. Ct. App. 1988).

[29] Frye v. United States, 293 F. 1013 (D.C. Cir. 1923).

[30] *See*, *e.g.*, United States v. Yee, 134 F.R.D. 161 (N.D. Ohio 1991).

Challenges to the use of DNA evidence have taken several forms:

> 1. It was argued that it was not legitimate to multiply allele frequencies together to compute genotype frequencies.[31] This simple procedure for calculating genotype frequencies from allele frequencies is an application of the so-called Hardy-Weinberg law (sometimes called the product rule). By multiplying the allele frequencies together, we assume that the combinations of alleles in each individual occur at random, and that each copy of each allele has the same chance of being passed on to the next generation, from parent to child. This assumption is not unreasonable considering the fact that men and women do not decide to have children based on their respective VNTR or STR genotypes. However, if the possession of certain genotypes, for example (7,35), provides an individual with increased resistance to an infectious disease or increased predisposition to childhood cancer, then the genotype frequencies may be higher or lower than those calculated by multiplying the component allele frequencies. Although the functions of VNTR and STR loci remain unknown, some loci have been described with functions having such effects.
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> 2. In a similar vein, it was argued that single locus genotype frequencies cannot be multiplied together because it is possible that certain combinations of VNTR and STR genotypes may also increase or decrease critical demographic variables, such as fertility and early mortality.[32]

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[31] *See*, *e.g.*, R.C. Lewontin & Daniel L. Hartl, *Population Genetics in Forensic DNA Typing*, SCI., Dec. 20, 1991, at 1745-50.

[32] J.E. Cohen, *DNA Fingerprinting for Forensic Identification: Potential Effects on Data Interpretation of Subpopulation Heterogeneity and Band Number Variability*, 46 AM. J. HUM. GENETICS. 358 (1990).

3. It was noted that racial and ethnic groups exhibit differences in the frequencies of individual alleles. It was therefore argued that the frequencies of DNA profiles for one group may not be valid for another. Data was quickly obtained for the three largest ethnic groups in the United States—African Americans, Caucasians, and Hispanic-surnamed individuals, and, consequently, this particular challenge has largely been muted.[33] Whereas the largest racial and ethnic groups show relatively minor differences in allele frequencies, some isolated and smaller ethnic groups may differ significantly in their allele frequencies.

4. Finally, the calculation of DNA profile frequencies from relatively small sample sizes (on the order of 500 to 1,000 individuals) was challenged. However, it was argued that it is not the frequency of the DNA profiles in the population sample that is of significance, but rather the frequencies in the population itself. The calculation of allele frequencies in the sample is an estimate of the allele frequencies in the whole population and therefore is subject to error. It was therefore acknowledged early on that DNA profile frequencies should be presented along with a range within which the "real" frequency (the population) lies. This range is expressed as a probability (usually 95%) and is known as the "confidence interval." Although the precise calculation

[33] The first National Research Council report, published in 1992, recommended that this problem be addressed by calculating the frequency of a DNA profile in the three largest ethnic groups in the United States—African Americans, Hispanic-surnamed, and Caucasians—and by presenting the largest of the values to the court. This so-called "ceiling" principle was employed for several years in many cases throughout the country. The practice was criticized, however, on the grounds that presenting a jury with only one number restricts the information available to the court. The recommendation was amended by the later committee, which suggested the current practice of presenting the court with the values for all three (or more) ethnic groups. *See* COMMITTEE ON DNA TECHNOLOGY IN FORENSIC SCIENCE, NATIONAL RESEARCH COUNCIL, DNA TECHNOLOGY IN FORENSIC SCIENCE (National Academy Press, 1992); NATIONAL RESEARCH COUNCIL, THE EVALUATION OF FORENSIC DNA EVIDENCE (National Academy Press, 1996).

> of this confidence interval was occasionally contested in court, it was rarely an issue. A more serious concern was whether the term "confidence interval" and its attendant probability measure, although a familiar concept in statistical circles, had the potential to raise doubts in the minds of jurors unfamiliar with statistical concepts regarding the reliability of the frequencies.

At a basic level, all of these challenges can be summarized by the question: Can the numbers be trusted? While rarely successful, these challenges have resulted in large expenditures of court time and resources.

Throughout the 1990s, the identification of additional STR loci in the human nuclear genome led to an increase in the number of STR loci that were incorporated into testing protocols. As a result, the estimated frequency of a DNA profile, based on increasing numbers of loci, decreased geometrically.[34] Consequently, the number of courtroom challenges questioning the reliability of the numbers has decreased significantly.[35] To dispense with these challenges and to counter the lack of credibility associated with extremely small frequencies[36] (such as 1 in 7.9 trillion), in 1997,

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[34] The FBI CODIS (*C*ombined *D*NA *I*ndexing *S*ystem) uses a set of 13 STR loci located on 12 chromosomes. The national and 50 state DNA databases are also based on these same 13 loci.

[35] For example, the frequency of a DNA profile in the order of 1 in 7.9 trillion could easily be 10 or even 100 times larger, and yet still be extremely small.

[36] Numbers of this magnitude inevitably raise the question of errors in testing. It is undoubtedly true that laboratory errors will occur more than once in 7.9 trillion tests. The simple confusion or cross-contamination of a defendant's sample with the forensic sample can result in an apparent positive match. It should be noted, however, that the DNA identification act mandated that testing laboratories be licensed and pass a series of stringent tests of their procedures. *See* Bruce Budowle et al., *CODIS and PCR-Based Short Tandem Repeat Loci: Law Enforcement Tools*, presented at the Second European Symposium on Human Identification (1998), *available at* http://www.promega.com/geneticidproc/eusymp2proc/17.pdf. Nevertheless, it may be prudent to require repeat testing where possible and feasible every time a positive match is seen.

the FBI advanced the concept of the uniqueness of DNA profiles.[37] At a press conference, the FBI stated, "'[i]f the estimated probability of a DNA profile is less than 260 billion, and it is seen in a person, then that person is the source of the sample.'"[38] Therefore, below this threshold frequency, no numbers need be presented. In one of the first FBI reports in which this principle was applied,[39] the FBI reported that, "'[b]ased on the results . . . specimen K39 (Clinton) is the source of the DNA obtained from specimen Q3243-1 to a reasonable degree of scientific accuracy.'"[40]

The value of such a statement, from the point of view of the prosecution, is that it marginalizes any arguments relating to the calculation of DNA profile frequencies. In addition, this statement is more easily digested by jurors, who are unaccustomed to thinking in terms of probability and statistics. On the other hand, these statements are dangerously tendentious. While appearing simply to be an extrapolation of a statistical argument, uniqueness is not a statistical concept. In its deceptive simplicity, the concept of uniqueness cannot take into account the fact that DNA profiles are almost certainly *not* unique when relatives are considered. Even though the expected frequency of a 13-locus DNA profile in a population of unrelated individuals is considerably less than 1 in 260 billion, about 3 in 1 million pairs of siblings would be expected to have the same thirteen-locus DNA profile.[41] As the following case illustrates, the failure to appreciate the genetic relatedness of family members can have serious consequences.

In 1988, following a trial by jury, James Fagan was convicted of criminal sexual conduct in the first degree in the first case in

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[37] *See DNA Fingerprinting Comes of Age,* 278 SCI. 1407, 1407 (1997).

[38] *See* B.S. Weir, *Are DNA Profiles Unique?*, presented at the Ninth International Symposium on Human Identification (1998), *available at* http://www.promega.com/geneticidproc/ussymp9proc/content/25.pdf.

[39] *Id.*

[40] *Id.*

[41] James F. Crow, *DNA Forensics: Past, Present and Future*, presented at the Tenth International Symposium on Human Identification (1999), *available at* http://www.promega.com/geneticidproc/ussymp10proc/content/01crow.pdf.

Michigan to admit DNA evidence.[42] The victim was the defendant's 14-year-old daughter, who had become pregnant and who subsequently elected to terminate the pregnancy. Tissue from the aborted fetus was preserved, and the defendant requested genetic testing in an attempt to demonstrate his innocence. Results from three VNTR loci showed that Fagan could not be excluded as the father. The results also showed that the probability of observing the three-locus profile was 1 in 13,680 in a random sample of North American African American males and 1 in 21,384 in a random sample of North American Caucasian males. The jury attached great importance to the genetic testing results in returning a guilty verdict. Fagan was sentenced to life imprisonment on October 5, 1988. A subsequent review by the Michigan Court of Appeals revealed, however, that: (a) at the time the victim became pregnant, Fagan was estranged from the family, and (b) the victim shared a bedroom with her brother, who was one year younger than she.

In this situation, the brother of the victim must be considered as an alternate defendant. The most appropriate statistical test in this case is the so-called likelihood ratio test—the likelihood that the brother impregnated the victim relative to the likelihood that Fagan impregnated the victim. This ratio is 1/8—a number[43] substantially larger than the probabilities presented to the jury.[44]

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[42] People v. Fagan, No. 113830 LC No. 86-36977FC, *appeal denied*, 447 Mich. 1039 (1994).

[43] It should be noted that the victim's mother refused to allow her son—the brother of the victim—to be genetically tested. Had he been tested and excluded as the father, the appropriate probabilities would indeed have been 1/13,680 and 1/21,384.

[44] After the Michigan appellate courts rejected Fagan's appeals, his habeas corpus petition was granted by the U.S. Court of Appeals for the Sixth Circuit, on the grounds that Fagan was denied effective assistance of counsel. Fagan v. Trippett, No. 96-1870, 1997 U.S. App. LEXIS 16403, at *1-2 (6th Cir. July 1, 1997). A petition for writ of certiorari to the U.S. Supreme Court was denied. Trippett v. Fagan, 522 U.S. 1008 (1997). Fagan was released after some eight years in prison after the prosecutor elected not to retry him.

B. Determination of the Frequency of a Mitochondrial DNA Profile

The theory behind the calculation of mitochondrial DNA profile frequencies is much simpler than that behind the calculation of nuclear DNA profile frequencies. Because the mitochondrial DNA genome is maternally inherited as a single unit, there is no possibility that new combinations of genotypes will be generated by the union of genetic material from the mother and father and by the independent behavior of the 23 pairs of nuclear chromosomes. Consequently, the frequency of a given mitochondrial DNA profile is defined simply by the number of times it appears in a sample divided by the size of the sample. Sample sizes are presently in the range of 5,000 to 10,000. While it is almost certainly true that sample sizes will increase, the degree of resolution achieved by increasing sample sizes will only increase arithmetically, whereas, for nuclear DNA, the degree of resolution will increase geometrically as more loci are considered. Therefore, the frequencies of mitochondrial DNA profiles are unlikely to change substantially in the near future.

When the minimum frequencies of mitochondrial DNA profiles are within the range of 1/5,000 to 1/10,000, their presentation in the courtroom raises two issues, which were vigorously debated during the early introduction of nuclear DNA evidence. As nuclear DNA profile frequencies dropped to astronomically low numbers, however, these concerns largely became irrelevant.

First, as with nuclear DNA, the relative frequencies of mitochondrial DNA profiles have been shown to vary among racial and ethnic groups. The magnitude of mitochondrial DNA frequencies, large in relation to those for nuclear DNA—even in the early 1990s—accentuates the importance of providing courts with appropriate information on population variation. Data are already available for the largest population groups, but these do not provide sufficient information for smaller, more isolated groups. For example, DNA profile frequencies in Native American tribes can depart significantly from those of Caucasian or African-American populations. The following early case in which

mitochondrial DNA testing was introduced illustrates a dilemma facing courts when issues of ethnic identity are raised.

In 1991, Arthur Passino was arraigned in Vermont and charged with homicide.[45] In a pretrial *Frye* hearing, the defense argued against the admissibility of DNA evidence. The defense established that Passino's paternal grandparents were Italian, his maternal grandfather was a full-blooded Abnaki, and his maternal grandmother was half French and half Abnaki.[46] In denying the introduction of DNA evidence, Judge R.F. Kilburn wrote, "It is unclear which if any of the FBI's databases is appropriate for calculating the probability of a coincidental match." [47] Professors Bruce Weir and Ian Evett questioned the ruling, arguing that because the defense claimed that Passino was innocent, his ethnicity should not be considered relevant. [48] The logic of their argument is inescapable. However, the ethnic composition of the pool of alternative suspects *is* directly relevant. In this regard, Professor Richard Lewontin noted that the victim, who was herself of Abnaki ancestry, was murdered in a trailer camp that housed many other residents of Abnaki ancestry. He thus argued that the Abnaki should be included as comprising "'the population' of potential suspects, of whom the defendant is only one."[49]

The second issue concerns the accuracy of courtroom testimony regarding DNA evidence. It is hardly necessary to state that the evidentiary value of DNA evidence is directly related to the frequency of a DNA profile. However, it is easy and unfortunately only too frequent for both the prosecution and the defense to make errors in presenting the information to the court. A correct statement would be, "the chance of obtaining this DNA

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[45] State v. Passino, No. 185-1-90 (Dist. Ct. Franklin County Vt.).

[46] The Abnaki Native American tribe and Abnaki-French Canadian families comprise a small, isolated, rural population group occupying an area on the US-Canadian border. They are mostly impoverished and many live in trailer camps.

[47] B.S. Weir & I.W. Evett, *Whose DNA?* 50 AMER. J. HUM. GENETICS 869 (1992).

[48] I.W. EVETT & B.S. WEIR, INTERPRETING DNA EVIDENCE 33 (Sinauer Associates 1998).

[49] R.C. Lewontin, *Which Population?* 52 AMER. J. HUM. GENETICS 205 (1993).

profile if the DNA in the forensic sample came from an individual other than the defendant is 1 in a million." Unfortunately, the prosecution often may say something like, "there is only a 1 in a million chance the defendant is innocent." To juries and the reader untrained in probability and statistics, these two statements may sound deceptively familiar. However, in the first example, the speaker is making a statement about the frequency of the DNA profile in the population, while in the second statement, the speaker is making a statement about the guilt or innocence of the defendant *conditional* on the frequency of the DNA profile of the forensic sample. This error has been termed the "Prosecutor's Fallacy," or in statistical terms, "the transposed conditional."[50] It is important to realize that the difference between the two statements is not just a matter of wording. In fact the second statement—"there is only a 1 in a million chance the defendant is innocent"—makes an implicit assumption concerning the likelihood of guilt or innocence of the defendant based on other, non-DNA evidence. Bayes' theorem, well known in statistics, allows the calculation of the probability of a defendant's guilt or innocence by incorporating data on the frequency of a DNA profile as well as a prior probability of guilt based on other information. Table 2 presents calculations using Bayes' theorem that incorporate a prior probability, or assumption that the defendant is guilty. These calculations show that the probability that a defendant is guilty, given a 50% prior assumption of guilt, is virtually identical to the frequency of the DNA profile in the population. In other words, by falling into the Prosecutor's Fallacy and interpreting the frequency of the DNA profile as the probability of guilt, the assumption is made that the defendant has a 50% chance of being guilty, even before the DNA evidence is examined.

Arguably, the importance of the difference between the two statements depends on the frequency of the DNA profile. Thus, if the frequency of the DNA profile is 1 in 7.9 trillion, even a prior probability of innocence of 0.99 will not change the *a posteriori* probability of guilt in a meaningful way. However, if the frequency

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[50] For further discussion of this issue, see EVETT & WEIR, *supra* note 48, at 30-31.

of a DNA profile is 1 in 10,000, as for mitochondrial DNA profiles, then Table 2 shows that the difference between the two statements becomes far more significant. For example, the odds that the defendant is innocent, given an *a priori* probability of guilt of only 0.001, are 1 in 11. In paternity cases, the court commonly is presented with a range of *a priori* probabilities together with the corresponding *a posteriori* probabilities. However, it is not clear to what extent courts will accept Bayesian statistics in other contexts.[51] Since mitochondrial DNA profile frequencies are unlikely to change significantly in the near future and since the temptation to succumb to the Prosecutor's Fallacy is frequently not resisted, it may be appropriate to advocate for the admissibility of Bayesian calculations in criminal litigation in which mitochondrial DNA evidence is introduced.

CONCLUSION

During the last fifteen years, major advances have been made in the development of techniques and statistical approaches for forensic applications of DNA information. The formidable probative power and reliability of DNA evidence is now fully recognized, and it is unlikely that DNA evidence will be superseded in the future by other technologies or approaches.

Nevertheless, we can expect to see changes or improvements in the following four areas.

1. *Technology for nuclear DNA*. While there is no need to improve upon the resolving power and reliability of information provided by nuclear DNA profiles, the biotechnological processes for analyzing DNA samples will undoubtedly improve. Techniques are already available for the multiplex analysis of DNA for several STR loci simultaneously. Technological advances are also under development that will greatly improve the speed of analysis and effect a concomitant decrease in costs, such that, within

[51] *See* Crow, *supra* note 41.

a few years, it may be possible to carry out analyses at the scene of the crime in less than an hour.[52] It is also possible that more sensitive biotechnologies may be developed, allowing nuclear DNA to be extracted for analysis from samples that hitherto contained only mitochondrial DNA in sufficient quantities.

2. *Mitochondrial DNA*. The forensic application of mitochondrial DNA information is the younger technology and, as such, offers more possibilities for improvement. While population sample sizes will increase, resulting in a proportional decrease in the frequencies of mitochondrial DNA profiles, they will never approach those of nuclear DNA. However, future research may allow for a better understanding of the nature and mechanisms underlying the generation of intra-individual variation (heteroplasmy), which may increase the probative power of mitochondrial DNA profiles. This research could potentially eliminate the "unable-to-confirm-or-exclude-a-match" category, described in Part III.B. above. Recently, there have been reports of a paternal contribution of mitochondrial DNA, so-called "paternal leakage," and of the formation of new chimaeral (recombinant) genomes incorporating both maternal and paternal sequences.[53] It is imperative that the nature and extent of these phenomena be confirmed and investigated. In the absence of further information, it is inevitable that these reports will stimulate new challenges to the admissibility of mitochondrial DNA evidence. On the other hand, the resolution of these issues should improve the acceptance of mitochondrial DNA in the courtroom.

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[52] J.M. Butler & P.M. Vallone, *High-throughput genetic analysis through multiplexed PCR and multicapillary electrophoresis*, in PCR TECHNOLOGY: CURRENT INNOVATIONS 111 (T. Weissensteiner et al. eds., 2d ed. 2004).

[53] Yevgenya Kraytsberg et al., *Recombination of Human Mitochondrial DNA*, 304 SCI. 981 (May 14, 2004); *see also* Budowle et al., *supra* note 16, at 131-33.

3. *Databases*. The last five to ten years have seen the development of DNA databases for nuclear DNA profiles in the United States, in most countries in Western Europe, and elsewhere. In the United States, there is a national database,[54] divided into three components: certain federal offenders,[55] D.C. offenders,[56] and military offenders,[57] as well as databases maintained by all fifty states.[58] In 2004, the combined size of these databases was approximately 1.6 million individuals. Fortunately, European databases use a subset of the CODIS set of 13 STR loci.[59] It has been argued that identification of individuals through database searches involves special statistical considerations. The second report published by the National Research Council of the National Academy of Sciences dealt with this issue in some detail, arguing that the match probability (frequency of the DNA profile) should be multiplied by the size of the database (the so-called "Bonferroni correction" in statistics).[60] Thus, the match probability will increase as the size of the database increases, such that, for a unique DNA profile and a database equaling the entire country, the match probability will be 1—a nonsensical result.[61]

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[54] Budowle et al., *supra* 36. *See also* Sandra J. Carnahan, *The Supreme Court's Primary Purpose Test: A Roadblock to the National Law Enforcement DNA Database*, 83 NEB. L. REV. 1, 6-7 (2004) (discussing the function and mechanics of the CODIS national database).

[55] 42 U.S.C.S. § 14135a (2004) (collection and use of DNA identification information from certain federal offenders).

[56] 42 U.S.C.S. § 14135b (2000) (collection and use of DNA identification information from certain District of Columbia offenders).

[57] 10 U.S.C. § 1565 (2004) (collection and use of DNA identification information from certain military offenders).

[58] Carnahan, *supra* note 54, at 4.

[59] Peter M. Schneider, *DNA Databases for Offender Identification in Europe—The Need for Technical, Legal and Political Harmonization*, presented at the Second European Symposium on Human Identification (1998), *available at* http://www.promega.com/geneticidproc/eusymp2proc/11.pdf (last visited Nov. 20, 2004).

[60] NATIONAL RESEARCH COUNCIL, *supra* note 21, at 5-9.

[61] EVETT & WEIR, *supra* note 48, at 221.

Professors Evett and Weir,[62] and Professors Peter Donnelly and Richard D. Friedman,[63] have proposed alternative approaches to handling database searches. There is still some disagreement, however, about how information from these databases should be treated.[64] Future resolution of these disagreements will undoubtedly prove beneficial.

The development of DNA databases for accused or convicted offenders has inevitably sparked discussion about the desirability of creating a database for all citizens.[65] Strong objections have been leveled on privacy and libertarian grounds by groups such as the ACLU. It is unclear at this point how this controversy will be resolved.

4. *Inclusion of additional information to complement DNA evidence.* Professor James Crow[66] has suggested that, in the future, other biological variables, such as the levels of gene expression, parasite loads, and immunity responses, may be used in combination with DNA to aid in the identification of even close relatives.[67] While this additional information is unlikely to supplant or supersede DNA information, it could add significantly to the information provided by DNA sequences.

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[62] *Id.* at 219-22.

[63] Peter Donnelly & Richard D. Friedman, *DNA Database Searches and the Legal Consumption of Scientific Evidence*, 97 MICH. L. REV. 931 (1999).

[64] James F. Crow, *The 1996 NRC Report: Another Look*, presented at the Ninth International Symposium on Human Identification (1998), *available at* http://www.promega.com/geneticidproc/ussymp9proc/content/24.pdf.

[65] Ben Quarmby, *The Case for National DNA Identification Cards*, 2003 DUKE L. & TECH. REV. 2, 5-7 (2003); John Wadham, *Five Reasons Against National ID Cards*, *available at* http://www.urban75.org/legal/id.html (last visited Nov. 20, 2004).

[66] *See* Crow, *supra* note 41.

[67] *Id.*

TABLE 1
CHARACTERISTICS OF NUCLEAR AND MITOCHONDRIAL DNA

Nuclear DNA	Mitochondrial DNA
~300 Million bases ("letters") in length	~16,569 bases in length*
Composed of 23 pairs of linear strands or "chromosomes"	Composed of one circular molecule
2 copies of each nuclear gene or locus/cell	50 - ~5,000 copies of each mitochondrial gene or locus/cell
Both parents contribute one gene copy to offspring	Maternally inherited; no contribution from father
Not necessary to sequence DNA to identify variants	Must sequence DNA to identify variants

* Some length variants are known

TABLE 2
CALCULATIONS ACCORDING TO BAYES' RULE

Prior Assumption of Guilt (Probability)	Odds that the defendant is innocent incorporating prior assumption of guilt and given a match between the defendant's DNA and the forensic sample	
	DNA Profile Frequency: 1/10,000	DNA Profile Frequency: 1/1,000,000
0.000	1	1
0.001	1 in 11	1 in 1,002
0.10	1 in 1,112	1 in 111,112
0.50	1 in 10,001	1 in 1,000,001
0.90	1 in 90,001	1 in 9,000,001
0.99	1 in 990,001	1 in 99,000,001

FIGURE LEGENDS

Figure 1: Shown diagrammatically is a pair of chromosomes, one possessing 17 copies of the motif AGAT, and the other possessing 12 copies of the motif.

Figure 2: DNA profiles for one VNTR locus from an actual case. Shown is an autoradiograph of DNA samples that have been labeled with a radioisotope. R – DNA profile of a reference individual. V – DNA profile of the victim. D_1 – DNA profile of one defendant. D_2 – DNA profile of a second defendant. F – the DNA profile of the forensic sample. Note that each lane contains two bands, corresponding to the two alleles of each sample. Note also the correspondence between the DNA profile of defendant 1 and the forensic sample.

The lanes designated by L (for "Ladder") show a series of DNA fragments of known sizes. These lanes are used for calibration as "molecular ladders."

FIGURE 1
Diagram of an STR Locus

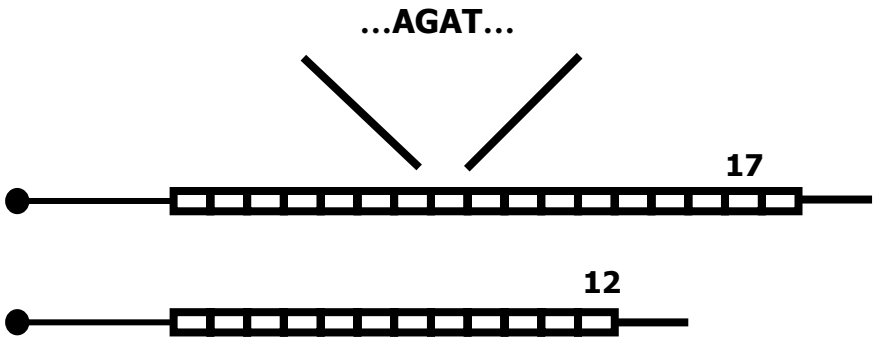

FIGURE 2