

DNA IN THE SERVICE OF JUSTICE

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SUMMARY: I. *Technological Advances in Forensic DNA Analysis*. II. *Identification*. III. *Individualization*. IV. *The Polymerase Chain Reaction (PCR)*. V. *STR Multiplex Kits*. VI. *Databasing*. VII. *Mitochondrial DNA Analysis*. VIII. *Y Chromosome STR*.

I. TECHNOLOGICAL ADVANCES IN FORENSIC DNA ANALYSIS

It is vital that we keep peace with the changing world. Crime continues to plague mankind and security is a critical issue for every citizen. Countries must seek to modernize their Criminal Justice systems not only by increasing productivity and efficiency but also by incorporating technology. We are facing new challenges today. Criminals have become more sophisticated in their planning and conduct of criminal activity and law enforcement must respond using science and technology. Forensic Science is a unique discipline that uses the principles and methods of the basic sciences, such as chemistry, physics, biology, etcetera to solve problems related to the law, both criminal and civil. Forensic scientists are not concerned with winning or losing a case in court. They are searching for truth. They must provide the judges and juries with factual information so that an informed decision of guilt or innocence of a defendant can be made.

Crime Scene Investigation

Science and technology have application not only in the forensic laboratory. It is important that a scientific approach is utilized in the investigation of the crime scene. When a violent crime has been committed, it is

the responsibility of the first responding police officer(s) to save the life of the victim and to protect the scene from unauthorized entry by allowing only emergency medical personnel and crime scene investigators into the scene. The first responder must call for detectives and contact the crime scene unit.

Once at the site, the crime scene investigators must walk through the scene, establish the point of entry and exit of the criminal. They must document the scene using photography, videotape where appropriate, and make schematic drawings. These drawings should contain a scale so that locations of objects can be described precisely at a later date. The investigator should walk through the scene to identify physical and biological evidence, and conduct a search of the scene using a geometric search pattern i. e. either a grid or pie scheme. Evidence must be identified and documented. It must be collected and packaged and transported to the laboratory for analysis.

In the United States, there are many examples of cases where crime scene investigation has not been properly conducted and as a result a case remains unsolved, a guilty person is found by a jury to be not guilty, and even worse, an innocent person is wrongfully convicted. Many people believe that the jury in the criminal trial of O. J. Simpson erred in finding him not guilty despite what the prosecution called a mountain of evidence pointing to him as the double killer of his wife Nicole Brown Simpson and Ronald Goldman. In this case, the crime scene investigation was severely criticized by defense attorneys. In the case of Scott Peterson, a hair on a pair of pliers convinced a jury to find him guilty of the murder of his wife, Laci Peterson and her unborn child, Conner. A search of the alleged murder scene failed to turn up any significant physical evidence. Jon Benet Ramsey was murdered in a Colorado home and her murderer has still not been caught. It is thought that poor crime scene investigation was to blame. Another example of an unsolved case is that of Chandra Levy. Her skeleton was found a year after she went missing. Despite a search of that same area early in the investigation, the police had found no body. As a result, any evidence present at the site or on her body was lost. Another well known case took place on the Caribbean island of Aruba where Natalee Holloway was declared missing. It is highly likely that she was murdered. Many have said that the initial investiga-

tion into her disappearance was not handled properly and therefore the case remains unsolved to this day.

At a crime scene, physical or biological evidence may take many different forms. Biological evidence includes:

- Blood: From victim/scene/suspect.
- Semen: From victim/scene/suspect.
- Saliva: From victim/scene/suspect.
- Hairs From victim/scene/suspect.
- Fibers: From victim/scene/suspect.
- Stains on clothing: From victim/scene/suspect.
- Biological substances found on Restraining Devices: Rope/tape/hand-cuffs.

Some forms of physical evidence include “impression evidence:”

- Footprints/shoeprints.
- Fingerprints/palm prints.
- Tire tracks.
- Bite marks.
- Tool marks.

Some forms of evidence concerns ballistics and gun shot residue and they include:

- Live ammunition.
- Cartridge cases.
- Fired bullets.
- Shot gun shell wadding.

Other forms of physical evidence include:

- Glass.
- Soil.
- Questioned documents.
- Charred documents.
- Sink drains.

- Septic systems.
- Garbage compactor.
- Rubbish containers.

Additionally, some forms of physical evidence can reveal information important not only to crime lab analysts but also to police investigators. For example:

- Newspapers: dates.
- Mail: dates, name and address of sender.
- Drugs: legal by prescription or illegal.
- Poison.
- Fly larvae.
- Answering machine tapes.

Weapons may be found at the crime scene as well and these may provide law enforcement with vitally important information about the perpetrator. Weapons may be found at the scene or weapons may be missing (taken) from scene. These include:

- Revolver/auto pistol/rifle/shotgun/machine gun/other firearm.
- Knife/ice pick/razor/scissors/machete/ax/hammer/tire iron/ jack handle.
- Club/bat/stick/bottle.

Since virtually anything can be used as a weapon we must include:

- Hands/fist/feet/shoes/boots/rope/cord/wire/pantyhoe/scarf/belt/chain.

When crimes are committed indoors, structures and environmental conditions then become important to the investigation. For example:

- Weather conditions.
- Temperature: Interior/exterior.
- Lighting fixtures: On/off/not working.
- Heating system: Type of system/on/off.
- Air conditioning: On/off/not working.
- Household appliances: On/off/not working.
- Doors/windows/hatchways: locked/unlocked/open/closed/forced entry.
- Clocks.

- Pets: feces.
- Laundry.
- Telephone/electric service lines: cut or disconnected.

When crimes are committed using a motor vehicle, important evidence may include:

- Engine: running.
- Ignition: on/off/jumped.
- Doors: locked/unlocked.
- Windows: up/down.
- Accessories: on/off.
- Fuel reading.
- Battery charged odometer reading.
- Vehicle damage.
- Trunk.
- Vehicle stolen.
- Radio station.
- Mirrors.
- Ash trays.
- Contents of vehicle.
- Light switch.

The focus of this section is on biological evidence. When biological evidence is found at a crime scene and it is brought to the laboratory for analysis, the criminalist first attempts to identify the substance. Subsequent to the identification, the analyst continues to try to individualize the substance, that is, to determine its source or origin. As mentioned above, sources of Biological evidence include blood, semen, saliva, urine, hair, teeth, bone, and body tissues such as muscle.

II. IDENTIFICATION

When a questioned substance is found at a crime scene, its identity is determined first using very sensitive but not highly specific presumptive tests. If the testing provides a positive result, then it is followed by confirmatory testing. For example, blood is first tested presumptively using leucomalachite green, phenolphthalin or luminol. If the results are positive, then the specimen is further tested to confirm that it is blood utiliz-

ing tests that are far more specific though less sensitive. Confirmatory testing includes crystal tests as well as spectrophotometric analyses. This is followed by species identification testing. Positive reactions identify the unknown stain as human blood. Technology has now made available a serological test that can prove a substance to be human blood in one step. The ABACard hematrace assay utilizes monoclonal antibodies to verify the presence of human hemoglobin. More laboratories are using this technology to fight crime.

III. INDIVIDUALIZATION

The analyst now tries to individualize the substance by determining the source of the stain. Before DNA came into use by forensic scientists, erythrocyte (red cell) antigens, enzymes, and non-enzymatic proteins would be analyzed to obtain genetic information about the biological substance on or in the evidence. These 'genetic markers' were studied because they are all polymorphic and can provide information as to the source of the evidence. Polymorphism means that multiple genes or alleles are present in the population for a particular genetic marker and that individuals can be differentiated by studying these systems.

For example, in the ABO blood typing system, three genes control one's inheritance and are responsible for determining if a person is a blood type A, B, AB, or O. If a person has type A blood, then it is impossible for that person to produce evidence which is type B. However, it is possible that evidence which is typed as A could have originated from this individual. What is important in deciding if he is the source, is how rare type A blood is in the population. If it is common (and it is), then it is not very probative. If it is rare, then it is an important piece of information for a jury to weigh in their decisions of guilt or innocence.

1. *The Development of DNA Technology-Individualization.* *RFLP Analysis*

DNA is polymorphic, stable, and its analysis has high discrimination potential. DNA testing is reliable, accurate, and economical. DNA is found in the cell's nucleus in the form of chromosomes, each of which is made up of a double stranded molecule. The double stranded DNA is synthesized from individual monomers called deoxyribonucleotide triphosphates (dNTPs).

The human genome contains lengths of DNA equivalent to 6 feet. This accommodates 3.1 billion basepairs of DNA parceled out to 46 chromosomes in the nucleus.

In 1985, the forensic community became aware of the importance of DNA in individualizing biological evidence. Prof. Alec Jeffreys developed the technique known as Restriction Fragment Length Polymorphism (RFLP) analysis for human identification. This procedure is based on studying variable nucleotide tandem repeat (VNTR) genetic markers (loci) in physical evidence. A VNTR is a segment of the human genome which contains repetitious DNA with the core repeating unit containing usually between 10 and 100 basepairs. A core repeat unit is shown herein. The DNA molecule is double stranded and both strands are shown.

ACTGCTGCTAAGACT
TGACGACGATTCTGA

If we allow this core repeat unit to be represented by an #, then the VNTR segment of DNA would appear as :

AGCT#####GCAGT
TCGA#####CGTCA

This segment containing a number of core repeats can be detected by using a probe, single stranded DNA which is complementary to the sequence of bases in the core. Since humans are diploid and we have 46 chromosomes in our genome, there are 22 pairs of autosomes and 2 sex chromosomes. Half our chromosomes come from mother, and half from father. Therefore, on a particular chromosome, at a particular locus, a VNTR “gene” would be represented twice, once on each homologous chromosome. Thus, the DNA fragments containing the VNTR on both chromosomes would constitute a persons genotype at this locus.

DNA Fingerprinting was first introduced into the courtroom in the United States in a 1988 Florida rape trial. The defendant, Tommy Lee Andrews was found guilty based on DNA testing. A major turning point in the history of forensic DNA analysis took place in 1989 when the FBI laboratory

came “on line” and began routinely testing casework evidence using RFLP technology.

In RFLP analysis, human DNA is isolated from a specimen, restricted (cleaved) with a “restriction enzyme,” separated by size using gel electrophoresis, transferred to a membrane using Southern blotting, hybridization with a labeled probe and then autoradiography to capture the image onto X-ray film.

Jeffreys would compare the genotypes of biological evidence and suspect at a number of different loci and determine if there was a match (inclusion) or a non-match (exclusion). The use of this technology for human identification started what has become known as the revolution of DNA technology in the criminal justice system. At the same time that Prof. Jeffreys was studying RFLP, Dr. Kary Mullis was at work establishing the extraordinary technology known as Polymerase Chain Reaction (PCR). PCR is a technique in which minute amounts of specimen DNA can be amplified. This is critical in casework where there is very little specimen to analyze. This technique could produce billions of copies of a target DNA sequence from a single molecule of template DNA. What has followed these pioneering “revolutionary” efforts has been referred to as an “evolution” of genetic identification technology. Over the past two decades we have seen more sensitive and more specific technology move from the research and development laboratory into the working crime laboratories. We now know that the difference between two human beings is 0.1% of the human genome. This means that, on average, 1 in 1000 bases will differ from person to person.

The chronological sequence of advancements in DNA testing, beginning in 1995 can be described as follows:

- DNA Fingerprinting – RFLP analysis.
- PCR-HLA DQA1 analysis.
- PCR-Polymarker analysis.
- PCR-AMPFLP-D1S80 (an amplified fragment length polymorphism) analysis.
- STRs- autosomal and Y chromosome short tandem repeat analysis.
- Mitochondrial DNA analysis.
- Single Nucleotide Polymorphism (SNP) analysis.

DNA analysis in a forensic context has been used primarily in rape cases. More than 2/3 of all DNA cases fall into this category. We are trying to determine if there is a match between the evidence and a suspect. It is also important to know the victim's DNA profile. In this way, we can determine the likelihood that a person accused of a rape actually committed the act.

The most challenging types of evidence include specimens that consist of mixtures of DNA. This would include mixtures of the *same* substance from more than one perpetrator, mixtures of *different* substances from more than one individual, or mixtures of DNA from the perpetrator and victim. In any case mixtures must be resolved using established protocols so that the interpretation is not subjective but follows specific rules.

Another challenge to the criminalist is when evidence contains DNA that is somewhat degraded. PCR will succeed in producing many copies of template DNA as long as the sample is not completely degraded. Still another challenge to the analyst is the existence of PCR inhibitors in the specimen under study. Such inhibitors have been identified in blood, soil, urine, denim jeans and create difficulties by preventing or inhibiting the PCR reaction.

The usefulness of DNA analysis for human identification cannot be overstated. In criminal matters, it helps link a suspect with biological evidence. In paternity matters, it is extremely powerful in identifying an alleged father as the biological father.

It has been useful in historical investigations including the identification of the Tsar's family who were massacred during the war. It is now being used in missing persons investigations and in mass disasters including plane crashes. DNA analysis has helped us to establish identification of deceased individuals and to link body parts. DNA analysis is used by the military for identification purposes. One of the more important uses has been in the establishment of a national convicted felon DNA database (See below).

The following is a brief history of some of the major events in the history of forensic DNA typing.

1980 Ray White describes the first polymorphic RFLP marker.

1985 Alec Jeffreys produces multilocus VNTR probes and applies them to RFLP for human identification.

1985 Kary Mullis published the first paper on the PCR amplification method.

1989 The FBI starts to accept DNA casework.

1991 The first STR paper is published.

1995 The Forensic Science Service (FSS) starts the UK DNA database.

1988 The FBI launches the CODIS national database.

Different kinds of tests require different amounts of evidentiary DNA. There are 4 basic types of DNA test including: RFLP, PCR based tests, Sequencing, and Single Nucleotide Polymorphism Analysis. PCR-based tests require only nanogram amounts of isolated DNA for success, whereas RFLP testing require 100 times as much DNA. The former requires single stranded DNA, while the latter requires double stranded, high molecular weight DNA. In order to understand how much DNA can be expected to be isolated from a specimen, the following table provides some approximate values.

2. *Sample DNA amounts*

- 1 CELL= 1/140 ng.
- 1 SHED HAIR=1 ng.
- 1 PLUCKED HAIR=300 ng.
- 1 DROP BLOOD=1,500 ng.
- 1 DROP SEMEN= 10,000 ng.

IV. THE POLYMERASE CHAIN REACTION (PCR)

PCR is a powerful technology for forensic casework since it can increase the amount of DNA found in an exceedingly small evidentiary specimen. In PCR, DNA is extracted from the forensic specimen, added to a PCR reagent mix which includes taq polymerase, buffer, MgCl₂, primers, and dNTPs, place inside a thermal cycler, and allowed to amplify over 28-32 cycles. The amplified products (amplicons) are then tested.

DNA must first be removed from the substrate upon which it was deposited. Thereafter, the DNA must be extracted so that it is purified from all cellular constituents such as lipids, proteins, carbohydrates, salts, et cetera. DNA extraction using the resin Chelex is a simple, rapid, and

economical procedure producing single stranded DNA template molecules. It not only works with samples of extremely small sample size but will still produce results for samples that are very old or partially degraded. The process lends itself to automation and does not use radioactive probes. There is also a rapid turn-around time with results available within 24 hours.

In PCR, a specific region of a chromosome, a polymorphic locus, is targeted for amplification. A locus is the site of a genetic system. The double-stranded DNA molecule is heat denatured by raising the temperature to 95°C, the temperature is lowered to 65 °C in order to allow binding of forward and reverse primers to each strand, and then raised to 72 °C so that the Taq polymerase enzyme can incorporate building block molecules and extend the newly made DNA from each primer. The enzyme then ligates the bases sequentially. In 32 cycles of amplification, assuming 100% efficiency, 1.07 billion copies of the targeted DNA region are created.

PCR can be used in the analysis of a variety of genetic polymorphisms including sequence polymorphisms which are typed by oligonucleotide hybridization and length polymorphisms which are typed by gel electrophoresis.

Although PCR has revolutionized forensic analysis, there remain potential problems that the analyst needs to be cognizant of and these include:

Potential problems

- Contamination.
- Mixed samples (differential lysis in sexual assault casework).
- Allelic dropout.
- Preferential amplification of small alleles.

Benefits

- Economical.
- Easy to perform.
- Non-radioactive probes.
- Can produce results with small and degraded samples.
- Multiplexing technique in which many markers can be copied simultaneously.
- Sensitivities to levels less than 1 ng of DNA.

V. STR MULTIPLEX KITS

The AmpFISTR Profiler Plus kit tests nine loci plus Amelogenin in a single PCR amplification reaction. These nine loci include: D3S1358, VWA, FGA, D81179, D21S11, D18S51, D5S818, D13S317, and D7S820. The Cofiler kit can analyze six short tandem repeat loci plus Amelogenin and identify the genotypes at each locus. These six loci include D3S1358, D16S539, THO1, TPOX, CSF1PO, and D7S820. Four different fluorescent dyes are used in these analysis. These are: Blue (5-FAM), Green (JOE), Yellow (NED), Red (internal lane standard). Fragment size is determined by using the ABI Prism Genetic Analyzer and the specific genes present at each locus, the genotypes, are identified.

The 13 CODIS core loci can be analyzed using the two multiplex kits, the Profiler Plus and Cofiler kits made by Applied Biosystems or the two multiplex kits manufactured by Promega, Powerplex 1.1 and 2.1. The PowerPlex 16 multiplex kit which was produced in 2000 can analyze 16 different loci in a single amplification and has a power of discrimination of $1 \text{ in } 2 \times 10^{17}$. Using an ABI Prism 310 Genetic Analyzer, human identity testing can take place in under 5 hours with a spot of blood the size of a pinhead. The probability of a random match is approximately 1 in 3 trillion. To demonstrate the statistical significance of a match, the analyst uses the product rule, multiplying the frequencies of each genotype over the 13 loci tested. Typically, the statistic shows the rarity of a genetic profile to be 1 in greater than 1 trillion regardless of the ethnic group from which the frequency data have been obtained. Ethnic groups as used in the United States include, Caucasians, Blacks, Hispanics, and Asians.

VI. DATABASING

The FBI launched its Combined DNA Index System (CODIS) DNA database in October 1998. It links all 50 states. It has been extremely effective for linking serial crimes and unsolved cases with repeat offenders. It requires the analysis of 13 core STR markers. These 13 loci are on different chromosomes with the exception of D5S818 and CSF1PO which are located on homologous chromosomes #5.

Currently there is a backlog of more than 600,000 samples in the United States and because of new legislation requiring more individuals to be placed into the database, this backlog will increase. For example, New York State has recently passed legislation requiring all those who commit misdemeanors to supply their DNA specimens to be placed on the database.

CODIS consists of a Local DNA index system, a State DNA index system and a National DNA index system. CODIS has a convicted offender database as well as a crime scene evidentiary database.

VII. MITOCHONDRIAL DNA ANALYSIS

Mitochondria contain a unique form of DNA different from nuclear DNA. Mitochondria are organelles responsible for cellular respiration and ATP synthesis. They contain a circular double stranded DNA genome containing 16,569 basepairs. Each molecule contains two hypervariable regions, HV1 and HV2 located in the control Region or D-loop of the genome. Mitochondrial DNA is maternally inherited. Segments of DNA within HV1 and HV2 are amplified and sequenced. These sequences are compared to a reference mitochondrial DNA sequence and differences are noted. Now the known specimen is compared to the unknown specimen and it is determined if differences are the same or different. In this way, one can determine if the former matches the latter or not.

VIII. Y CHROMOSOME STR

The structure of the Y chromosome consists of 60 megabases of total DNA sequence.

It is therefore, one of the smallest chromosomes (only chromosome #22 is smaller).

2.5 megabases on the tips of the Y chromosome can recombine with X (pseudoautosomal) regions. But 35-36 megabases consists of euchromatin.

In this form of forensic analysis, STR loci on the Y chromosome are studied. Statistics have shown that 98% of violent crime is performed by males. It is used primarily for sexual assault cases to identify the perpetrator and is especially powerful when there are multiple male assailants. The analysis can isolate the male component without differential extraction which attempts to separate the male and female cells and DNA. Ho-

wever, Y chromosome STR analysis is also used for genealogical studies and anthropological studies of population migration patterns. All loci on the Y chromosome are linked and paternally inherited. The genetic profile of loci on the Y chromosome is called the individual's haplotype. Thus a male will pass his Y chromosome to all his male offspring and they will pass their Y chromosomes onto their male offspring. A large number (almost two dozen) of Y chromosome loci can be simultaneously studied by multiplex amplification.

In the case of mitochondrial DNA analysis and in the case of Y chromosome STR analysis, statistical calculations following a determination of a match, cannot be performed using the product rule since all loci studied are linked. Therefore, the analyst must use the counting method. Thus if 5000 specimens have been examined and the next specimen is found to be unique, then the statistic for this sample is 1 in 5,001.

What remains to be done for Y STR forensic analysis is that population studies need to continue to evaluate the diversity of haplotypes. Genetic variation at multiple points along the Y chromosome is combined to form a Y haplotype for a sample. This haplotype constitutes a genetic profile for the Y chromosome. Suspects and evidence are compared by examining Y chromosome haplotypes of each. There is also a need to develop a robust assay for the accurate characterization of Y markers.

It is critical that laboratories adopt quality control procedures and maintain quality assurance. Accreditation of Laboratories by international groups can benefit forensic science casework by assuring that the laboratory has achieved a minimal level of quality standards. Furthermore, Certification of Lab Personnel, and Proficiency Testing can help to assure that the analysts possess the knowledge and skills to perform their work accurately and reliably. Failure to incorporate quality control and quality assurance in the laboratory can result in an array of ethical issues confronting the laboratory director.

Finally, I would like to make the following recommendations for the improvement of forensic science in the future.

1. Accredite all crime laboratories.
2. Make crime laboratories independent of law enforcement and prosecutors to avoid the potential for bias of experts.
3. Certify analysts-Test them for their skills and knowledge of the discipline in which they work.

4. Use blind proficiency testing to determine if they are producing reliable results.
5. Have a separate budget for the laboratory independent of the agency in which the laboratory is placed.
6. Require more complete laboratory reports.