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DNA FINGERPRINTING

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CHAPTER-1: DNA FINGERPRINTING TECHNIQUES

DNA fingerprinting has arguably been called the greatest tool in the history of forensic science. This chapter investigates why this technology is so powerful, and describes the various ways of performing these fingerprints. Unlike the case with normal digital fingerprints where it has been estimated that 3-4 individuals may have the same fingerprint, every person has a distinctive DNA fingerprint, except for identical twins. An individual's normal fingerprint can be erased by filing down, however an individual's DNA fingerprint cannot be changed through any known method. Also a DNA fingerprint (DNAF) can be produced from a rather small amount of DNA from tissue, skin, semen, blood or hair. When accompanied by current polymerase chain technology (PCR) for amplifying DNA, there is enough DNA in one hair root to create a DNAF (Betsch, 2006).

Examples of Applications of DNA Fingerprinting

There are many applications of DNAF such as identification, forensics, paternity testing, and diagnosis for inherited disorders. The armed forces have started to collect DNA from all of its members. In the event of a fatality the DNAF will help to identify the remains with more precision than the current methods, such as dental records and normal fingerprints. Forensics allows biological criminal evidence to be coupled to the DNAF of the perpetrator of the crime. Many difficult crimes have been solved and cases decided using DNAF. There is also the FBI's Combined DNA Index System (CODIS) a national criminal DNA data bank, which is created by linking county, state and federal DNA databanks (Adams, 2002; CODIS, 2004). Paternity testing tells who the legitimate parents of a child are. Which is helpful in reuniting separated

families, and settling custody and child support issues. Inherited disorders in newborns and pre-newborns can be detected using DNAF. If such disorders can be identified early, there can be a "head start" to identify risks and prepare for special treatment. If the disorder can be identified, perhaps in the future a genetic treatment can be created (Betsch, 2006).

DNA Background

DNA; deoxyribonucleic acid, is known as the molecule of inheritance. DNA directs the growth, function, organization and operation of every cell. DNA is found in almost every cell of every living organism. DNA consists of bases whose names are abbreviated A, C, G, and T. The sequence of these bases differs between any two individuals. The DNA is contained in the Chromosome (wikipedia, 2006). The chromosome is located in the nucleus of the cell and is visible with a normal microscope, the DNA is not visible (Betsch, 2006). A genotype is the inheritable instructions carried by living organisms. These instructions control all aspects of life (Blamire, 2000). Various locations in the DNA are called loci. The current DNA test used by the FBI begins with an analysis of 13 core loci, which have been determined over the years to have a high probability of differing between individuals.

Types of DNA Fingerprints

There are three main ways to run a DNAF: RFLP, VNTR and STR. Restriction Fragment Length Polymorphism (RFLP) describes the difference in lengths between specific DNA fragments cleaved with restriction enzymes. Restriction enzymes cut DNA at specific sequences of bases, for example the enzyme EcoRI cuts DNA at the sequence GAATTC. If the DNAs from two different people are cut with EcoRI, and the specific fragments at a core

forensic locus are compared, their lengths are different. Such differences in DNA fragment lengths can be caused by DNA insertions and deletions in bases (Davidson College, 2001). Variable Number of Tandem Repeats (VNTR) describes one way restriction fragments can vary their lengths. In this instance specific restriction fragments from two people may differ in length due to different numbers of tandemly repeated bases, i.e. one individual may have GATC-GATC-GATC-GATC, while another individual may have GATC-GATC (Melcher, 2000). To identify specific restriction fragments in a RFLP or VNTR type fingerprint, the DNA is cut with restriction enzymes that cut at specific locations (Figure-1, 3rd panel), then the fragments are separated by size using electrophoresis (Figure-1, 4th panel). Because DNA is negatively charged, it moves towards the positive anode with the smaller fragments moving through the sieving material faster. Then the DNA fragments are blotted to membrane to allow hybridization to a probe (Figure-1, 5th panel). The probe hybridizes or sticks only to a specific fragment (whose sequence is complementary to it), allowing only specific fragments to be visualized (Figure-1, 8th panel).

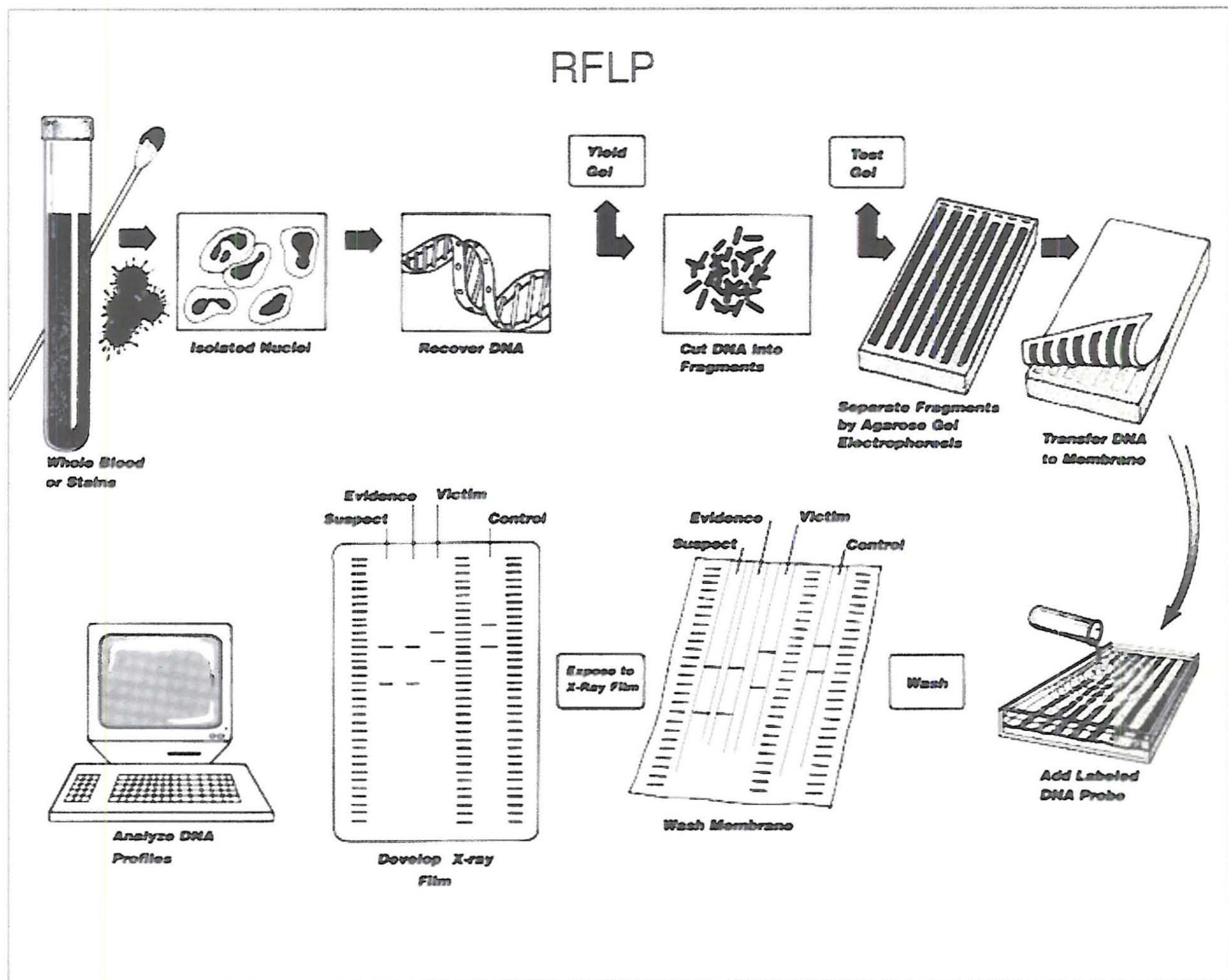

Figure-1: Diagram of an RFLP-Type DNA Fingerprint (Student Web Projects, 2006).

Figure 2 shows an RFLP from an actual investigation. The blood found on the defendant's clothing (center of the diagram) is clearly not the defendant's blood (lane D). However there is a match with the blood taken from the victim (lane V). This indicates the victim's blood is found on the defendant's clothing.

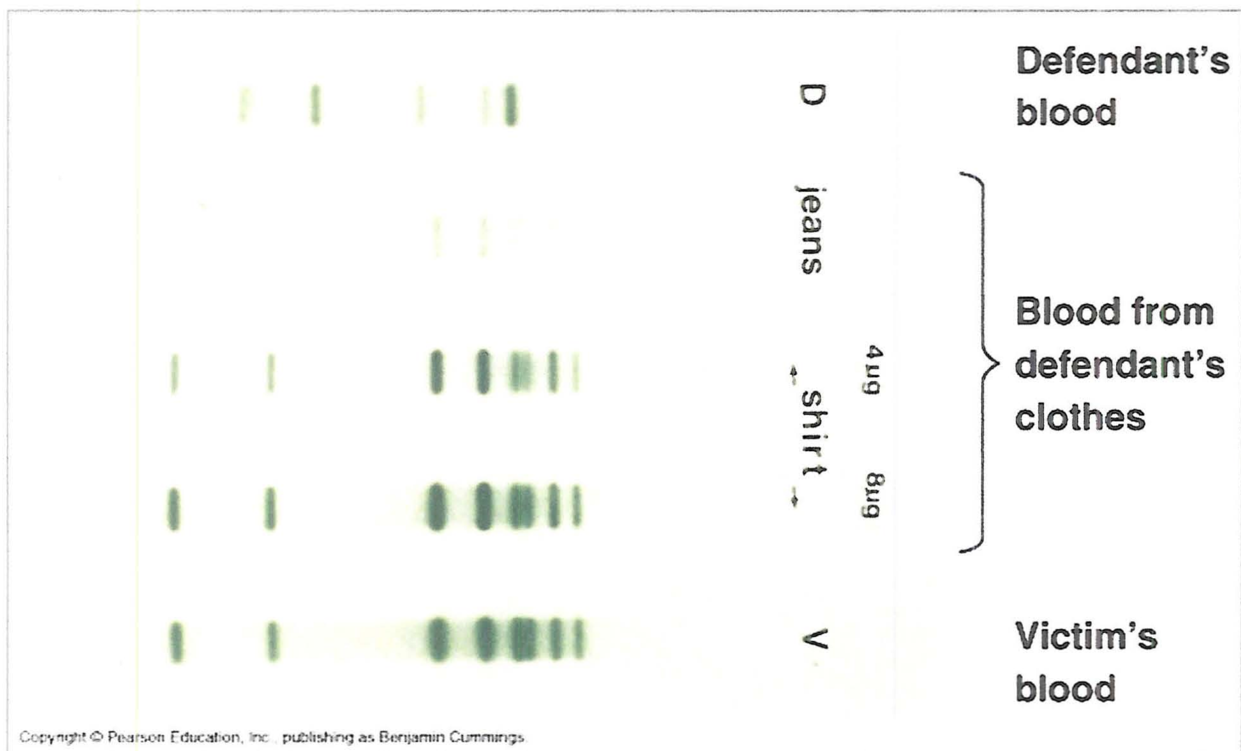

Figure-2: Example of a VNTR Fingerprint for a Crime scene Analysis (University of Miami, 2006).

Short Tandem Repeats (STR) are short sequences of DNA (from two to five base pairs) repeated in a head tail manner (The Biology Project, 2000a). STRs are so short they can be amplified millions of times in a process called Polymerase Chain Reaction (PCR) (Dolan DNA Learning Center, 2006). Thus the combined use of PCR/STR for DNA analysis is highly sensitive. Currently the FBI analyzes 13 core loci in the DNA. PCR amplification (Figure 3) is exponential, theoretically twenty PCR cycles would yield over two million copies of the original sequence. Scientists were even able to extract enough DNA from an eighty million year old insect trapped in pine pitch to use for PCR amplification.

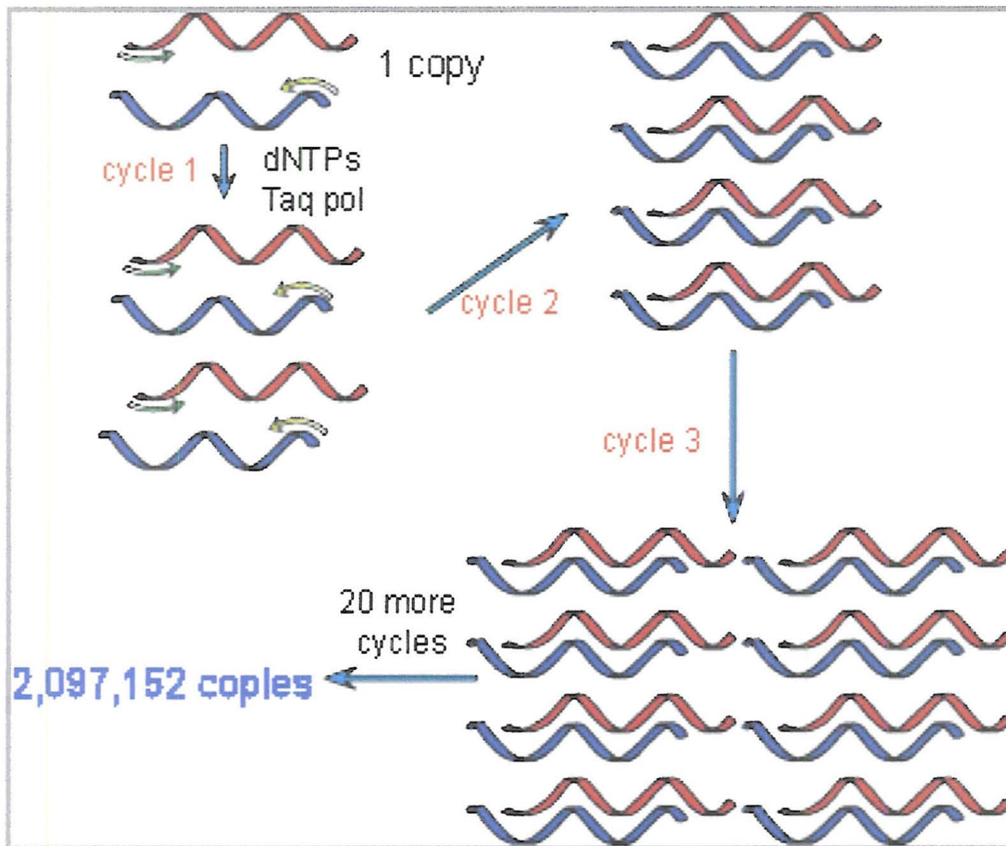

Figure-3: Diagram of PCR Amplification of DNA (Bioteach, 2006).

PCR requires three main steps, each occurring at a different temperature (Figure 4). First the DNA sample is separated into strands from heat (second panel in the figure). The separated strands are now able to accept a primer. Excessive amounts of a primer are added to the separated strands, and as the temperature is lowered (third panel in the figure) the DNA strands anneal to the primer. Because primer is in 10-fold excess, they anneal first to the DNA, instead of allowing the DNA strands to self-anneal. Now a Taq DNA polymerase enzyme is added to the mixture which synthesizes the DNA in opposite directions (fourth panel). The Taq enzyme is normally found in hot springs, its ability to survive in extreme temperatures allow the DNAF to be created (Brown, 1995).

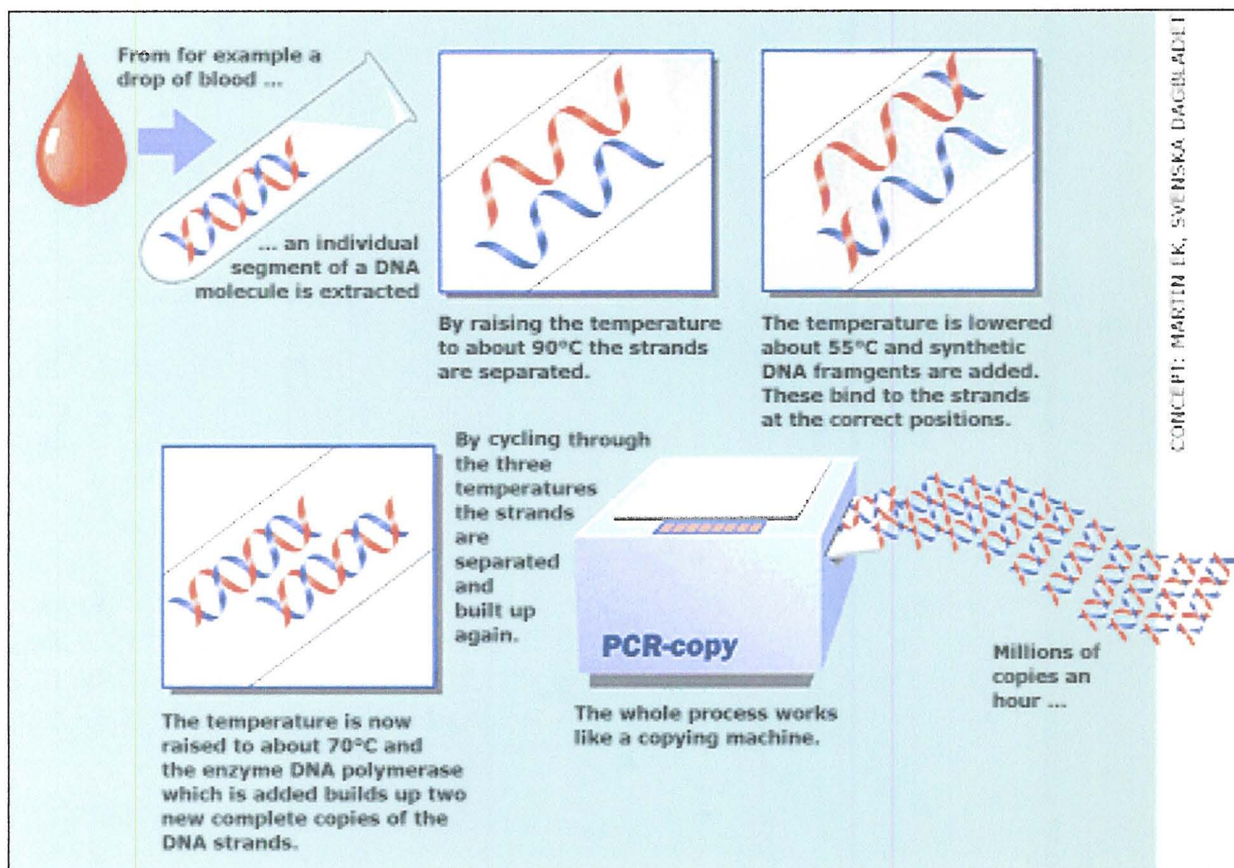

Figure 4: The Three Main Temperature Steps of PCR (Nobel Prize, 2006).

Now that the DNA sample has been multiplied exponentially, a STR can be used. As stated previously, the current STR procedure analyzes 13 core loci. By identifying the genotype at each of the thirteen loci (Figure 5) and running a frequency analysis the sample can statistically be identified as excluded from or identical to another sample. (The Biology Project, 2000b).

Locus	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818
Genotype	15, 18	16, 16	19, 24	12, 13	29, 31	12, 13	11, 13
Frequency	8.2%	4.4%	1.7%	9.9%	2.3%	4.3%	13%

Locus	D13S317	D7S820	D16S539	THO1	TPOX	CSF1PO	AMEL
Genotype	11, 11	10, 10	11, 11	9, 9.3	8, 8	11, 11	X Y
Frequency	1.2%	6.3%	9.5%	9.6%	3.52%	7.2%	(Male)

Figure 5: Example of an STR DNA Analysis on 13 Core Loci Plus an XY Analysis (University of Arizona, 2006).

Each core locus is identified by a code, i.e. D3S1358. The genotype at this locus is given by the numbers 15 and 18, which indicate the type of STR found at this location. The frequency for each genotype in the general population is represented by the percent number underneath the locus name. For example the locus vWA has the genotype "16, 16" this genotype is shared with about 4.4% of the population. The frequency for this particular profile for all thirteen loci is one in 7.7×10^{15} or one in 7.7 quadrillion. This rises to the level of statistical certainty that two samples either came from the same person except for identical twins, or are from different individuals (The Biology Project, 2000b).

Advantages and Disadvantages of Each DNA Analysis Technique

Each DNA analysis process has advantages and disadvantages. When using RFLP's or VNTR's, there is less worry about contamination because small amounts of contamination will not show up on the final analysis. There is no amplification of the contaminating DNA relative to the original source DNA. However a relatively large amount of DNA is needed to create a RFLP or VNTR type DNAF, and the process (requiring radiation during probe hybridization) can take two to three days. With the STR method, a much smaller sample can be analyzed in a matter of hours without radiation. But the STR is more susceptible to contamination because only a small sample is needed any contaminant of the sample could be amplified by the PCR method instead of the sample itself. PCR cannot be used with the RFLP and VNTR methods.

As a matter of practice, usually samples are first analyzed using the STR/PCR method for convenience and rapidity, then later analyzed by RFLP or VNTR if time allows or if there are any contamination issues. Different situations call for different methods of creating a DNAF. When a quick result is needed, or there are large numbers of samples to analyze, the STR is used.

When there is a hit from the STR if enough DNA was obtained, the more proven reliable RFLP or VNTR methods are used to verify the STR. If there is only a small DNA sample, the STR method is the only way to create a DNAF using PCR amplification. STR fingerprints are stored in electronic format, which simplifies collaboration in databases such as CODIS.

Examples of DNA Fingerprinting Applications

DNA fingerprinting has been used to both convict the guilty, and to exonerate the innocent. There are many instances of exoneration from conviction of a crime through DNAF's. Two women were abducted at knifepoint from a mall parking lot and raped, in two separate instances. In 1987 Glen Woodall was sentenced to two life terms in prison without parole plus an additional 203 to 335 years. The prosecution had strong evidence against Woodall. A state police chemist testified that a sample of Woodall's blood matched a sample of semen taken from the crime scene. Hair found in the victim's car was consistent with Woodall's. One of the victims had made a partial identification, including that of clothing found in Woodall's home. At the time of the pretrial hearing, DNA testing was a new science and not admissible, but in 1989 PCR amplification was run on semen samples taken from vaginal swabs from the two victims. The results indicated the same perpetrator committed both of the rapes, however the DNA was not a match for Woodall. In 1992 a RFLP-type fingerprint was used to verify that Woodall was innocent (President's DNA Initiative, 2006a).

In another case, Ronald Cotton was convicted of rape and sentenced to life in prison. While in prison an inmate bragged about how he committed the crime that Cotton was convicted of. There was a retrial and both of the victims testified against Cotton who received two life

sentences plus 55 years in prison. In 1994 Cotton learned about DNA testing, and in 1995 PCR was used to amplify DNA from a small amount of semen found on a vaginal swab from the victim. The DNA profile did not match Cotton's DNA. After almost ten years Cotton was released from prison (President's DNA Initiative, 2006b).

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CHAPTER-2: DNA FORENSICS

DNA forensics allows biological evidence to be collected, stored, and eventually linked directly to a specific person either as a suspect, victim or witness. Problems with the improper handling of DNA evidence have caused the evidence to be thrown out in many court cases. This chapter documents some of the currently approved DNA handling procedures concerning sources of DNA at a crime scene, prevention of DNA evidence contamination, DNA collecting and packaging methods, storage transport and documentation of DNA evidence, and several examples of DNA forensics at work.

Sources of DNA at a Crime Scene

Different types of tissues and body fluids contain different amounts of DNA. There are certain types of tissue that are preferable to others for the collection of DNA to create a DNAF. Blood is a good source for DNA. The DNA is present in white blood cells, red blood cells have no nuclei therefore have no DNA. One of the best sources of DNA as evidence of a sexual assault is the sperm head, the sperm head contains ten times as much DNA per cell as blood. DNA can also be found in saliva extracted from a multitude of places such as cigarette butts, bite marks, postage stamps or anything that would leave a trace of saliva. Hair follicles on hair pulled from the body not cut or broken also contain DNA. Any un-degraded body tissue is a good candidate for a DNA sample. One of the best sources for DNA in a decomposed case is bone and teeth, incongruously these are the parts that can outlast other body parts by thousands of years (Biology Project, 1997). The table in Figure 1 shows

examples of crime scene evidence, the likely location of the DNA on the evidence, and the biological sources of DNA.

Evidence	**Possible Location Of DNA on the Evidence**	**Source of DNA**
baseball bat or similar weapon	handle, end	sweat, skin, blood, tissue
hat, bandana, or mask	inside	sweat, hair, dandruff
eyeglasses	nose or ear pieces, lens	sweat, skin
facial tissue, cotton swab	surface area	mucus, blood, sweat, semen, ear wax
dirty laundry	surface area	blood, sweat, semen
toothpick	tips	saliva
used cigarette	cigarette butt	saliva
stamp or envelope	licked area	saliva
tape or ligature	inside/outside surface	skin, sweat
bottle, can, or glass	sides, mouthpiece	saliva, sweat
used condom	inside/outside surface	semen, vaginal or rectal cells
blanket, pillow, sheet	surface area	sweat, hair, semen, urine, saliva
"through and through" bullet	outside surface	blood, tissue
bite mark	person's skin or clothing	saliva
fingernail, partial fingernail	scrapings	blood, sweat, tissue

Figure-1: Table of DNA evidence sources (National Commission on the Future of DNA Evidence, 1999).

Approved DNA Handling Procedures

Keeping the sample protected and documented is important for the DNA to have merit as evidence. The sample must be protected sometimes for decades to prevent environmental or malicious damage. Documentation of the manner in which the DNA is identified, preserved, collected, packaged, transported, and stored is of extreme importance for the evidence chain. Procedures have been developed to help seamlessly follow the sample and the DNA from the crime scene to the courtroom. Also protocols have been established by the President's DNA Initiative, for the protection and prevention of DNA evidence contamination, collection and packaging and storage, transport and documentation of a DNA evidence collected at a crime scene for the use of DNA as evidence.

Protection and Preventing of DNA Evidence Contamination

Law enforcement personnel can cause crime scene contamination; hair, skin, sweat and possibly blood can accidentally be left at the crime scene. Whenever two objects come in contact with each other trace evidence is exchanged. Each time an investigator enters a crime scene, they not only possibly leave trace evidence behind, but also evidence may be taken away from the crime scene. Considering this as more personnel are exposed to a crime scene there is a greater risk for contamination and evidence to be inadvertently carried out.

There are pre-secured crime scenes and post-secure crime scenes. Challenges to law enforcement for a pre-secured scene include the potential for contamination, and destruction of evidence. The actions that took place in between the time the crime was committed and the time the scene is secured cannot be fully accounted for. Public pre-secured scenes are more

likely to be contaminated than private pre-secured scenes because of greater access of the general public. In the private pre-secured scene it is generally the investigating personnel who cause contamination. Post-secured scenes would ideally prevent any further contamination of the crime scene. Again this is easier in a private residence, outdoors and public crime scenes offer many points of access that are difficult to secure. Some of the reasons are large areas, difficult terrain, and weather factors. Often limited law enforcement personnel are available, leaving the scene unsecured from intrusion (Baldwin, 2005).

Regarding the chance of an infection transferring between a collected DNA sample and the collector, in practice all samples are considered to be contagious (Presidents DNA Initiative, 1999a). Contact with the sample should be kept to a minimum. Gloves are worn at all times, and measures are undertaken to prevent aerosolization of the sample. Because DNA can be extracted from a very small sample, contamination can occur when DNA from a different source gets mixed with the DNA from the forensic sample (Presidents DNA initiative 1999c). By touching the sample or coughing into it, the individual who is collecting the sample could inadvertently cause contamination through direct or indirect contact.

Figure-2: Photo of a technician in protective clothing (Presidents DNA Initiative, 1999k).

Touching anything with bare skin leaves skin cells behind, and a misdirected sneeze could easily contaminate a small drop of blood (Presidents DNA initiative 1999d). The use of latex gloves, shoe covers, gowns and facial masks reduce the risk of contamination (Fig 2) (Presidents DNA initiative 1999e).

Collecting and Packaging DNA Evidence

There are general procedures for collecting and packaging samples for shipment to the lab. The evidence should be allowed to dry if possible, and each piece of evidence should be collected and packaged individually, and properly labeled (Presidents DNA initiative 1999b). To help prevent contamination while collecting evidence, an order of collection has been established; hairs and fibers are collected first, followed by biological fluids, tool marks, visible fingerprints or footwear patterns (Baldwin, 2005).

Several techniques are implemented in the collection of evidence for DNA analysis. Cotton tipped swabs are used to collect DNA evidence from crime scenes. Either the swab is directly used to collect fluids, or for dry the swab is slightly moistened with clean water and worked into the stain absorbing evidence. Extreme care is exercised to prevent sample-to-sample contamination. The swab is then air dried and placed individually in a clean properly labeled paper container. Another method used specifically to collect dried blood uses tape much like collecting a traditional fingerprint. Tape is pressed onto a dried bloodstain to attain a good adhesion of the evidence. The tape is then placed evidence side down on a clean paper card and placed in a clean properly labeled paper container (Kramer, 2002).

Either the entire item can be sent to the lab for analysis, or items that are too large can have a sample cut from them, or a swab can be used to collect evidence along with a control sample (Presidents DNA Initiative, 1999f). The control samples are collected near the sample sources but do not contain any obvious fluid evidence. Control samples are analyzed along with the DNA sample to see if there are any effects of the sample processing on the DNA sample (Presidents DNA Initiative, 1999g). Evidence such as paper and clothing should not be folded to help prevent cross contamination (Fig 3) (Presidents DNA Initiative, 1999h).

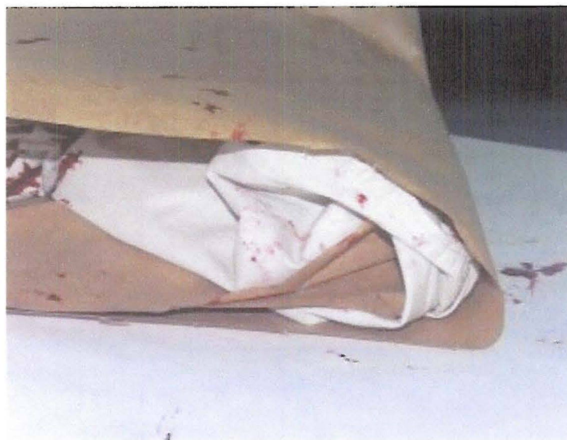

Figure-3: Photo of improper evidence collection techniques (Presidents DNA Initiative, 1999l).

Storage, Transport, and Documentation of DNA Evidence

DNA should be stored and transported in a cool environment. Sunlight and heat may cause the degradation of the DNA (Presidents DNA Initiative, 1999g). Plastic bags should only be used for very short-term storage, if left for too long bacterial growth becomes a problem that could render the sample more difficult or impossible to get DNA from (Presidents DNA Initiative, 1999h).

The proper storage of biological evidence is important to reduce the risk of damage to what could be possibly the only evidence in a criminal case. Harmful substrates like tanic acid

treated leather can destroy a biological sample. A dried bloodstain on a smooth coffee mug could fall off, and the potential for a DNA analysis could be lost. Anytime a physical object containing biological evidence is at risk a swab or other method should be used to collect a sample from the evidence and placed into controlled storage.

Although your common sense says to store DNA in a freezer, DNA evidence is stable at room temperature. Freezer storage can result in hydration over time and degradation. Evidence should be kept at room temperature until the analysis is complete. A preservative can be added to liquid blood samples to extend the stability of refrigerated samples over an extended period of time. For long-term storage, add bloodstain drops onto paper cards and place in a freezer. Clothing with biological stains should also be placed in a freezer to best preserve the evidence (Spear, 2004).

Every sample should have the proper documentation of every person who comes in contact with the crime scene or the sample (chain of custody), the time and date the DNA was collected, who collected the sample, where the sample was collected, how the sample was collected, possible locations and sources of other DNA, whether or not the sample was wet or dry, and other factors relevant to the collection of the sample. A full documentation and adhesion to the collection protocols is imperative for the DNA to have credibility (Presidents DNA Initiative, 1999i).

Examples of DNA Forensics at Work

On May 25, 2003, a Yakelev-42 Spanish military airplane crashed into Turkey on a return flight from Afghanistan. On board were sixty-two Spanish soldiers returning home from a peacekeeping mission. Thirty of the bodies had a DNA analysis, and were documented as unidentified by the Turkish and returned to Spain. The Spanish without completing a further identification to each of the remains returned them to the families. The following year a DNA analysis was performed correlating DNA data from the Turkish with DNA reference samples taken from the soldier's families. The analysis revealed that each of the thirty families received the wrong remains. The remains were exhumed and reanalyzed against the family reference samples. Through the proper documentation on behalf of the Turkish forensics team, the errors could be corrected and each of the families were able to receive the proper remains (Alonso, 2005).

On July 25, 1984, Dawn Hamilton was viciously raped and murdered in Maryland. In March 1985 without any physical evidence Kirk Bloodsworth was arrested, convicted, and sentenced to death. In 1992 Bloodsworth and his attorney requested a DNA analysis on the evidence from his trial, Hamilton's shirt and underpants. Two DNAF tests were conducted one by the Forensic Science Associates and the other by the FBI. Each agreed that the DNA on the underpants was not the same as Bloodsworth. On June 28, 1993 Bloodsworth was released from prison and compensated $300,000 by the state of Maryland. However prosecutors were not convinced that Bloodsworth was innocent, he lived for ten years under a shroud of suspension. On September 5, 2003, Kimberly Shay Ruffner a convicted sex offender was implicated as the rapist and murderer of Hamilton and charged with first-degree murder. The

prosecutors who previously refused to accept Bloodsworths innocence went to his home to personally apologize to him (NACDL, 2003). The preservation of the evidence from this closed case not only allowed an innocent man to be un-imprisoned, but also allowed the conviction of the perpetrator of the crime. This case illustrates the importance of the preservation of evidence from the moments after a crime is committed to years or even decades following.

May 30, 1995, the O. J. Simpson trial was in its nineteenth week. The Defense (Sheck) was questioning Colin Yamauchi a police crime scene technician on the manner in which he collected and analyzed the evidence. Sheck tried to show that Yamauchi did not change his gloves between collecting and analyzing different pieces of evidence, failed to properly document blood testing and avoided safe-guards established to prevent contamination of evidence. Sheck tried to illustrate that Yamauchi may have transferred some of Simpson's blood inadvertently from a vile to a glove collected as evidence. Supposedly Yamauchi spilled some of Simpson's blood onto his plastic glove. Then without knowing the blood was on his plastic glove Yamauchi picked up the evidence glove to label it, in the process transferring Simpson's blood to the glove. If proper documentation existed with the time each piece of evidence was handled it could have been determined whether or not Yamauchi handled the glove before or after the vile of Simpson's blood (CourtTVnews, 2004). The importance of properly collecting and documenting evidence used for DNA analysis cannot be overstated. Haphazard collection and documentation can render the evidence useless. This not only wastes valuable law enforcement resources, but also could let potentially dangerous people free to walk the streets.

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