DNA in the Courtroom: The 21st Century Begins

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In 2004 at the 50th anniversary of the discovery of the structure of DNA, one of the speakers at a "black-tie" gala at the Waldorf Astoria in New York City was Marvin Anderson. After having served 15 years of a 210-year sentence for a crime that he did not commit, he became one of only 99 people to have been proven innocent through the use of DNA technology[1]. As he walked off the stage, he embraced Dr. Alec Jeffreys,[2] the man who discovered forensic DNA analysis.[3]

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[1] Innocence Project, Marvin Anderson, http://www.innocenceproject.org/case/display_profile.php?id=99 (last visited (December 14, 2006).

[2] Professor Sir Alec John Jeffreys, FRS, is a British geneticist, who developed techniques for DNA fingerprinting and DNA profiling. DNA fingerprinting uses variations in the genetic code to identify individuals. The technique has been applied in forensics for law enforcement, to resolve paternity and immigration disputes, and can be applied to non-human species, for example in wildlife population genetics studies. Jeffreys refined his technique by developing DNA profiling based on highly variable minisatellites in the human genome. With highly automated and sophisticated equipment, the modern-day DNA fingerprinter can process hundreds of samples a day. *Alec Jeffreys and The Birth of Forensic DNA*, http://home.iprimus.com.au/dna_info/dna/JA_DNA_LegSci_2.html (last visited December 14, 2006).

[3] LAWRENCE KOBILINSKY ET AL, DNA: FORENSIC AND LEGAL APPLICATIONS xii (Wiley-Interscience Publishers 2005).

DNA is one of the most significant discoveries in the field of forensic evidence yet it remains underutilized in the courtroom setting. This article provides an introduction to the scientific principles, structure and composition of DNA in an effort to make DNA more accessible to the judicial process.

HISTORY OF DEOXYRIBONUCLEIC ACID

In 1859, Louis Pasteur[4] finally proved that things which were too small to be seen by the naked eye were indeed alive when he disproved the theory of spontaneous generation; a theory previously held to be true by the great philosopher, Aristotle, and most people up to that time.[5] This date, 1859, will serve as a "time-marker" for appreciating where we are at any given spot in the history of the forensic uses of DNA.

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[4] Louis Pasteur (1822 - 1895). Called a chemist and not a microbiologist at the time, Louis Pasteur saved the French wine industry by determining that bacteria in the air could live in wine and make the wine sour. By developing the process now known as 'pasteurization', he demonstrated that living entities (incorrectly known as germ and now known as bacteria) could be killed by boiling and then cooling the wine. Pasteur then set about proving that these bacteria came from the air and did not arise spontaneously from the wine itself. He demonstrated this by sealing a quantity of a liquid in an airtight jar and leaving another quantity exposed to the air. Using the conceptual framework first put forth by Edward Jenner, he developed a process of vaccination against the killer disease, smallpox. Pasteur went on to discover vaccinations for chicken pox, cholera, diphtheria, anthrax and rabies. Pasteur believed that his germ theory could be used to explain how vaccination worked and diseases were spread among people or animals. Pasteur.fr, Louis Pasteur-Biography, http://www.pasteur.fr/pasteur/histoire/histoireUS/BioPasteur (last visisted December 14, 2006).

[5] RENE DUBOS, PASTEUR AND MODERN SCIENCE 54 (DOUBLEDAY AND CO, INC. 1960).

In approximately 1862, Gregor Mendel's[6] work with pea plants began our understanding of inheritance and the rules by which it worked.[7] This was a crucial step in making our present technology even a possibility. Specifically, if we are able to say something accurately about the mechanism of inheritance we then may have enough evidence to say that this suspect is not associated with this evidence, victim or crime scene.

In 1869, Friedrich Meischer[8] first described DNA with no realization of its significance to cell function, by recognizing that Nucleic Acid as a distinct component of living matter. Meischer thought that this was a curious material, but suspected that there was too little of it to be of much importance in cell function.[9]

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6 Gregor Mendel (1822-1884) developed the basic theories of heredity based on his work with pea plants. Mendel was the first person to trace the characteristics of successive generations of a living thing. His experiment was "designed to support or to illustrate Lamarck's views concerning the influence of environment upon plants." He found that the plants' respective offspring retained the essential traits of the parents, and therefore were not influenced by the environment. This simple test gave birth to the idea of heredity. The notion that traits were inherited in certain numerical ratios led to the concepts of dominance and gene segregation. From his studies, Mendel derived certain basic laws of heredity: hereditary factors do not combine, but are passed intact; each member of the parental generation transmits only half of its hereditary factors to each offspring (with certain factors "dominant" over others); and different offspring of the same parents receive different sets of hereditary factors. Mendel's work became the foundation for modern genetics. Gregor Mendel, *The Beginning of Biomathematics*, http://mendel.imp.ac.at/mendeljsp/biography/biography.jsp (last visited December 14, 2006).

7 ROBIN MARANTZ HENIG , THE MONK IN THE GARDEN:THE LOST AND FOUND GENIUS OF GREGOR MENDEL, THE FATHER OF GENETICS 2 (Houghton Mifflin Company 2000).

8 Letter from Dr. I.O.I. Davies, MRPharmS, to The Pharmaceutical Journal (March 4, 2000), at, http://www.pjonline.com/Editorial/20000304/letters/davies.html.

9 HORACE F. JUDSON, THE EIGHTH DAY OF CREATION: MAKERS OF THE REVOLUTION IN BIOLOGY, (1996 Expanded Edition), *at* http://www.fmi.ch/members/marilyn.vaccaro/ewww/eighth.day.creation.htm.

In 1933, Thomas Hunt Morgan[10] won the Nobel Prize for Medicine for his work on the chromosomal theory of inheritance.[11] This achievement was a major step forward because it established an understanding that inheritance was indeed linked to chromosomes. This discovery allowed for a more focused view for subsequent research. In the 1940's, Oswald Avery[12] and Linus Pauling,[13] among others, studied

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[10] Thomas Hunt Morgan (1866 – 1945) was an American geneticist and embryologist. By studying mutations in the fruit fly, *Drosophila melanogaster*, Morgan was able to demonstrate that genes are carried on chromosomes and are the mechanical basis of heredity. Nobelprize.org, *Thomas Hunt Morgan*, http://nobelprize.org/nobel_prizes/medicine/laureates/1933/morgan-bio.html (last visited August 28, 2006).

[11] Thomas Hunt Morgan, *Sex Limited Inheritance In Drosophilia*, 32 SCIENCE, 120-122 (1910).

[12] Oswald Avery (1877-1955) began his research by developing immunological and chemical methods to differentiate strains of bacteria. One of the first molecular biologists, he was a pioneer in immunochemistry, but he is best known for his discovery in 1944 with his co-workers Colin MacLeod and Maclyn McCarty, that DNA is the material of which genes and chromosomes are made. They determined that bacteria are able to transfer genetic material through a liquid medium to other bacteria, transforming (giving new genetic characteristics to) them. Oswald Avery – Biography, http://www.britannica.com/eb/article-9011425/Oswald-Avery (last visited December 14, 2006).

[13] Linus Pauling (1901 - 1994), major interest was in the nature of the chemical bond. This led him to publish more than about 350 publications in the fields of experimental determination of the structure of crystals by the diffraction of X-rays and the interpretation of these structures in terms of the radii and other properties of atoms; the application of quantum mechanics to physical and chemical problems, including dielectric constants, X-ray doublets, momentum distribution of electrons in atoms, rotational motion of molecules in crystals, Van der Waals forces, etc.; the structure of metals and inter-metallic compounds, the theory of ferromagnetism; the nature of the chemical bond, including the resonance phenomenon in chemistry; the experimental determination of the structure of gas molecules by the diffraction of electrons; the structure of proteins; the structure of antibodies and the nature of serological reactions; the structure and properties of hemoglobin and related substances; abnormal hemoglobin molecules in relation to the hereditary hemolytic anemia; the molecular theory of general anesthesia; an instrument for determining the partial pressure of oxygen in a gas; and other subjects. In 1954, he was

the nature of protein. Influenced by Oswald Avery and Edward Tatum's[14] work on the transforming ability of DNA, Joshua Lederberg[15] proved in 1946 that bacterial conjugation, the exchange of DNA material between two bacteria, occurred, ending the controversy over the existence of genes

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given the Nobel Prize in Chemistry for his work on protein bonding. In 1963, he was awarded the Nobel Peace Prize. Nobleprize.org, *Linus Pauling*, http://nobelprize.org/nobel_prizes/chemistry/laureates/1954/pauling-bio.htm.

[14] Edward Lawrie Tatum (1909 –1975) was an American geneticist who shared half of the Nobel Prize in Physiology or Medicine in 1958 with George Wells Beadle for showing that genes control individual steps in metabolism. The other half of that year's award went to Joshua Lederberg. Beadle and Tatum's key experiments involved exposing the bread mold *Neurospora crassa* to x-rays, causing mutations that, in turn, caused alterations in enzymes involved in metabolic pathways. These experiments supported the concept known as the "one gene, one enzyme" hypothesis. Later, Tatum developed the understanding the basis of the metabolism of the amino acid, tryptophan in *Escherichia coli*. Nobelprize.org, *Edward Lawrie Tatum*, http://nobelprize.org/nobel_prizes/medicine/laureates/1958/tatum-bio.htm.

[15] Joshua Lederberg (1925 -) Between 1946 and 1952, Joshua Lederberg almost single-handedly reshaped the field of bacterial genetics. His work to prove that bacteria could serve as a powerful experimental system, with widespread application in genetic research gave crucial insights into the chemical mechanisms of gene action and helped explain the evolution and adaptation of microorganisms. Lederberg together with George W. Beadle and Edward L. Tatum, used nutritional mutants of Neurospora that were genetically blocked in the synthesis of growth factors such as a particular amino acid or vitamin, to reach their famous "one gene, one enzyme" hypothesis. Their hypothesis stated that genes were specific sections of the genetic material that direct the synthesis of particular proteins. Lederberg was able to show that the reversal of this phenomenon was caused by a reverse mutation, and that the mutation and the reversion were allelic, meaning that they occurred in the same place on the chromosome. Lederberg also proved that, under certain circumstances, bacteria display sexual behavior, that is, reproduce by a recombination of their genes using a process he called conjugation. Nobelprize.org, *Joshua Lederberg*, http://www.nobelprize.org/nobel_prizes/medicine/laureates/1958/lederberg-bio.htm.

in bacteria. Lederberg, along with George Beadle and Edward Tatum, received the 1958 Nobel Prize in Physiology or Medicine for this discovery. In 1954, while at Cambridge University, James Watson,[16] Francis Crick and Rosalind Franklin determined the structure of DNA.[17] In 1962, James Dewey Watson and Francis Crick, while Franklin had since passed, received the Nobel Prize in Physiology or Medicine for these advances, which completed the thought process about inheritance. Indeed by this time, scientists had begun to appreciate the whole picture of how one human is "coded" in all the specificity that presumes. This created the basis for being able to say that this person is the one and only human

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[16] James Dewey Watson KBE (Hon) (1928 -) is one of the four discoverers of the structure of the DNA molecule. Watson, Francis Crick, and Maurice Wilkins were awarded the 1962 Nobel Prize for Physiology or Medicine, for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material. Watson was aware of the work of Oswald Avery that suggested that DNA was the genetic molecule. In 1951 the chemist, Linus Pauling, published his model of the protein alpha helix, a result that grew out of Paulings' relentless efforts in x-ray crystallography and molecular model building. Watson thought that x-ray diffraction experiments might determine the structure of DNA. Crick soon solved the mathematical equations that govern helical diffraction theory; Watson knew all of the key DNA results of the Phage Group. Watson and Crick constructed an incorrect molecular model of DNA in which the phosphate backbones were on the inside of the structure. Rosalind Franklin asserted that the phosphates almost certainly were on the outside not the inside. Watson and Crick eventually came to see that she was right and used this information in their final determination of the helical structure Watson's key contribution was in discovering the nucleotide base pairs that are the key to the structure and function of DNA. This key discovery was made in the Pauling "tradition", by playing with molecular models. Chargaff had already suggested the pairing, which, in Watson's mind, were the "big" two-ring A and G technically referred to as purine structures being paired with the "small" one-ring T and C, known as pyrimidines. Nobelprize.org, *James Dewey Watson*, http://www.nobelprize.org/nobel_prizes/medicine/laureates/1962/watson-bio.html. *Nobelprize.org*, *Francis Crick*, http:// nobelprize.org/nobel_prizes/medicine/laureates/1962/crick-bio.htm.

[17] A. Klug, *Rosalind Franklin and the Discovery of the Structure of DNA* 219 NATURE 808 - 844 (August 24 1968).

likely to be associated with this material, and to say so with some certainty.

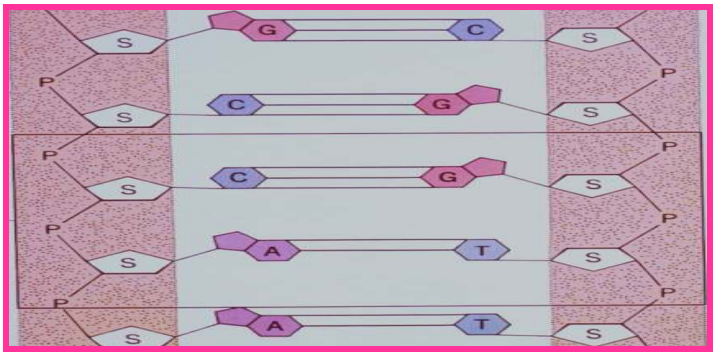
DNA: A NEW PARADIGM

From the simple dominance and recessive inheritance of traits as described by Gregor Mendel, the understanding of the structure and function of DNA has changed the way one thinks about the cell, how it caries out its various activities, and the impact of inheritance on health and disease.

THE BASIC STRUCTURE OF DNA

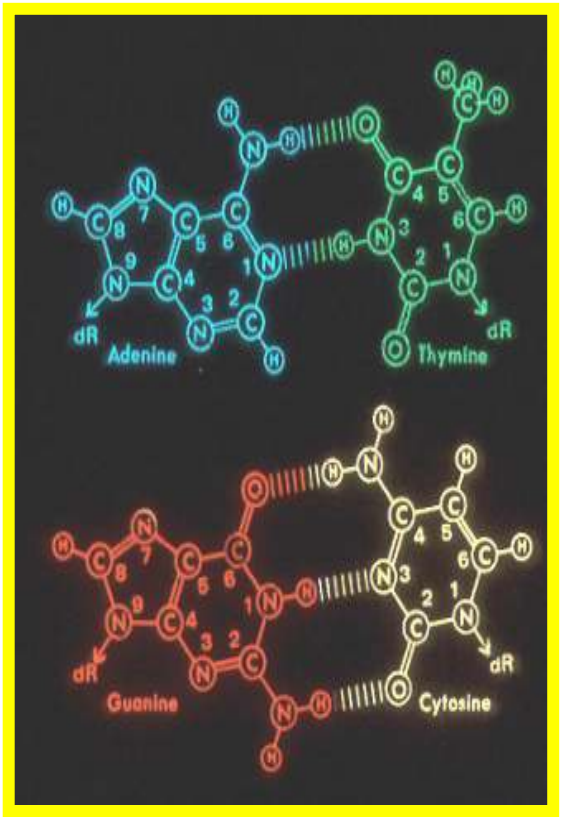
DNA is composed of two long strands of deoxyribose, a sugar, which is linked together in such a way that there is a phosphate backbone. Nucleotide bases, the basic building blocks of nucleic acids, in DNA are the purines adenine and guanine, and the pyrimidines, cytosine and thymine. These bases are strung in between these two strands and are held together by comparatively weak hydrogen bonds. DNA is frequently described as a ladder in which these nucleotide bases form the rungs of the ladder while the sugars and phosphates form the long parallel members. (See figure #1)

Figure #1: A depiction of the DNA molecule showing the double helix formation with the deoxyribose strands forming the coils and the nucleotide bases forming the bridges between the two strands.



The bases are usually designated as A, C, G, and T. (See figure #2)

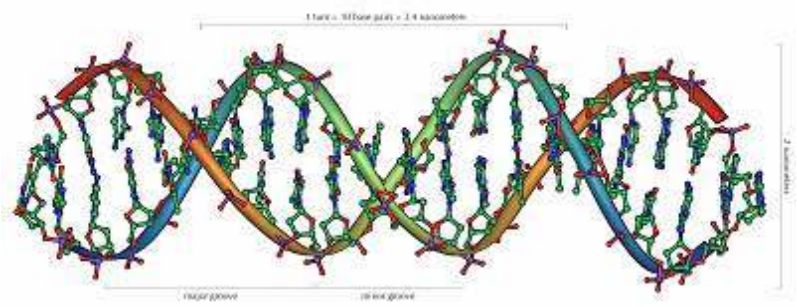
Figure #2: The four nucleotide bases and their relative symmetry.



Because of their chemical characteristics, cytosine will only bind to guanine while adenine will only bind to thymine. In RNA (Ribonucleic Acid), the companion molecule to DNA, adenine will bind to uracil, because there is no thymine in RNA. Regardless of molecule, these pairs of bases are both 10.7 Angströms, a unit of length used to measure molecules,

in width, thus giving DNA a remarkable symmetry.[18] One Angström is equal to 10-10 or 0.0000000001 meters. (See figure #3).

Figure #3 A drawing of an X-ray crystallography of DNA showing the helical structure.



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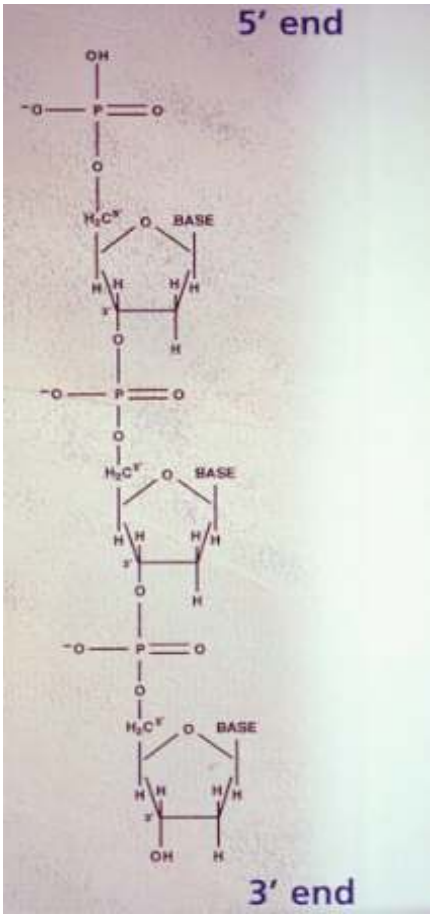
The molecule has another characteristic called directionality. The deoxyribose can bind to another deoxyribose at two places. They are numbered as 3' (three prime) or 5' (five prime) binding sites. (See figure #4)

[18] Alexander Rich, *The Double Helix: A Tale of Two Puckers,* 10, 4 NATURE STRUCTURAL BIOLOGY 247 - 249 (2003).

[19] At http://en.wikipedia.org/wiki/Image:DNA_Overview.png#filehistory

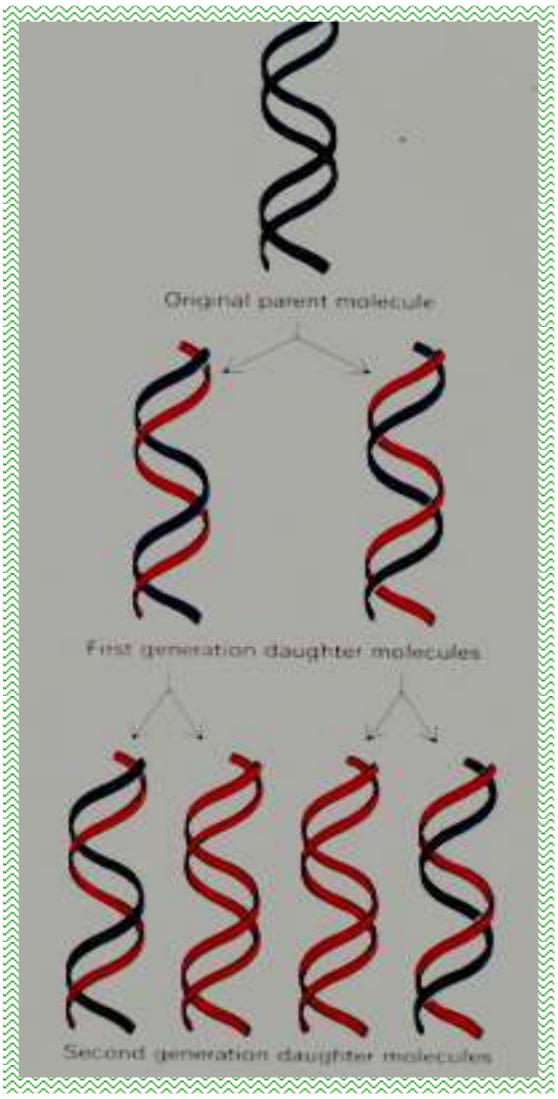
Figure #4 Deoxyribose with the 3' and 5' binding sites identified.



The 3' binding site of one deoxyribose will chemically bond to the 5' binding site of the second molecule. When two-deoxyribose molecules bind to each other, one sugar has a free 3' end while the other has a free 5' end. In the double stranded DNA, the two strands have opposing directionality.

While a DNA molecule is most often seen in the double stranded structure, the two strands actually separate during a process known as replication. The process by which one double stranded DNA molecule can become two double-stranded DNA is called semi-conservation replication in that one strand from the former molecule remains in each of two successor molecules. (See Figure #5)

Figure #5 Semi-conservative replication is performed by using one of the two parent DNA strands as a template upon which to build a new strand. This method assures a high level of accuracy from generation to generation



Next, the parent DNA molecule opens to form two single stranded molecules. Through the use of specific enzymes, a second strand of DNA that is complementary to the parent strand is constructed.

In the cell, DNA is twisted and twisted again to form super coiled packets. In the first twisting phase, DNA is coiled around a protein known as a nucleosome, and then these nucleosomes are further twisted together to form chromosomes. A cell is then programmed to undergo mitosis to produce two identical daughter cells.

NON-SEXUAL INHERITANCE OF DNA

DNA allows for the passage of characteristics from one generation to the next. It does this by using a code that incorporates the nucleotide bases. For every three-nucleotide bases of DNA, its companion molecule, RNA, can identify an amino acid. RNA strings a number of amino acids together and forms a protein. Proteins are essential for life and serve three, among other, important functions. Proteins control the hemoglobin inside of red blood cells that bring oxygen to the tissues, hormones that control growth, and enzymes that form blood clots.

This process, where the body grows and replaces cells, is called mitosis. The period of time between mitotic activities is called interphase, and it is during interphase that the cell doubles its DNA through semi-conservative replication. During interphase, little activity can be visualized in the nucleus, the major organelle of a cell. The first stage of mitosis is called prophase and is characterized by the chromosomes becoming visible and forming pairs. During the second stage or metaphase, the paired chromosomes move to the cell's place of division. During metaphase, the chromosomes are pulled apart to opposite sides of the cells. In the final stage of mitosis, or telophase, cell walls are manufactured to create two independent cells that contain identical DNA.

SEXUAL INHERITANCE OF DNA

A second process called meiosis is used in situations of sexual reproduction. Different from mitosis, this process creates gamete cells, which contain only one-half of the original cell's DNA. When two gametes, sexual cells such as ovum and sperm, merge, the total number of chromosomes is identical to the original cell, but the content of the chromosomes is now a mixture from two parents and not an identical offspring. This alteration results in the RNA identifying different amino acids for certain proteins and these different amino acids result in different proteins being made. For example, a blood group may be identical to only one parent or to neither. (See table #5). Hair color and skin pigmentation are other examples.

Table #5: Possible ABO blood groups found in the offspring of one parent with blood group A and the other parent with blood group B. Genes that determine blood groups A and B are expressed while the genes for blood group O are suppressed. Parent #1 has a genotype or collection of genes A and O that produce the A blood group, while parent #2 has the genotype of B and O that produce the B blood group. The offspring then could have AB, B, A, or O blood groups.

	Parent 2: A	Parent 2: O
Parent 1		
B	AB	BO or group B
O	AO or group A	OO

MITOCHONDRIAL INHERITANCE OF DNA

While the major issues of inheritance are concerned with nuclear DNA, it is important to remember than DNA is also found in mitochondria. Mitochondria are structures in the cell that contain maternal DNA. They are responsible for specific

activities necessary to maintain life such as certain forms of energy production. Because the portion of sperm that merges with the ovum in humans usually does not contain mitochondria, the analysis of mitochondrial DNA has been limited to providing information concerning the maternal line.

TECHNOLOGY BRIDGE

DNA's composition and importance has expanded from ideas to actual usefulness in an amazingly short period of time. The following is an overview of the major types of testing that are based on the molecular understanding of genetics and inheritance.

IMMUNOASSAY

Immunoassay, the earliest form of DNA testing, was actually testing for the products, or the specific biological substance of DNA. The products of DNA coding frequently are large molecules which are identifiable by the body's immune system as "self." Self-proteins, when given to another, are recognized as "non-self" and the body's immune system manufactures antibodies to defend against them. These antigens can range from anywhere from viral particles such as measles, to markers found on the membranes of cells such as those tested for in organ transplantation. In 1900, Karl Landsteiner[20] identified the common blood groups A, B,

[20] Between 1901-1903 Landsteiner discovered that a reaction may occur when blood of one human is mixed with the blood of another human being. In turn, this might be the cause of the subsequent shock, jaundice, and haemoglobinuria identified from earlier attempts at blood transfusions. His suggestions received little attention until 1909 when he classified the blood of human beings into the well known ABO blood group system. Also between 1901-1903, Landsteiner suggested that characteristics determining the blood groups are inherited and the blood groups may be used to decide instances of doubtful paternity. While in New York, Landsteiner helped discover the Rh-factor in blood.

and O using a process known as antigen-antibody testing.[21] By using a known antibody against an unknown antigen, one can determine the presence or absence of its specific antigen. In reverse, if one uses a known antigen; it is possible to determine the presence or absence of a specific antibody.

DNA PROBE TECHNOLOGY

This significant technological advance was eclipsed by the development of "DNA Probes." Where the "immunoassay" involved any antigen looking for its corresponding antibody, the "DNA probe assay" involved a short nucleic acid sequence (probe) specifically known to be associated with a particular desired biological entity (and no other) looking for its corresponding DNA sequence in the specimen. The target here could be "genomic DNA," which is derived from the nucleus of the target cell, or "mitochondrial DNA," derived from the cytoplasm of the cell, or any one of a few other types of nucleic acids associated with biological entities.

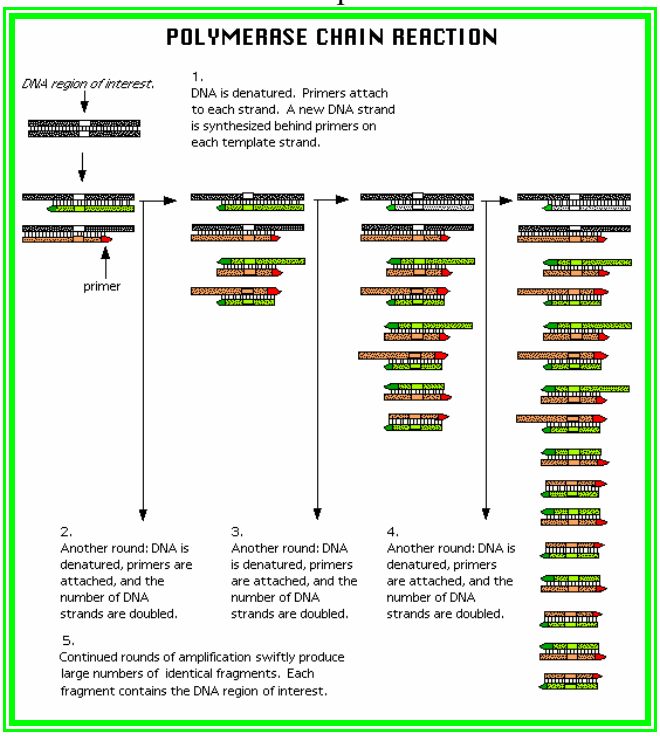
In this regard, all "probes" would be synthetic versions of the nucleic acid sequence that was initially found to always be associated with a particular biologic entity (and no other). The probe's target in the testing process could be the naturally occurring complementary sequence found in the specimen, where this technology could "find" one in one billion (1 x10*9*). Even this level of seeking was eventually eclipsed by the development of "amplification" processes, most notably the Polymerase Chain Reaction (PCR), which was developed in 1985.

Nobelprize.org, *Karl Landsteiner*, http://www.nobelprize.org/nobel_prizes/medicine/laureates/1930/landsteiner-bio.htm.

[21] Akihiko Kimura et al , *Production and Characterization of Monoclonal Antibodies Against Tissue Specific Epitopes on ABO Blood Group Substances in Saliva,* 104 INTL. J. OF LEGAL MEDICINE 1437-1596 (July 4, 1991).

In the basic PCR protocol, the target nucleic acid sequence is either "extracted" from the specimen (blood, tissue, etc.) or "hybridized" onto a fixed spot in a tissue. The amplification process then proceeds via the separation of nucleic acid strands (DNA) using gentle heat, targeting the specific sequence, and attaching "primers," then polymers, then facilitating the "copying" process many times over. (See Figure #6)

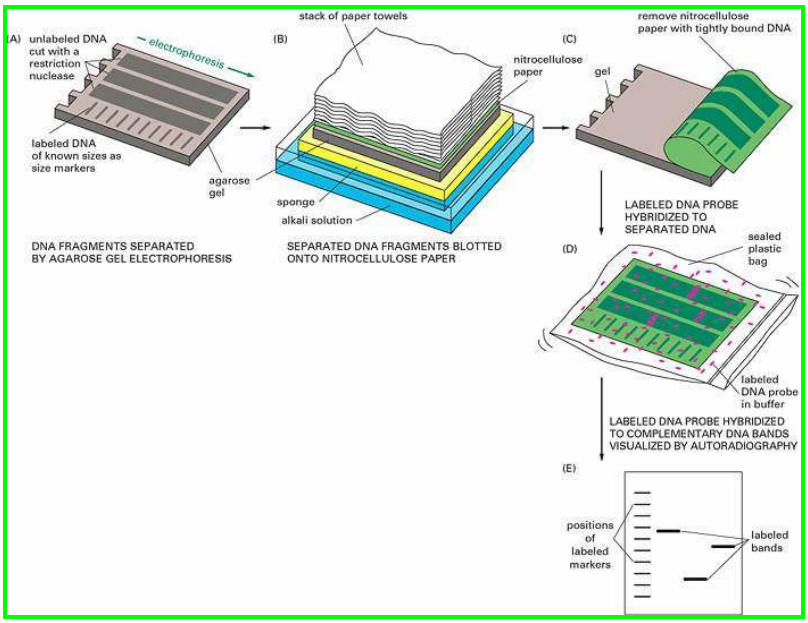
Figure #6 The principle of PCR. By using techniques to increase the number of DNA molecules of interest, one strand of DNA can be multiplied millions of times.



PCR TECHNOLOGY

The Southern Blot is an example of a current technology that can detect a specific DNA fragment in a complex sample.[22] Procedurally you would cut all DNA with Restriction Endonucleases, separate by Electrophoresis, transfer to nylon or other flat surface membrane, hybridize with labeled probe and then detect the hybridized product. (See Figure #7).

Figure #7. The Southern Blot Technique.



As with all technology, there are some limitations. The whole process is costly in terms of set up, maintenance, and performance. It is technically demanding with complex quality control issues. It is also labor intensive with

[22] P.C. TURNER et al., MOLECULAR BIOLOGY 159 (Springer Verlag 2000).

sophisticated training issues. Finally, there is the concern for contamination throughout the procedure, from specimen collection to final analysis.

Traditionally these procedures require a separate laboratory facility including dedicated lab coats, chemicals, solutions, equipment and environmental controls for reagent preparation (filtered air circulation, etc.). Appropriately, similar facilities are required for specimen preparation as even the smallest bit of DNA from one specimen will be amplified and appear as a completely legitimate finding in any other specimen. Additional separate and dedicated facilities must also be made available for the amplification and detection phase of the process.

FORENSIC USES OF DNA

DNA "Fingerprinting"[23] has its origins beginning in 1985 as Dr. Alec Jeffreys of Leicester University observed in a curious article on a DNA sequence in the myoglobin gene where some DNA regions are repeated, and the number of repeats vary somewhat by individual.[24] Jeffreys referred to these repeating sequences as Variable Number of Tandem Repeats (VNTRs) and could identify them by using Restriction Fragment Length Polymorphism (RFLP), a technique that uses a restriction enzyme to cut DNA around the VNTRs.

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[23] DNA Fingerprinting: A barcode like occurrence which can distinguish the uniqueness of one individual from another. It is most often used to determine the presence of a particular time by matching their fingerprints at a crime scene.

[24] A.J. Jeffreys & V. Wilson & S. L. Thein, *Individual-Specific 'Fingerprints' of Human DNA,* NATURE 316, 76 - 79 (4 July 1985).

The first forensic use of this idea was in an immigration case where the suspect was exonerated using this process. On another occasion, Dr. Jeffreys was approached by the Leicester Police Department regarding unsolved cases involving the rape and murder of two local teenage girls, one in 1983 and one in 1986.[25] In an attempt to use Prof. Jeffreys' new technique, the police department had every male in the town tested. They were all initially exonerated. Later on, after an overheard conversation in a pub, one male, Colin Pitchfork, who had evaded the first round of testing by having another man stand in for him, was tested a second time. As a result of this second round of testing, Pitchfork was indicted after a positive result was found. Mr. Pitchfork, a cake decorator, was ultimately convicted in 1987 as the first person ever to be indicted and convicted using forensic DNA evidence. Since then, forensic DNA has been described as the most useful investigative tool in the past 100 years since the use of quasi-genetic fingerprinting. (See Table #1)

Table #1 - A more complete listing of sentinel events leading to our current exploitation of this technology.

1977	DNA Sequencing (Frederick Sanger)
1985	PCR first described (Kary Mullis)
1986	First public DNA testing (Cellmark, Lifecodes)
1988	FBI begins using Single locus RFLP
1989	First gel Silver stain, Slot Blot & Dot Blot
1990	DQ□ Beginning of the HGP

[25] JOHN M.BUTLER, FORENSIC DNA TYPING: BIOLOGY, TECHNOLOGY, AND GENETICS OF STR MARKERS 3 (2nd ed. 2005).

1995	O.J. Simpson
1998	CODIS (13 very good markers, all on introns)
2000	FBI stops using RFLP, changes to Multiplex STR HGP finished !!!
2001	Consortium of Biomolecular Computing (CBC), Weizmann Institute

The first major U.S. case involving the use of forensic DNA evidence occurred on August 26, 1999. While in the early morning, a University of Virginia student awoke with a gun pressed against her head. She, and a male friend who was sleeping, were terrorized. She was subsequently raped and sodomized. The assailant eventually stole money and beer from the student's dorm room as he left the premises, apparently completely unnoticed. The victims were unable to provide a useful description to the police during the investigative process. However, crime scene investigators were able get saliva from an empty beer can as well as invisible semen from bed sheets. The Virginia Division of Forensic Services and the Charlottesville Police Department used the minimal tools at their disposal, including the Sex Offender Registry and the Convicted Offender Registry, and on Oct. 5, 1999 a suspect, Montaret D. Davis of Norfolk, VA., was identified by the DNA evidence collected at the crime scene and was indicted. On April 18, 2000, Davis was convicted of rape, forcible sodomy, abduction and is currently serving a 90 year sentence in a Virginia state correctional facility.[26]

In just over 20 years since Dr. Jeffreys' discovery, there are hundreds of public DNA forensic laboratories in the U.S. There are several dozen private paternity testing labs, with hundreds of thousands of DNA tests performed annually utilizing various developed and tested techniques such as single-locus Probe RFLP, Multi-locus Probe RFLP, and PCR.

[26] *Id.* at 1.

The decrease in the time needed for analysis has dropped from eight weeks to mere hours, while at the same time, the sensitivity has increased to a level to be contributory in real-time to the investigatory process.

An unanticipated, but celebrated, example of the ubiquity of these processes occurred relative to FBI Spec. #Q3243, a dress, and Spec. #K39, DNA from President Bill Clinton. Using the HaeIII digest, high molecular weight DNA was identified to contain evidence of the following genetic loci: D2S44, D17S79, D4S139, D5S110 and D7S467, thus linking President Clinton to the garment of Monica Lewinsky. [27] These developments placed forensic DNA squarely in the list of great forensic techniques.

CLASSICAL FORENSICS of DNA

Fingerprints, or Dactyloscopy, is one of the most notable classic forensic techniques, This process uses inked surfaces to take impressions from papillary ridges on the fingertips, or the palms of hands, or the soles of feet.[28] These reveal different patterns that are considered to be unique per person and remain unchanged during life. (See table #2)

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[27] RICHARD SAFERSTEIN, CRIMINALISTICS: AN INTRODUCTION TO FORENSIC SCIENCE 363, 365 (2001).

[28] *The Science of Fingerprinting,* UNITED STATES GOVERNMENT PRINTING OFFICE 1990.

Table 2

Fingerprints

- NCIC (National Crime Information Center)
  - 20 characters (2/finger)

01-49, ulnar loop	CI, central pocket whorl - inner tracing
51-99, radial loop	CM, central pocket whorl - meet tracing
AA, plain arch	DO, double whorl - outer tracing
TT, tented arch	DI, double whorl - inner tracing
PI, plain whorl - inner tracing	DM, double whorl - meet tracing
PO, plain whorl - outer tracing	XI, accidental whorl - inner tracing
PM, plain whorl - meet tracing	XO, accidental whorl - outer tracing
CO, central pocket whorl - outer tracing	XM, accidental whorl - meet tracing

Source: Cole, S.; ***Suspect Identities: A History of Fingerprinting and Criminal Identification***, Harvard University Press, Cambridge, Massachusetts, 2001

In the 1970s, a process called Automated Fingerprint Identification System (AFIS) computerized these fingerprint files, by storing, classifying, sorting, and retrieving them.[29] Currently, the National Crime Information Center (NCIC) uses twenty characters, two per finger, as the indexing technique. Similar to forensic DNA, there are several limitations to identification due to differing qualities of technique. For example, a comparison of two inked prints may have specific identification points, however, latent prints taken at a crime scene are more likely to have far less. Current jurisdictional and practice standards usually require seven or eight matching points for identification.

In the big scheme of things, the now classified "old wisdom," was that you needed a big bloodstain to retrieve a sufficient DNA sample. The "new" wisdom is that you need only one cell. In ABO blood grouping, one of the standby technologies, advantages included the procedure being simple, fast and inexpensive. Some of the limitations,

[29] *Id.*

however, were that there were only four possible results, A, B, O, AB, and that 83% of the U.S. population is either A or O. Newer approaches, those utilizing mitochondrial DNA, Restriction Fragment Length Polymorphism, Multiplex Short Tandem Repeats and identification of the gene locus, DQ□, avoid these limitations and can be quite "inclusive."[30]

USE OF DNA IN COURT

The key to using DNA in the courtroom is the specificity and selectivity that may be brought to bear. To illustrate this problem, consider the following hypothetical problem. The post office gets a letter from any address in the country addressed to any other address in the country. The post office will solve this problem by dividing all 300 million Americans into small groups, by Zip Code; and then by further dividing those into smaller groups, the nine digit zip code; and finally, by dividing those people into smaller groups, by street address. Forensic DNA testing works on this principle.

If we assume that gene loci[31] are independently inherited at the time of gene recombination, then the idea of the alleles,[32] at one place being independently inherited from any other would create the mathematics of the "zip code" example above. However, we need to have enough variation at each locus and enough loci to multiply together. When we multiply the genotype frequency by the number of loci (according to "Product Rule" mathematics),[33] the result of such arithmetic is referred to as a DNA profile frequency. A simple and pre-forensic DNA era example would be the use of ABO blood groups.

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[30] Catherine Theisen Comey et al., *PCR Amplification and Typing of the HCA DQ Alpha Gene in Forensic Samples*, 32, 8 J. FORENSIC SCI. 1-11 (1993).

[31] Gene Loci: Collections of genes at a particular place.

[32] Allele: A specific gene located at a lone place.

[33] HANDBOOK OF MATHEMTAICAL FUNCTIONS WITH FORMULAS, GRAPHS, AND MATHEMATICAL TABLES, 11 (Milton Abramowitz & Irene A. Stegun, eds., Dover Press, 9th ed. 1972).

ABO blood groups contain 3 Alleles, A, B and O, which can be sorted into six possible Genotypes, AA, BB, OO, AO, BO and AB. One of the multiplier consequences of forensic DNA technology would occur if we had some locus that had 10 alleles, vs. three in the ABO example. This would sort to fifty-five genotypes, and a locus with twenty alleles, for example, could account for 210 genotypes. This is a number much bigger than six, using the ABO example, but still far short of identifying a suspect or, more difficultly including the suspect as the one and only human who could be associated with the evidence or crime scene. Thus, inclusive mathematics, even with DNA, can only come from the use of these alleles and loci, in a process called multiplexing. For example, if we could find ten loci with ten alleles each, this would produce a power of inclusion of 2.5 x 10^{17}genotypes, resulting in a number greater than the total population of the earth by a factor of a trillion.

EVIDENCE TO INCLUDE OR EXCLUDE DNA

Ultimately, science is used in this regard to support the inclusion or the exclusion of a suspect or evidence while some aspect of a case being investigated. In the simplest terms, we are trying to establish an association between the biologic evidence and the source.[34] In terms of forensic DNA testing, we look for alleles in the evidentiary specimen by comparing them to alleles in the exemplar, or known specimen. With the recent, but past technology, criminalists used gene-coded polymorphic products, Glycoproteins, Lipoproteins and Glycolipids, coded by genetic alleles.[35] Examples would include the blood groups ABO (A, B, O, AB and H), Lewis (Le^a and Le^1) which could be measured by the then existing technology of serologic testing. In fact any detectable polymorphic product of a gene could have been a candidate for forensic testing. (See Table #6.)

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[34] KOBILINSKY, *supra* note 2.
[35] *Id.* at 48.

Table #6. Gene Products that have been used by DNA Each of the loci have multiple variations (alleles) of the gene at that site.

PGM	Phosphoglucomutase
EAP	Erythrocyte Acid Phosphatase
EsD	Esterase D
AK	Adenylate Kinase
ADA	Adenosine Deaminase
6-PGD	6-Phosphogluconate dehydrogenase
G-6-PD	Glucose - 6 – Phosphate Dehydrogenate
	3 major variations
	> 400 others
Tf	Transferrin

All of these constituted "indirect genetic tests." In general, these were better for exclusion than inclusion (typical for forensic techniques), and had comparatively poor stability but an adequate degree of polymorphism. In addition, there was the difficulty of finding an adequately large enough specimen to test. This was almost always the case with victim specimens since enzymes are labile to environmental conditions in the dead body. However, using current technology, there is enough DNA in one cell to provide specimen adequacy. In human non-reproductive cells, there are forty-six chromosomes of which forty-four are autosomal chromosomes and there are two "gender" chromosomes. All in all about six feet of DNA fitting in each one one-thousandths of an inch cell weighing about 6.1 picograms (6.1x10-*12*g.).[36] This is enough material for approximately one hundred to two hundred thousand genes, but there are probably only twenty to thirty thousand genes that result in expressed proteins, and these expressed genes or exons likely represent ~ 5.2% of the genome. They cause

[36] Turner, *supra* note 21, at 33.

hundreds of thousands of proteins to be formed due to re-used motifs or lengths of codes that are used by more than one protein. The 3.1 billion bases have somewhere in the range of 3.1 million variances, seeming on the one hand to not be minimal and great at the same time. In the end, it results in humans being about 99.9% the same, with regards to DNA, as one another, to put it in context worldwide there are 6.2 billion of us.[37] For forensic purposes, we are trying to use DNA as a search-engine hoping that we can find loci with more alleles that are more stabile so that even smaller amounts can be amplified using a PCR or similar technique, in the hope of identifying a victim or excluding a suspect.

OBJECTS TO BE TESTED

Unique sequences that are usable for forensic study are found in most enzymes structures, since structural proteins are usually not the targets of forensic DNA testing for forensic purposes. Of the ones we are looking to discover, there are moderately repetitious sequences. They are the VNTRs and the STRs.

There are also highly repetitious sequences. Some of these make RNA needed for translation into proteins and would include Short Interspersed Nuclear Elements (SINEs).[38] These recently discovered short segments of DNA are a polymorphic group of alleles that are about three hundred base pairs long. There may be as many as one billion of these in the human genome. One allele garnering much interest is the Alu insertion.

Some individuals will have an Alu at a specific place, and others will not. Therefore, these are examples of biallelic (vs. multi-allelic) loci. Although this is generally not the best polymorphism for these purposes, there are huge numbers of them that may contribute the selectivity we need in future

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[37] U.S. CENSUS BUREAU (March 1, 2004).

[38] Arian F.A. Smith & Arthur D. Riggs, *MIRs Are Classic RNA-Derived SINEs That Amplified Before the Mammalian Radiation,* 23(1) NUCLEIC ACIDS RESEARCH 98-102 (January 1, 1995).

forensic applications. An Alu's increased length of insertion locus can be detected by PCR. While there is not much forensic use yet, it will be only a matter of time before that does occur.

Ultimately, the person-to-person variation (the 0.1% part of cellular DNA unique to one person) contains several types of polymorphisms. At the smallest level, the actual sequence of nucleotide bases can vary. For example, sequence - - - AGACTAGACATT - - - as opposed to sequence - - - AGATTAGGCATT---. The number of repeated sequences may also vary. For example, the sequence - - - (AATG) (AATG) (AATG) - - - has three repeats while the sequence - - - - -(AATG) (AATG) - - - - - has only two repeats.

Current usage looks for the very common "tandem repeats." Long repeats are thousands of base pairs in length. They tend to be "Satellite DNA" that clusters around chromosomal centromeres.[39] Medium-length Repeats or so-called "Minisatellite" DNA would include the VNTR originally described by Prof. Jefferys in the Collin Pitchfork case. These are sixteen to forty-one repeat units of some ten to one hundred base pairs in length. Short Repeats or "Microsatellite" DNA contain STR loci used frequently in the current technology. They are two to six base pairs in length and are very quickly amplified by PCR.

DNA FINGERPRINTING

Thousands of polymorphic STRs in humans, approximately one out of every ten thousand nucleotides, are used in DNA fingerprinting.[40] The current use looks for sequence polymorphism via techniques such as the reverse dot-blot procedure, and length polymorphism via amplified

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[39] *Satellite DNA*, *available at* ttp://en.wikipedia.org/wiki/Satellite_DNA.

[40] T. M. Clayton et al,. *Analysis and Interpretation of Mixed Forensic Stains Using DNA STR Profiling*. 91,1 FORENSIC SCI. INT. 55-70 (Jan. 9, 1998).

fragment-length polymorphism (AmpFLP).[41] Mitochondrial DNA (mtDNA) polymorphisms can be found in the hyper variable displacement (D) loop of the mitochondrial genome and are considered a mostly reliable measure of maternal inheritance.[42] Similarly, Y Chromosome polymorphisms are considered a mostly reliable indication of paternal inheritance.[43]

In current usage, STRs with four nucleotide bases are most commonly used and of those GATA and AGAT are the most common tetranucleotide repeats used in forensics. (See Table #8) The advantages of tetranucleotide STR loci in forensic typing vs. VNTR minisatellites or di- and trinucleotide repeat STRs include a narrow allele size range that allows for less technical issues and because they decrease the loss of alleles when attempting to amplify smaller alleles. Small PCR products help in recovery from degraded specimens by decreasing "stutter product" (3 instead of 4 bases as the result of strand slippage during PCR).[44] The stutter product is an artifact that results in a minor peak during the PCR process. This peak comes from "slippage,"

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[41] Mark R Wilson et al., *Recommendations for Consistent Treatment of Length Variants in the Human Mitochondrial DNA Control Region*, 129,1, FORENSIC SCI. INT. 35-42 (Sept. 10, 2002); S. Cosso & R. Reynolds, *Validation of the AmpliFLP D1S80 PCR Amplification Kit for Forensic Casework Analysis According to TWGDAM Guidelines*, 40,3 J. FORENSIC SCI. 11 (1995).

[42] S. Anderson et al., *Sequence and Organization of the Human Mitochondrial Genome* 290, 5806 NATURE 457-465., (April 9, 1981); M. Casanova et al., *A Human Y-linked DNA Polymorphism and Its Potential for Estimating Genetic and Evolutionary Distance.* 230, 4732 SCIENCE 1403-1406 (Dec. 20, 1985).

[43] Alice R. Isenberg & Jodi M. Moore, *Mitochondrial DNA Analysis at the FBI Laboratory*, 1,2 FORENSIC SCIENCE COMMUNICATIONS (1999), *available at* http://www.fbi.gov/hq/lab/fsc/backissu/july1999/dnatext.htm; E.K. Hanson & J. Ballantyne, *A Highly Discriminating 21 Locus Y-STR "Megaplex" System Designed to Augment the Minimal Haplotype Loci for Forensic Casework,* 49,1 J. FORENSIC SCI. 12 (2004), *erratum in* 49,6 J. FORENSIC SCI. 5 (2004).

[44] P. Sean Walsh et al., *Sequence Analysis and Characterization of Stutter Products at the Tetranucleotide Repeat Locus vWA*, 24, 14 NUCLEIC ACIDS RESEARCH 2807 – 2812 (1996).

causing incorrect base pairing, of the polymerase enzyme during the extension phase of amplification. The polymerase "jumps-over" a repeat unit and continues processing the template. The missed unit usually ends up being a loop, thus it usually appears as a minor peak.

(Table #8) Specific STR sequences include:

Mononucleotide repeats

A, C

Dinucleotide repeats

AC, AG, AT, CG

Trinucleotide repeats

AAC, AAG, AAT, ACC, ACG, ACT, AGC, AGG, ATC, CCG

Tetranucleotide repeats

AAAC, AAAG, AAAT, AACC, AAGG, AACT, and 27 others

Pentanucleotide repeats

102 combinations

Hexanucleotide repeats Or up to 350 other combinations

Pentanucleotide STR loci are becoming more frequently used as technology advances. This has resulted in some useful advantages to be exploited.[45] (See Table #9) In general, PCR and its many variations has replaced RFLP as the preferred approach to forensic DNA analysis. (See Table #10)

[45] Jeff Bacher & James W. Schumm, *Development of Highly Polymorphic Pentanucleotide Tandem Repeat Loci with Low Stutter*, 2,2 PROFILES IN DNA 3-6 (1998).

Table #9 The advantages and disadvantages of pentanucleotide STR loci use.[46]

Advantages	Limitations
STR advantages generally apply	Same (STR) disadvantages as well
Simpler and more precise interpretation due to τ stutter band artifacts	More rare in the genome than tetranucleotide STRs
Some loci have high degree of heterozygosity (few microvariants)	
Longer repeat length with few microvariants allows more flexibility in separation technique	

Table #10. Several reasons why PCR is the current preferred technique for DNA analysis.

Characteristic	RFLP	PCR
Time required to obtain results	6-8 weeks with radioactive probes, 1 week with chemiluminescent probes	1-2 days
Amount of DNA needed	50-500 ng.	0.1-1.0 ng.
Condition of DNA needed	High molecular weight intact DNA	May be highly degraded
Capable of handling sampling mixtures	Yes (single-locus probes)	Yes

[46] Source: National Commission on the Future of DNA Evidence, 2000.

Allele identification	Binning required	Discrete alleles obtained
Automated and capable of high-volume sample processing	No	Yes
Commonly used DNA markers	D1S7, D2S44, D4S139, D5S110, D7S467, D10S28, D17S79	DQ□1, D1S80, PolyMarker (LDLR, GYPA, HBGG, Gc, D7S8), TH01, VWA, numerous STR loci

Approaches in the early 1990's included RFLP, HLA-DQ*a*, AmpliType PM, "Polymarker," and D1S80.[47] One major advantage of the newer STR Multiplex kits is specimen size. STR kits need one to two nanograms, one-billionth of a gram, as opposed to the RFLP methods that require fifty to one hundred nanograms.[48.]

DNA TESTING PRINCIPLES

The current basic process involves the purification of the DNA sample by extraction, quantification, amplification, and detection.[49]

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[47] John S. Waye et al., *A Simple and Sensitive Method for Quantifying Human Genomic DNA in Forensic Specimen Extracts,* 7, 8 BIOTECHNIQUES 852-855 (1989).

[48] J.W. Hicks, *DNA Profiling: A Tool for Law Enforcement,* 57(8) FBI LAW ENFORCEMENT BULLETIN 1-5 (1988).

[49] PCR TECHNOLOGY: PRINCIPLES AND APPLICATIONS FOR DNA AMPLIFICATION 3-9 (Henry A. Erlich, ed, Stockton Press 1989).

EXTRACTION TESTING

Forensic specimens are not like clinical laboratory submissions or research specimens in that they are likely to contain a lot of contaminating material. Therefore, it is all the more imperative to get the DNA out of the specimen as well as out of the cells. There are three common extraction techniques used in forensic DNA work.[50]

Organic extraction techniques are the oldest and can be used as a preparation step for RFLP or PCR. This technique generally recovers high molecular weight DNA using a chemical called Sodium Dodecl Sulfate (SDS) to crack open the cells. Once the cells are disrupted, Proteinase K is used to separate protein from the DNA The addition of a solution of Phenol, Chloroform, and Ethanol separates the DNA. This process is also known as dialysis.

The Chelex™ technique developed in 1991 is faster and has fewer steps than the organic extraction technique, but can be used as a preparation for PCR only.[51] Although it is a limitation given the frequency with which PCR is used, it is not considered to be a serious limitation. The Chelex™ technique generally recovers single stranded DNA (ssDNA) using a procedure that adds a "chelating-resin" suspension directly to the specimen (blood, bloodstain, semen for example) followed by an ion-exchange resin that binds the polyvalent metal ion, magnesium. In the last step, the metal is taken away bringing other material with it.

In the 1980s, a third technique was used. In this technique, the crime scene investigator uses FTA Paper, an Australian technique, to store DNA.[52] The great advantage

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[50] NAT'L RESEARCH COUNCIL, THE EVALUATION OF FORENSIC DNA EVIDENCE, (Nat'l Academy Press 1996). No pages only electronically available.

[51] P.S. Walsh et al, *A Rapid Chemiluminescent Method of Quantitation of Human DNA* ,20,19 NUCLEIC ACIDS RESEARCH 5061 - 5065 (Oct. 11 1992).

[52] L. BURGOYNE ET AL., PROCEEDINGS FROM THE FIFTH INTERNATIONAL SYMPOSIUM ON HUMAN IDENTIFICATION 11-17 (Promega Corp. 1994).

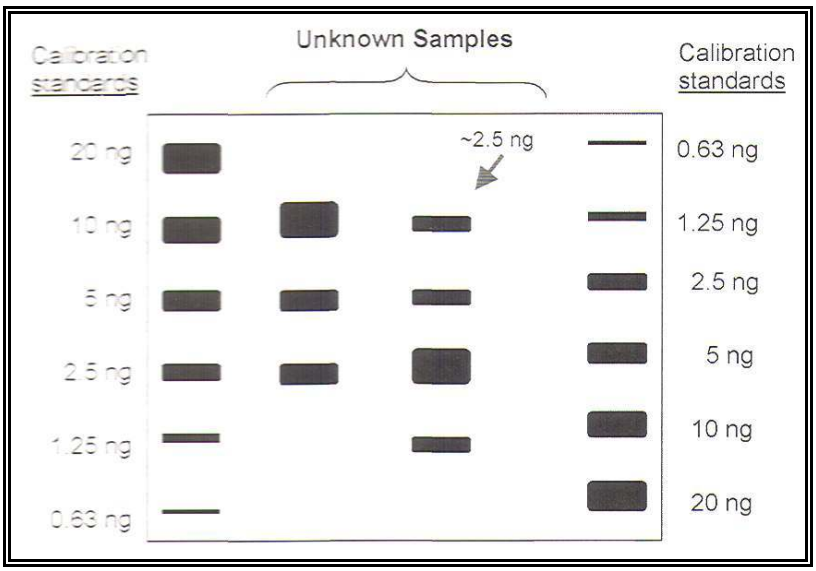
here is that the DNA can then be stored at room temperature for several years. The stabile specimen can thus be used as a preparation base for PCR and can also be used in automated protocols for analysis. Essentially a spot of blood or other fluid is placed on the paper and allowed to dry. Any cells are disrupted or lysed upon contact and white blood cell DNA is immobilized in the FTA matrix. At the analysis stage a small 'punch' in the paper is made and inserted into a tube for washing with an appropriate solvent. PCR can then be performed on the resulting sample.

QUANTIFICATION TESTING

The next step after the portion of a specimen likely to contain target DNA has been extracted is to quantify how much of it is there. An example is a common slot-blot standardization.[53] This is used to have calibration for the results generated.

Figure # 9 An example of the quantification phase of DNA analysis. By comparing the darkened areas of precipitate to the internal standard and the base pair measurement, one can determine the quantity of the DNA from the unknown specimens.

[53] P.S. Walsh et al., *Preferential PCR Amplification of Alleles: Mechanisms and Solutions,* 1(4) PCR METH. APPLIC. 241-250 (1992).



AMPLIFICATION TESTING

There are automated, high throughput, systems (ABI Profiler Plus, Cofiler Multiplex STR, Promega STR) that specify 1-2.5 nanograms of template DNA. They use a forty base pair probe that is complementary to a primate-specific satellite DNA sequence. The object of amplification is to increase the amount of DNA to a concentration sufficient for testing. By a process of healing and cooling, those DNA segments of interest will undergo semi-conservative replication for an amount of time sufficient to increase the total DNA by a factor of over 100,000 times.

DETECTION TESTING

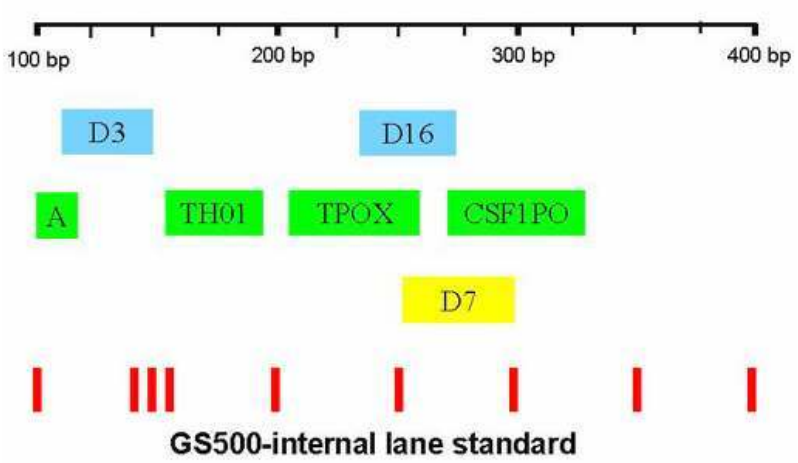
Once the complementary pairing is complete, a D17Z1 Yield-Gel electrophoresis process then separates the results of the base pairing. High molecular weight DNA in known amounts of one to two hundred picograms, one-trillionth of a gram, is used as a standard to quantify the evidentiary and known DNA. They are placed into cut wells in agarose gel alongside the DNA isolated from the evidence and the

exemplar DNA. With the cathode near the origin and the anode at the opposite end, an electric field is established and the segmented DNA is moved through the gel in proportion to their electrical charge. Due to the differences in weight and charge, the DNA will travel various distances towards the anode end where it will precipitate. The whole gel track is then exposed to ethidium bromide and a fluorescent dye is applied. Through a process known as intercalation, the dye intertwines itself through the lattice of the double-stranded DNA. The fluorescent bands that occur at the site of DNA precipitation cause the banding pattern to appear. Multiple dyes with different colors are often used in this process.

The fluorochrome dyes each produce a wavelength when excited by the correct light (in the ABI PRISM = Argon laser, excitation 488 nm, 514 nm).[54] The detector has filters that minimize overlap and the instrument uses a "matrix file" to tell each color apart. The matrix file should be reset often-due to potential misalignments. Internal software then calculates the percentage of each emitted color. (See Figures #10 and 11) When the assay is complete, most forensic laboratories will use the 13 CODIS Loci system to make comparisons and matches. This will provide the basis for conclusions used at trial or elsewhere in legal proceedings.

[54] P. Koumi et al., *Evaluation and Validation of the ABI 3700, ABI 3100, and the MegaBACE 1000 Capillary Array Electrophoresis Instruments for Use with Short Tandem Repeat Microsatellite Typing in a Forensic Environment,* 25(14) ELECTROPHORESIS 2227-2241 (2004).

Figure #10 Example of a typical banding pattern seen with multiple fluorochrome dyes on a DNA specimen.



ANALYSIS TESTING

Figure #11 - ABI Prism 7700. Many other high throughput DNA quantification techniques are in common use in forensic laboratories throughout the nation, some of the best using the PicoGreen Assay

This system has been adopted by the FBI and includes: chromosome number, most common alleles, the number of

possible or detectable types and Amelogenin.[55] (See Figure #13) Amelogenin is a locus for "tooth bud" development in the fetus first described in a study of the "X-linked" disease Amelogenesis Imperfecta.[56] It does not constitute an STR locus, but the allele on the "Y"chromosome is slightly longer than the allele on the "X" chromosome. Therefore, PCR can distinguish it via capillary or gel electrophoresis. These genetics also permit its measurement to be used reliably as an indicator of gender. One technical limitation in the interpretation phase is that the primers available are non-human and therefore several animals can give a positive result.

Figure #13. The CODIS Loci System used in most DNA comparisons

Locus	Chromosome Location	Repeat Pattern	Number (Alleles) Names	Common # of Types
CSF1PO	5q33.3-34	TAGA	10 (6-15)	55
D13S317	13q22-q31	TATC	8 (8-15)	36
D16S539	16q22-24	GATA	9 (5-15)	45
D18S51	18q21.3	AGAA	21 (9-26)	231
D21S11	21p11.1	TCTA TCTG	+22 (24.2-253 38)	
D3S1358	3p21	TCTG TCTA	+8 (12-19)	36

[55] Armando Mannucci et al., *Forensic Application of a Rapid and Quantitative DNA Sex Test by Amplification for the X-Y homologous gene amelogenin,* 106,4 INT'L. J. LEGAL MED 190-193 (1994).

[56] Medline Plus, *Amelogenesis Imperfecta*, http://www.nlm.nih.gov/medlineplus/ency/article/001578.htm.

D5S818	5q21-q31	AGAT	10 (7-16)	55
D7S820	7q11.21-22	GATA	10 (6-15)	55
D8S1179	8q24.1-24.2	TCTA TCTG	+12 (8-19)	78
FGA	4q28	CTTT	14 (17-30)	105
THO1	11p15-15.5	TCAT	7 (5-10)	28
TPOX	2p23-2pter	GAAT	8 (6-13)	36
VWA	12p12-pter	TCTG TCTA	+11 (11-21)	66
Amelogenin X, Y				

The standard technique involves commercial multiplex STR kits to get a profile. These commercial kits have various capabilities at selectivity or discrimination. Ultimately, the power of discrimination is tied to the use of forensic DNA in legal proceedings. If we needed to exclude everyone in Massachusetts except the suspect, we would need a power of discrimination of $1:6.4 \times 10^{6}$, to exclude everyone on the planet $1:6.5 \times 10^{9}$. (See Figure # 14)

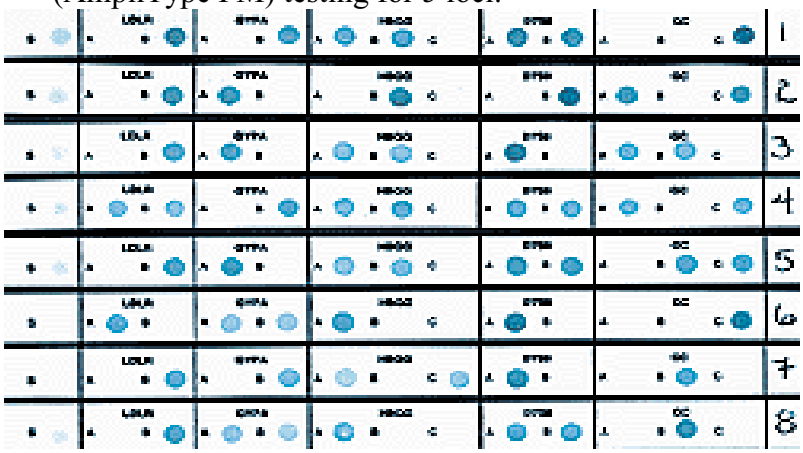
Figure #14. The powers of discrimination available for use through examples of high throughput instruments.[57]

Multiplex Kit	Power of Discrimination*
Profiler Plus	$1:9.6 \times 10^{10}$
Cofiler	$1:8.4 \times 10^{5}$
PowerPlex 16	$1:1.8 \times 10^{17}$
Identifier	$1:2.1 \times 10^{17}$
	* Calculation for Caucasian Population

[57] Source: National Commission on the Future of DNA Evidence, 2000.

One example that has been through several variations is the AmpliType PM (Polymarker), which has measured the eight alleles of HLA-DQa and HLA-DQa1.[58] This system works and has been categorized for many populations. There is also a multiplex version (AmpliType PM), with 5 loci (LDLR, GYPA, HBGG, D7S8, Gc). In this system, AmpliType PM (Polymarker), the probes are covalently bound to nylon substrate, an amplified specimen is added, and a reverse dot-blot procedure is conducted (See Figure #12D). The advantages of such a PCR based system are that it is quick, uses visually interpreted results, and can be used for small and/or degraded specimens. It is limited by the lack of discrimination of VNTRs and STRs and has few alleles and loci that cause difficulties in identifying component mixtures.

Figure #12. An example of the multiplex version (AmpliType PM) testing for 5 loci.[59]



[58] CETUS CORP., AMPLITYPE USER GUIDE FOR HLA DQα FORENSIC DNA AMPLIFICATION AND TYPING KIT 17 – 20 (Cetus Corp. 1990).

[59] Source: Applied Biosystems

THE NEXT STEPS

Emerging forensic techniques include the analysis of SNPs (Single-Nucleotide Polymorphisms).[60] These occur at a frequency of approximately one per one kilobase or one in one thousand bases, which means that there are millions per genome. This would greatly exceed the discovered or estimated number of STRs. While SNPs are less informative (taken one at a time) because they are biallelic (2 alleles/locus) thirty to forty SNPs can produce discriminatory power equal to CODIS using thirteen 13 loci. (See Table #15).

Table #15 The advantages and disadvantages of SNPs as a marker for DNA testing.

Advantages	Limitations
SNPs are found in abundance in mammalian genomes	Most SNPs are biallelic. Even sites with as many as three alleles are individually of limited discriminatory significance
There are many methods available to detect SNPs	The limited number of alleles makes analysis of mixed samples more complicated
Robust multiplex amplification is relatively easy to achieve	

MITOCHONDRIAL DNA

Mitochondrial DNA (mtDNA) is inherited maternally as an "SNP"[61] This fact has been used as the basis of "lineage determination" around the world for anthropological

[60] KOBILINSKY, *supra* note 2, at 161-162.

[61] A.A.M..MORRIS & R.N. LIGHTOWLERS, MITOCHONDRIAL DNA RECOMBINATION 941 (Lancet; 2000).

purposes.[62] It can also be used in a forensic sense. (See Table #16)

Table #16 The advantages and disadvantages of mitochondrial DNA in forensic situations.[63]

Advantages	Limitations
Extremely small amounts are useful due to multiple mitochondria/cell. PCR	Can not be used for siblings or maternal inheritance
mtDNA molecules are small, do not degrade as fast as nuclear DNA	Discrimination is limited by database size
Useful in tracing lineage	Heteroplasmy (more than 1 type of mitochondria) can complicate analysis
Discrimination power is better due to haplotypes being uncommon in databases	Discrimination power is limited by database size. mtDNA is inherited as a unit.

The history of this particular use of technology originated in a 1987 article in Nature that purported to trace human ancestry back to a "Mitochondrial Eve" of 140,000 - 280,000 BCE.[64] This notion challenged the then assumed "multiregional" base of human origins in favor of one worldwide replacement model. The "Mitochondrial Eve" model supposes that *Homo erectus* and "Neanderthal" completely disappeared. It also assumes that mtDNA is inherited clonally, faithfully copied from mother to egg, and that there are no recombinations. Rapid mutations of mtDNA

[62] C. Saccone, *The Evolution of Mitochondrial DNA,* 4(6) CURR. OPIN, GENET. DEVELOP 875–881 (1994).

[63] Source: National Commission on the future of DNA Evidence, 2000.

[64] Rebecca L. Cann et al., *Mitochondrial DNA and Human Evolution,* 325 NATURE 31-36 (Jan. 1 1987).

are the basis of the "Molecular Clock" aspect of the basic idea used to estimate time into the past, with molecular analysis using the non-coding mtDNA D-loop.

The scientific debate about the utility of the "Molecular Clock" aspect of this analyte is material to its use for forensic purposes. In 1981, the Anderson sequence, a 16,569 base pair human mitochondrial genome was described at Cambridge University. In 1987, Cann, Stoneking and Wilson drew from this work and proposed the "Mitochondrial Eve" idea. Sykes and Paabo of Oxford University generally supported this in 1991. They worked on the celebrated "Ice Man" and "Cheddar Man" furthering the veracity of mtDNA as a path to establishing maternal lineage.[65] In 1995, Cavalli and Sforza of Stanford University and Smith from Sussex University suggested that mtDNA is not trustworthy.[66] In 1996, they indicated that as many as one in twenty-five children do not have their mother's mtDNA. Further, they stated that recombinations do happen. Sykes in 1999 commented on these criticisms saying "don't panic, this is still a useful and reliable approach".

Also in 1999, Smith and Hagelberg stated that some mtDNA is paternally inherited. Hagelberg eventually changed his mind, but Smith has not. Smith stated that "there is too much mtDNA variety to have arisen by chance." In 2002, Marianne Schwartz from the University Hospital Rigshospitaler in Denmark wrote a paper commenting on a 28-year-old patient who presented as a "mtDNA mosaic." This patient had mitochondrial DNA inherited from the patient's mother in some tissues (blood, hair, other tissues) and from the father in others (skeletal, muscle). This should not happen because embryos have a fairly efficient system to get rid of sperm mitochondria. However, in this patient it did happen. In 2003, Sykes authored the "Seven Daughters of

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[65] Oliva Handt et al., *Molecular Genetic Analyses of the Tyrolean Ice Man,*264,5166 SCIENCE 1775-1778 (June 17,1994).

[66] LUIGI LUCA CAVALLI-SFORZA & FRANCESCO CAVALLI-SFORZA, THE GREAT HUMAN DIASPORAS: THE HISTORY OF DIVERSITY AND EVOLUTION (1995).

Eve," which includes the observation that more than 90% of "Y" chromosome does NOT undergo mutation.*67* The Y chromosome is also thought to be inherited paternally as an "SNP" and has some advantages of its own in forensics.

Table #17 The advantages and disadvantages to Y chromosome investigations in forensic study.[68]

Advantages	Limitations
Useful in tracing male family relationships. Male progeny get "Y" as a unit	Discriminatory power limited by size of database. No recombination among loci
Can be used to trace relatedness of individuals in a geographic area	

The Y chromosome has various STR positions that are useful for various purposes. These and other yet to emerge areas of exploitation of DNA for forensic purposes still need to have a basis in criminalistics but follow from classic assumptions long established in the courts. Using Locard's Exchange Principle, any of the following methods can be used in the collection of evidentiary DNA.[69] A suspect's DNA may be deposited on the victim's body or clothing, on an object, or at a location. The victim's DNA may be deposited on the suspect's body or clothing, on an object or at a location. A witness' DNA may be deposited on the victim

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[67] BRYAN SYKES, THE SEVEN DAUGHTERS OF EVE 1-223 (2001).

[68] Source: National Commission on the Future of DNA Evidence, 2000.

[69] E. LOCARD, LA POLICE ET LES METHODS SCIENTIFIQUES, (2d ed. Paris: Payot), W. Jerry Chisum & Brent E. Turvey, *Evidence Dynamics: Locard's Exchange Principle & Crime Reconstruction* 1, 1, J. BEHAVIORAL PROFILING 1-10 (2000), *see also* SAFERSTEIN, *supra* at,4,5.

or the suspect, on an object, or at a location. Many different sources have been used in legal proceedings.

Table #18 The following have been used as credible DNA evidence sources.

(1988)	Hair with Root
(1993)	Debris from Fingernails
(1994)	Bones
(1995)	Semen & Semen Stains
(1995)	Hair shaft
(1995)	Blood & Blood Stains
(1996)	Urine
(1996)	Feces
(1996)	Teeth
(1996)	Personal Items (razor blade, chewing gum, wrist watch, ear wax, toothbrush)
(1991)	Cigarette Butts
(1994)	Postage Stamps
(1997)	Envelope Sealing Flaps
(1997)	Saliva with Nucleated Cells
(1997)	Fingerprints
(1998)	Dandruff
(1998)	Muscle Tissue

PROFESSIONALIZATION

The American Academy of Forensic Sciences (AAFS), is a nonprofit professional society organized in 1948. It is devoted to the improvement, the administration, and the achievement of justice through the application of science to the law.

Members of the AAFS include physicians, criminalists, toxicologists, attorneys, dentists, physical anthropologists, document examiners, engineers, psychiatrists, educators, and others who practice and perform research in the many diverse fields relating to forensic science. These members reside in all fifty of the United States and its possessions, as well as Canada, and in more than fifty other countries. As a professional society the AAFS is committed to the promotion of education and to the elevation of accuracy, precision, and specificity in the forensic sciences and other evolving matters. This state of professionalism has contributed to the cooperative efforts to validate the utility of DNA evidence in the courts. This is important to the legal system in helping to determine admissibility of evidence. The current status of these efforts is disseminated in various ways, including the International Symposium on Human Identification, the annual meeting of the American Academy of Forensic Sciences, the biannual Congress of the International Society of Forensic Haemogenetics and the triennial meeting of the International Association of Forensic Sciences.

Other validation efforts include the National Institute of Justice's evaluation of rigorous scientific tests to justify fingerprints, the National Integrated Ballistic Information Network (NIBIN), and various efforts aimed at creating a human DNA bank from birth. While this would make any DNA specimen found at a crime scene confirmable within hours, it also creates MANY legal, ethical and moral dilemmas.

COMMERCIALIZATION

Any such development with this kind of utility and demand base is sure to attract the attention of "for-profit" enterprises and almost from the beginning this has been the case for the arena of forensic DNA testing. In 2001, Orchid Biosciences acquired LifeCodes Inc. and merged it with Cellmark in Germantown, Maryland as well as Microdiagnostics in Nashville, Tennessee. These three companies are now integrated into Orchid Cellmark Forensics, a truly national resource for obtaining forensic DNA services. LabCorp, a national clinical laboratory testing organization which tests 300,000 specimens daily also has a forensic DNA division.

DNA IN NATIONAL AND GLOBAL CRIME

Perhaps a purpose for DNA technology not envisioned by Dr. Jefferys includes sorting out heinous acts in the global and national conflicts that have arisen since the end of the "Cold War." During the Argentinean "Dirty War" of 1976 – 1983, more than 10,000 people went missing. HLA testing was used to help determine "grand-paternity" for some of the children of the missing and dead. So many adults were killed during this period, creating so many orphaned children that in some cases their grand parents (now surrogate parents) had to be determined by DNA information.[70]

In 1993, the International Tribunal for the former Yugoslavia (ICTY) was created to address the issues of the 200,000 people who died from "Ethnic Cleansing."[71] In the United Nations safe zone of Srebrenica alone, 7,500 Muslim

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[70] Lisa Butler and Reuben Granich, *The Search for Argentina's Disappeared*, *at* http://www.hrcberkeley.org/dna/printreport_argentina.htm.

[71] Fact Sheet on the International Criminal Tribunal of Yugolslvia, http://www.un.org/icty/cases-e/factsheets/generalinfo-e.htm; *see also World Leaders sign Bosnian Accord*, U.S.A. Today, Feb. 14, 1996, *at* http://www.usatoday.com/news/index/bosnia/dec95/nbos112.htm.

men and boys were killed.[72] There was evidence that Bosnian Serbs tampered with evidence about this atrocity. Therefore, DNA testing had to be employed to sort it out.[73] In 1994, the International Criminal Tribunal for Rwanda (ICTR) was involved in the Rwandan Civil War. The war, which left 800,000 Tutsi dead, has been described as the "fastest, most efficient killing spree of the 20th Century."[74] In this instance, mtDNA was used to track "biological warfare" using HIV and Hutu rapists of 250,000 Tutsi women.[75] In 2002, the U.N. established the Permanent International Crime Court (PICC), which will use DNA evidence extensively as subsequent matters arise.

Nationally, the Innocence Project uses forensic DNA testing to the overturn the convictions of people who were wrongfully convicted of crimes. Despite the best efforts and practices of our judicial system, unfortunately there are such people. As of May 1, 2006, approximately 200 people have been exonerated through DNA testing. On the national level, two organizations are associated with this effort: Centurion Ministries of Princeton, New Jersey. and the Innocence Project of the Benjamin N. Cardozo School of Law in New York, N.Y. In addition, all states except Hawaii and South Dakota have a local Innocence Project.

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[72] Alix Kroeger, *Srebrenica Gets Multi-ethnic Police,* BBC News Online, Nov. 9, 2001, *at* http://news.bbc.co.uk/2/low/world/europe/1646949.stm.

[73] *Bosnia to Bury Its Victims on Massacre Anniversary,* The International News, http://www.thenews.com.pk/print1.asp?id+14652.

[74] Samantha Power, *Bystanders to Genocide*, THE ATLANTIC ONLINE, September 2001, http://theatlantic.com/doc/print/200109/power-genocide.

[75] Robyn Dixon, *For RapeVictims, Rwanda's Horrors Live on Through AIDS,* Boston Globe, May 5, 2004*, at* www.boston.com/news/word/Africa/articles/2004/05/05/for_rape_victims_rwandas_horrors_live_on_through_ aids?mode=PF.

TECHNOLOGY ON THE HORIZON

Technology which makes it increasingly difficult to disguise or disable evidence in the commission of a crime is beneficial for the common good. Some technology on the horizon includes photolithography and chemical etching. Additional improvements may decrease the time of STR testing up to minutes or even seconds. Still other improvements may cause the demise of STR testing in favor of SNP testing and SINE testing. Better techniques, more loci, smaller targets, and more complete databases all will characterize developments in the near future.

CONCLUSION

In the end, the moving from not knowing that there were living things too small to be seen to the current level of discrimination of forensic DNA testing in only 150 years is to be marveled at. Yet the future, even the near future, holds even more promise. Although all is not certain, surety has been established at a useful level. It has been said even of the law itself that "… legal rules are never clear, and, if a rule had to be clear before it could be imposed, society would be impossible."[76] Scientists will drive the matter as far as knowledge permits at any given time, but it remains for both scientists and progressive scholars of the courts to advance these good works to serve the interests of justice.

[76] EDWARD H. LEVI, AN INTRODUCTION TO LEGAL REASONING (1949).