THE DNA MANUAL

An Official Publication of the
Child Protection Unit Network

In collaboration with
The DNA Analysis Laboratory
Natural Sciences Research Institute
University of the Philippines

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OBJECTIVES

- Advise child protection specialists and other physicians of their role in the DNA forensic process
- Provide a brief review of DNA, its uses and its value as a criminal investigation tool
- Update practitioners on the current state of DNA forensic testing and analysis in the Philippines
- Provide practical knowledge to assist both physicians and the legal community in understanding reports issued from DNA laboratories
- Familiarize the legal community with the strengths and limitations of DNA as evidence

USERS OF THIS MANUAL

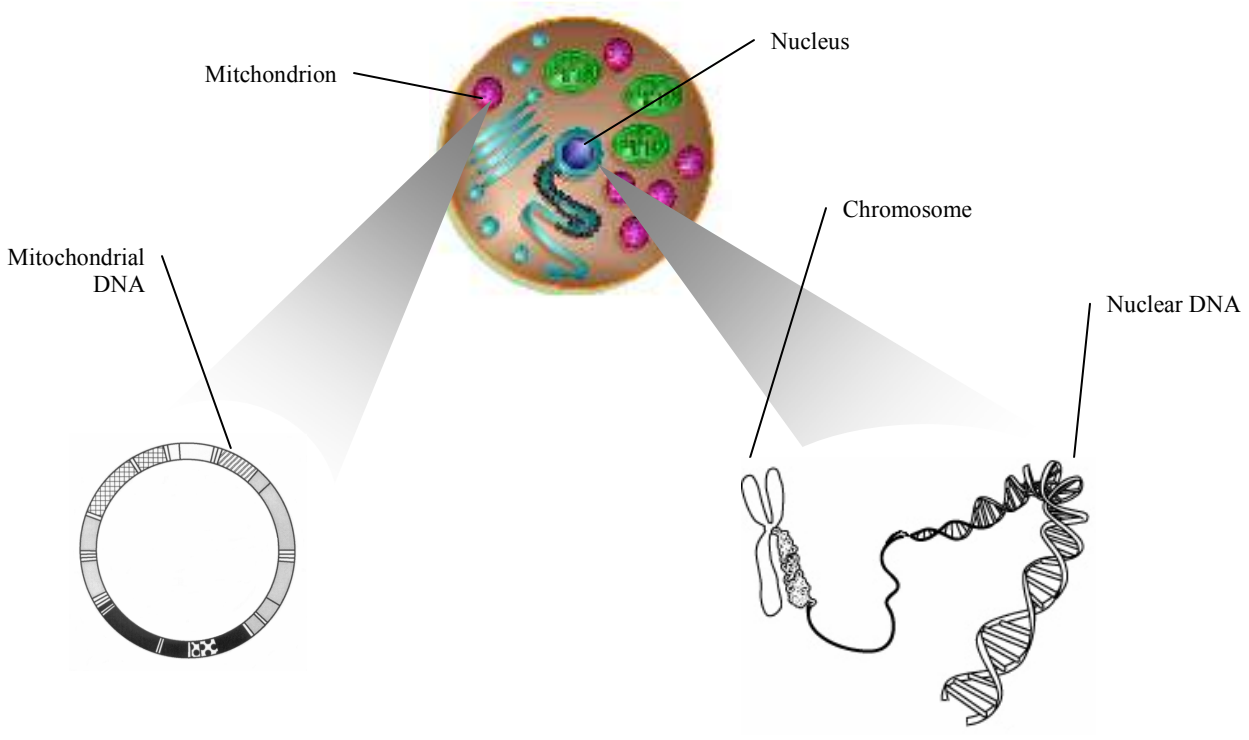
This manual was designed primarily to benefit child protection specialists, pediatricians and emergency room physicians in their treatment of child abuse patients. Judges, lawyers and police may also find this manual helpful in their own respective disciplines, as each plays an important role in the criminal investigation and prosecution of child abuse cases.

PART I: INTRODUCTION

What is DNA?

Deoxyribonucleic acid, or DNA, is the fundamental building block of all living matter. The "blueprint of life," DNA contains the inherited information determining how an organism is built and organized. DNA is a component of virtually all the cells of the body, and is identical in each of those cells.

A group of DNA molecules that, together, perform a specific function is known as a **gene**. Most genes are located on chromosomes that are then tightly packed within the nucleus of a cell (**nuclear DNA**). Genes may also be found in mitochondria, which are located outside of the nucleus of the cell. The DNA comprising genes found in the mitochondria are referred to as **mitochondrial DNA**.



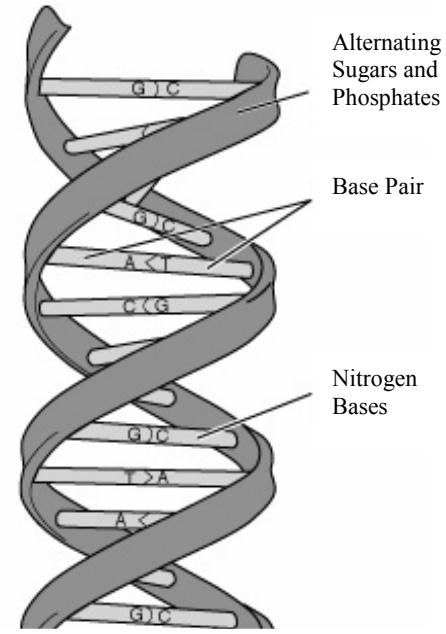
Structurally, nuclear DNA is a double helix: two strands of genetic material spiraled around each other like a twisted ladder. The sides of the ladder are made up of alternating sections of phosphates and sugars.

The inner strands (like rungs of the ladder) are pairs of four **bases**:

- **Adenosine (A)**
- **Cytosine (C)**
- **Guanine (G)**
- **Thymine (T)**

These letters comprise the DNA alphabet forming the basis of the genetic code. The bases of each strand bind to the opposite strand, holding the entire molecule together, much like the stairs on a spiral staircase. These bases bind specifically to their complementary mate; for example, A binds only with T, C only with G, and vice versa. Two bases binding together are referred to as a **base pair**.

Structure of Nuclear DNA



The genetic variation between individuals lies in the sequence of the base pairs comprising a particular gene or the length of certain genetic markers. Each person has a unique DNA sequence; consequently, an individual's personal **DNA profile** serves as his own "DNA fingerprint" that may be used to identify him.

Uses for DNA

DNA has been a part of mainstream medicine for years, ranging from diagnostics to preventative medicine. No longer a novel and unproven area of research, DNA is now an accepted science. Our increased understanding of its form and function has already greatly benefited several fields of study, including:

- **Health Care:** improved diagnosis of disease, particularly genetic disorders, and earlier detection of patients' predisposition to certain illnesses
- **Pharmaceutical Research:** pharmaceutical drugs designed to target specific sections of DNA that are responsible for the disease
- **Evolution:** comparison of DNA profiles of ethnically diverse populations to determine patterns of evolution and migration
- **Forensics:** increased capacity to identify potential criminal suspects, victims of crime and mass disasters, and resolve paternity disputes

Identification Methods

British scientist Alec Jeffreys invented the concept of identifying an individual using DNA in 1984 at the University of Leicester. His patented technique was called Restriction Fragment Length Polymorphism (RFLP). Since then, several methods of DNA identification have been developed.

1. Restriction Fragment Length Polymorphism (RFLP)
RFLP was the first analytical procedure used for human identification, and is a patented method under the name 'DNA fingerprinting.' In RFLP, DNA is extracted and cut by enzymes into smaller fragments. These fragments are sorted by length, using electrophoresis, and then radioactively tagged to reveal a unique pattern. Variations in the patterns are analyzed and compared with the reference DNA provided by the suspect or victim.

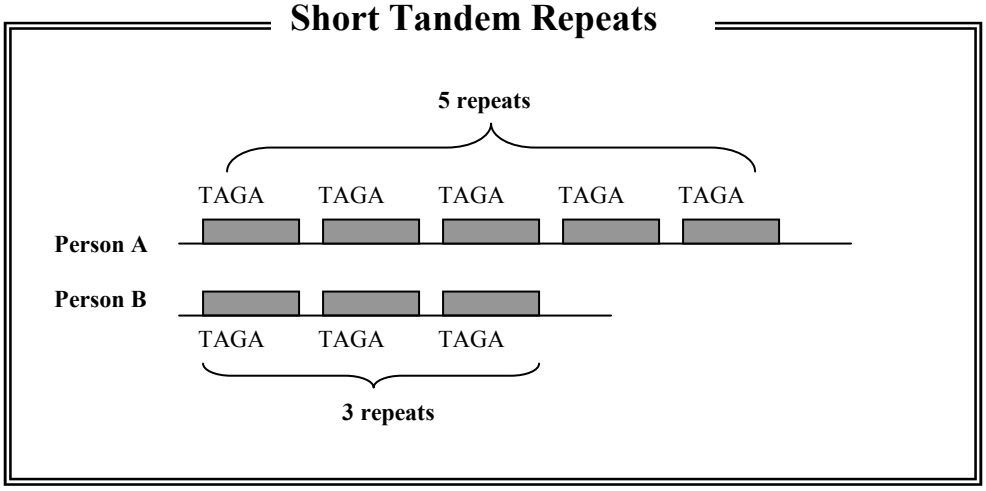
Due to the relatively low sensitivity of RFLP fragment analysis compared to other methods, its requirement for high quality DNA which limits its utility in analyzing degraded samples, and the lengthy amount of time required for the conduct of a test, this method has fallen out of favor in criminal investigations. Notably, **RFLP is not used** by any of the DNA laboratories in the Philippines.

2. Reverse Dot Blot Method
The reverse dot blot method utilizes polymarkers, such as the human leukocyte antigen DQ (HLA-DQ) and low density lipoprotein receptor (LDLR). Nucleotide bases in specific parts of a gene are detected using single-stranded DNA probes fixed onto a matrix. The appearance of a blue color indicates the presence of DNA complementary to a specific probe of a known gene type, allowing the genotype of the sample to be identified.

HLA DQA1-Pm loci analysis relies on expensive kits, the cost of which is prohibitive for laboratories in the Philippines. More importantly, this type of DNA analysis has limited use when dealing with mixed stains, such as vaginal swabs containing the DNA of both the victim and the perpetrator (Takahashi et al. 1995).

3. Mitochondrial DNA (mtDNA) Analysis
Mitochondrial DNA (mtDNA) analysis targets the DNA found in the mitochondria of a cell. This type of DNA is inherited through female ancestry, and allows identification of individuals by comparing their mtDNA with that of their mother, their mother' siblings and their grandmothers. Mitochondrial DNA analysis may be conducted on samples that are unsuitable for PCR or RFLP testing, such as dried bones or teeth, or hair without the root; consequently, mtDNA analysis is especially valuable in identifying victims in mass disasters (Holland et al. 1993).

4. Short Tandem Repeat (STR) Analysis via the Polymerase Chain Reaction
Short tandem repeats (STRs) are short sequences of DNA (normally 2 to 5 base pairs) that are repeated numerous times. Individuals are distinguished from one another by the number of repeats of STRs within a particular region.



Which Method Do We Use?

STR analysis is currently the method of choice in most forensic laboratories, including those in the Philippines, due to its short processing time and relatively low cost. It also allows the use of several types of reagents, a feature that is very important in the Philippines due to the limited availability and prohibitive cost of many molecular reagents.

PART II: DNA EVIDENCE IN CRIMINAL INVESTIGATIONS

Origin of DNA Forensic Evidence

Forensic DNA evidence was first used in the investigation of the rape and murder of two British schoolgirls in November 1986. During the initial investigations, semen samples that were isolated from the victims' bodies were compared with the initial suspect's DNA. The samples did not match, thus excluding the suspect as the perpetrator of the crime. In an attempt to locate the offender, police investigators requested all males in the prescribed area, aged 17 to 34 years old, to voluntarily submit blood samples. Investigators processed over 4000 reference samples for comparison with the DNA pattern of the semen samples. Eventually, the DNA profile from the semen samples matched that of a man named Colin Pitchfork. Pitchfork later confessed to the crime and was subsequently convicted for the rape and murder of the two girls. The success of DNA fingerprinting in resolving this rape and murder case initiated the use of forensic DNA technology by other law enforcement agencies.

Forensic DNA evidence is a powerful tool in criminal investigations. A suspect's DNA may be compared with DNA recovered from the victim or the crime scene; if the samples match, the suspect has been placed at the scene of the crime. Thus, DNA evidence can either link a suspect to a crime or eliminate him from suspicion.

DNA can be especially useful in the investigation of violent crimes, such as child sexual abuse. According to Edmund Locard's **Principle of Exchange**, "when any two objects come into contact, there is a transfer of material between them." Because of the intimate nature of child sexual abuse, assailants are likely to leave traces of biological material, either at the crime scene or on the victim specifically.

The strength of DNA as evidence, however, is based entirely on the reliability of the process used to collect and analyze it. Each step of the process is essential and must be performed accurately; the most technologically advanced laboratory equipment is useless without proper evidence collection and preservation. Mishandling or human error at any point could destroy the evidence or render it inadmissible.

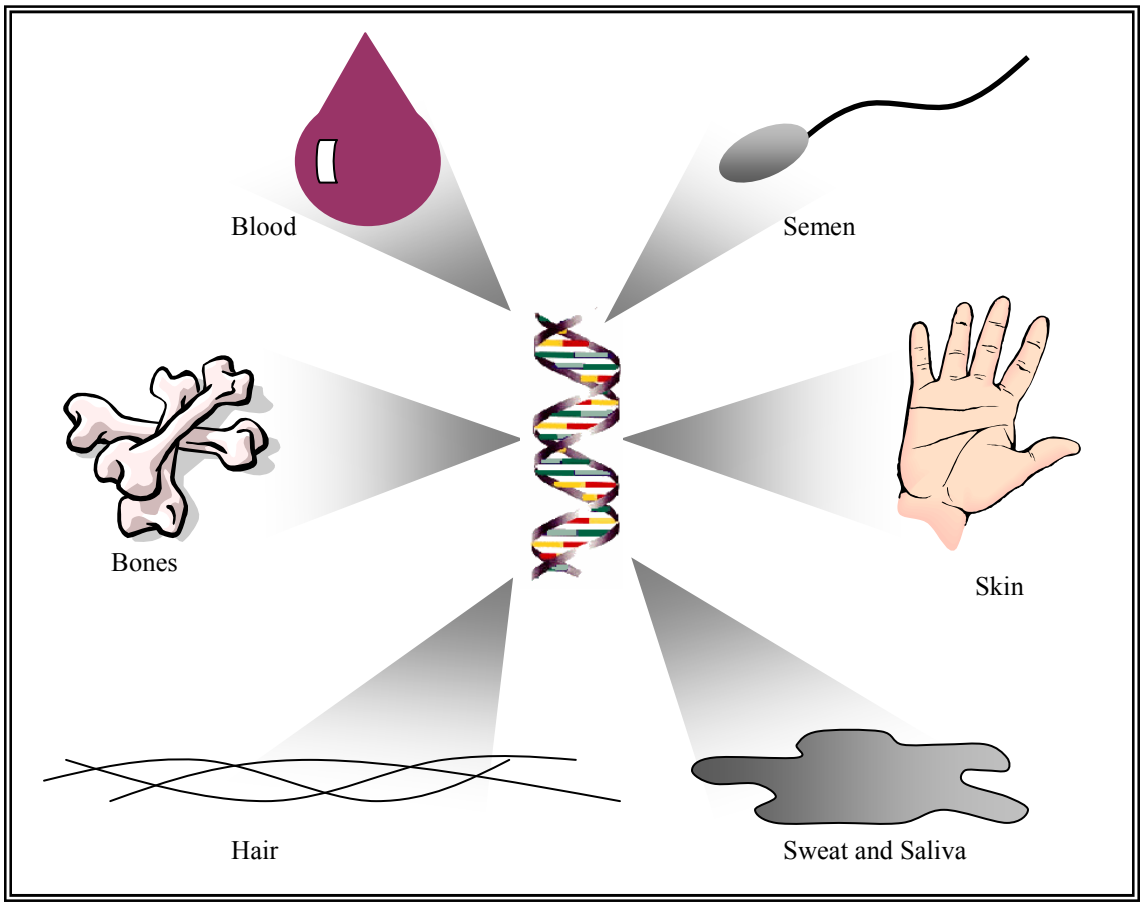
The forensic process is comprised of the following stages:

- Evidence Collection and Preservation
- Transfer of Evidence
- Laboratory Analysis
- Interpretation of Results

Evidence Collection and Preservation

In cases of child sexual abuse, victims come to child protection units for treatment and medico-legal evaluation. During examinations, physician will document the extent of the injuries and collect physical evidence, which may contain the suspect's DNA. Only physicians should collect evidence on or inside the victim's body.

DNA may be found in the following types of biological material commonly recovered in child sexual abuse cases:



The best evidence samples are those found on the patient and her clothing immediately after the crime: the skin of the assailant may be found under the patient's fingernails, a hair strand belonging to the assailant may be clinging to her clothing, and most importantly, semen may remain on the patient's clothing or within the vaginal region.

Additionally, DNA evidence may also be recovered from the bedding where the assault took place. Semen stains, sweat, hair strands, blood and saliva may all be collected from sheets or other bedclothes, just as with clothing.

The evidence collection process is facilitated by sexual assault evidence kits, or "rape kits," containing materials for collecting and preserving samples found on the patient's body and clothing. The rape kit guides the clinician through the evidence collection process, serving as a checklist and ensuring that all available types of evidence are gathered. Although variations do exist, the use and contents of rape kits are fairly similar throughout the world, consisting of paper bags, swabs, envelopes, glass slides and sterile tubes. Physical evidence must **never** be placed in plastic as this promotes fungal growth due to locked moisture when evidence is not fully dried prior to storage.

Contents of a Typical Rape Kit

• **Large paper envelope**	• **To contain all kit materials**
• **Small paper envelopes**	• **To hold pubic hair combings, foreign materials, and reference samples of public, head and body hair**
• **Paper bags of assorted sizes**	• **To contain each item of victim's clothing separately**
• **Larger size paper bags**	• **To contain smaller bags of clothing and paper from examination table**
• **Sterile swabs**	• **For vaginal, oral, rectal and penile swabbing (additional swabs for other secretions found on body)**
• **Glass slides**	• **For swabs**
• **Tubes or envelopes**	• **To contain used swabs**
• **Manicure sticks**	• **To collect fingernail scrapings**
• **Plastic comb**	• **To comb pubic hair for foreign material**
• **Sterile filter paper**	• **For saliva reference sample**
• **Tubes**	• **For blood sample collection**

Since rape kits are not yet commercially available in the Philippines, the UP-NSRI DNA Analysis Laboratory, in collaboration with the UP-PGH Child Protection Unit, is testing a prototype model for evidence collection. In the initial phase of development, the prototype kit includes components for collecting samples only from areas where DNA is likely to be found, such as swabs for internal and external genitalia, anus and mouth. Separate packages may also be used for submitting clothing and other types of evidence, if any is available. Each kit includes instructions and sample forms to facilitate the proper and efficient collection of samples from a victim. A copy of the documentation included in the rape kit may be found in Appendix C.

Unfortunately, evidence collection has a time limit. Although DNA as a chemical compound is fairly stable, the biological material containing it (e.g., pubic hair, blood or seminal stains) may be lost if medical examination of the victim is delayed. For example, sperm cells containing the assailant's DNA may remain in the female reproductive organs for up to 72 hours after the assault. This period shortens, however, if the victim bathes, urinates or defecates during that period. Moreover, in children younger than ten years old, swabbing the body for evidence more than 24 hours after the assault yields little evidence; physicians should instead focus on the child's clothing and bed linens (Christian et al. 2000). Consequently, if proper evidence is to be collected, patients must be encouraged to seek medical attention as soon as possible after they have been assaulted. For the same reason, crime scene investigation must also occur promptly to prevent the loss of evidence.

The manner in which DNA evidence is collected and preserved is also critical to the success of subsequent DNA testing. If evidence is collected or stored improperly, the DNA may become contaminated or may be destroyed completely.

Contamination

The evidence becomes contaminated when DNA from another source gets mixed with the DNA relevant to the case. The worst sources of contamination are those individuals handling the samples. For example, if someone sneezes or coughs over the evidence, or touches his mouth, nose, or other part of the face and then touches the area that may contain the DNA to be tested, that person may inadvertently introduce his own DNA into the sample. Handlers of DNA evidence must always wear gloves and should be careful to avoid any activities that may potentially contaminate the evidence. In contrast, environmental contamination, such as DNA from most animals and plants, do not interfere in the analysis of human DNA.

Degradation

Direct sunlight, warm temperatures and moisture are all damaging to DNA evidence. Consequently, evidence samples must not be kept in direct sunlight or in locations that get hot, such as rooms or vehicles without air conditioning. Further, evidence samples should always be placed in properly labeled paper bags or envelopes, rather than plastic bags, which retain damaging moisture and allow bacterial growth. These environmental factors will not change the DNA from one type to another, making the DNA from one person appear to belong to a different person; rather, the degradation only changes the DNA from a sample the can be typed into a sample that cannot; the degradation.

Despite the risks of contamination and degradation, one of DNA's greatest strengths is its stability. Provided it has not been exposed to adverse environmental conditions or contaminants, DNA samples can remain viable for years.

Guidelines for Collecting Evidence

To avoid contamination of evidence that may contain DNA, always take the following precautions:

- **Wear gloves. Change them often.**
- **Use disposable instruments or clean them thoroughly before and after handling each sample. Ideally, all instruments must be sterilized prior to use.**
- **Do not touch the area where you believe DNA may exist.**
- **Do not talk, sneeze and cough over evidence. Masks should be worn while handling evidence.**
- **Do not touch your face, nose or mouth when collecting and packaging evidence particularly when already wearing gloves. Change gloves as needed.**
- **Air-dry evidence thoroughly but away from direct sunlight before packaging.**
- **Put each piece of evidence into new individual paper bags or envelopes, not into plastic bags.**

*Photocopy and post in patient examination areas

Transfer of Evidence

Once the evidence has been secured in paper bags or envelopes, it should be sealed, and properly labeled. Individual organizations vary in their requirements but labels affixed to evidence should typically include the following information:

- Date
- Case number
- Patient's name
- Age of patient
- Physician's name
- Source and nature of sample: e.g. anal swab or vaginal swab
- Date of assault

The UP-NSRI DNA Analysis Laboratory prototype rape kit includes detailed instructions and a simple checklist for relevant information specially formulated to facilitate the efficient collection of samples from child victims (see Appendix C). Physicians should also fill out any appropriate paperwork for transferring the evidence from the hospital to the DNA analysis laboratory.

The integrity of the sample's origins is just as important as the integrity of the sample itself; that is, if the sample's source is questioned, the evidence becomes useless. Proper documentation by every individual who handles the evidence – from physician to messenger to laboratory technician – ensures that the evidence has not been tampered with or switched. This documentation process is referred to as the **chain of custody**.

The chain of custody can be thought of as the unbroken sequence of events documented for a piece of evidence, from the time of its discovery to its subsequent presentation in court. Every link in this chain must be recorded, including evidence collection, storage, laboratory analysis, return to storage and the transfer to court. Proper documentation includes:

- Date
- Time
- Name of handler or custodian
- Activity performed by that individual

If the evidence cannot be accounted for in even one step of its journey from crime scene to courtroom, it may be rendered inadmissible in court.

Laboratory Analysis

After collection, evidence samples are sent to a DNA laboratory for analysis. There are currently four independent DNA laboratories operating in the Philippines:

- The DNA Analysis Laboratory at the Natural Sciences Research Institute of the University of the Philippines, Diliman (UP-NSRI)
- The National Bureau of Investigation, Taft Avenue (NBI)
- The Philippine National Police Headquarters at Camp Crame (PNP)
- St. Luke's Medical Center

While the NBI and PNP laboratories are devoted primarily to crime-related DNA testing, the UP-DNA Analysis Laboratory accepts evidence from criminal and civil cases for DNA testing, as well as conducting research in population genetics and in developing DNA analytical methods for application in the Philippines. Many hospital laboratories, particularly in developing countries, do not have the equipment and trained manpower to conduct DNA analysis. The specialized nature of DNA testing requires sophisticated and expensive equipment and staff with the proper training and education in molecular biology and genetics. In the Philippines, St. Luke's Medical Center is the only hospital that conducts its own DNA testing, and only for civil cases involving paternity.

Y Chromosome STR Analysis

The UP-NSRI DNA laboratory now has the capability to perform an accurate and less expensive screening process for samples submitted from suspected child abuse cases. Swabs taken from child abuse victims will be analyzed for the presence of male DNA, the STRs from Y chromosomes. If Y chromosomes are detected, their presence alone is definitive evidence that child sexual abuse occurred (if found on the child's body) and it now becomes cost-effective to proceed with the full DNA analysis. (Delfin et al. 2003)

Once at a DNA analysis laboratory, DNA from the evidence samples will be compared with DNA provided by the suspect (**reference sample**). Reference samples may come in the form of buccal cells swabbed from the suspect's cheek or from the suspect's blood. DNA profiles will be generated from evidence and reference samples, and then the two profiles will be compared to determine if any association between the two individuals exists.

The laboratory process takes place in the following stages:

Extraction: Samples are soaked in liquid buffer, where enzymes break down the proteins and other cellular material without harming the DNA. The solution is then centrifuged to separate the DNA from the remaining cellular material.

Amplification (The PCR Process): Polymerase Chain Reaction (PCR) is a laboratory process in which a particular segment of DNA is repeatedly duplicated, in order to increase the amount of DNA for analysis. The process focuses on several specific locations along the DNA strand where the number of STRs varies considerably between individuals. PCR takes place in a small instrument called a thermocycler and requires only hours to complete.

Separation and Profiling: Gels are used to sort the amplified DNA according to size. Segments of DNA with a greater number of STRs will be separated from those with fewer STRs. The results are then compared against DNA molecules of known size in order to determine the final DNA profile.

PCR is extremely sensitive, permitting analysis of minute amounts of DNA. It is this sensitivity that makes the technique vulnerable to contamination. When a sample of DNA is submitted for testing, the PCR process will copy whatever DNA is present in the sample; it cannot distinguish between a suspect's DNA and DNA from another source, such as that of a human handler. As a result, if the sample was contaminated with outside DNA, the PCR will replicate the foreign DNA as well as the evidence DNA.

Interpretation of Results

Once processed, the independent DNA profiles of the evidentiary and reference samples are compared. The results of DNA testing may be inconclusive or result in the exclusion or inclusion of the suspect as the source of DNA found in the evidentiary material.

Inconclusive

DNA testing may not produce information allowing the suspect to be either included or excluded, rendering the results inconclusive. This may result from improper collection, handling and storage. Inconclusive results occur when:

- The quantity of intact DNA obtained is insufficient
- The sample contains a complex mixture of DNA from several individuals

Exclusion

If the DNA profile from the evidence sample does not match the profile of the suspect's reference sample, the suspect is "excluded" as a source of the evidence.

Inclusion

When the DNA profile of the suspect matches that of the evidence sample, the suspect is "included" as a potential source of that DNA.

It is important to note that **exclusion does not always equate with innocence**. For example, DNA analyzed from a hair found on the victim may belong to someone who had contact with the victim but was not involved with the crime, such as an acquaintance or family member. If the DNA evidence recovered is not that of the suspect, it does not automatically indicate that the suspect did not commit the crime – *only that the substance tested did not come from the suspect*. Nonetheless, the presence of DNA other than the victim or any known possible contaminant (such as human handlers of the sample) indicates the involvement of an unnamed individual with the victim around the time of the assault and should be investigated.

Similarly, the **inclusion of a suspect does not automatically determine the suspect's guilt**. The likelihood of the evidence sample belonging to the suspect depends, in part, on the number of DNA locations examined and the statistics measuring how often that particular DNA profile is found in the general population. A profile occurring rarely in the population would more convincingly implicate the suspect than would a more common DNA profile. Consequently, by increasing the number of DNA locations tested, the combined DNA tests becomes more discriminating and have a greater capacity to distinguish between a true match and a match simply due to chance. This leads to weightier conclusions.

Statistical analysis of matching DNA profiles (such as the comparison of an evidence sample with a suspect's reference sample) estimates the significance of the match. Calculations are conventionally reported as **random match probability**, the probability that an innocent individual, unrelated to the suspect and chosen randomly from the population, will match the DNA profile taken from the crime sample. For example, if the random match probability is one in ten million (**1:10,000,000**) in a Philippine population of seventy-five million, then there are approximately seven other people (aside from the suspect) who could possess the DNA profile found in the evidentiary sample. These eight people are not equally likely to have committed the crime, however; non-DNA evidence, such as geographic proximity to the crime and physical appearance from eyewitness testimony, must also be considered. Further, simply because statistics estimate that seven other people in the nation *may* possess the same profile does not mean there actually *are* seven other people with that profile.

An alternate (and arguably simpler) method for statistically expressing the weight of the match is the **likelihood ratio**, the ratio of the probability that the DNA profile in the evidence sample came from the defendant and the probability that the DNA came from a random, unrelated person. Essentially, this is the ratio of the probability of a match given that the defendant is guilty to the probability of a match given that the defendant is innocent (random match probability).

$$\frac{\text{Probability that the crime sample profile would match the defendant if the sample had actually come from the defendant}}{\text{Probability that the crime sample profile would match the defendant if a random, unrelated person had left it at the scene}} = \frac{1}{\mathbf{1:10,000,000}} = \mathbf{10,000,000}$$

It is imperative that, in these statistical calculations, the appropriate population genetic databases are utilized. Different populations vary in the frequency distribution of alleles (or gene types) of specific genes. Gene types that are common among Americans Caucasians, for example, may not be common in Filipinos, and vice versa; consequently, the use of a published American Caucasian database for statistical analysis of cases in the Philippines, is inappropriate. The use of an unsuitable population genetic database was shown to increase the possibility of false positives and/or negatives in statistical estimation of matching DNA profiles in disputed parentage cases (De Ungria et al. 2002). The DNA Analysis Laboratory of the University of the Philippines Diliman has established a genetic database of the National Capital Region using nine autosomal gene markers (Halos et al. 1999; Tabbada et al. 2001).

At the conclusion of analysis and statistical interpretation, the DNA laboratory will issue a report on the results, including the following information:

- Samples tested
- Controls used
- Whether the DNA profile of the evidence sample is consistent with the suspect's DNA profile (from the reference sample)
- Statistics regarding the probability that the evidence sample came from the suspect
- Conclusion based on the results of the tests

A sample DNA report is presented on the following page.

Sample DNA Report

DNA Analysis Laboratory
NATURAL SCIENCES RESEARCH INSTITUTE
University of the Philippines
Diliman, Quezon City

Case no:
Nature of DNA Analysis: DNA profiling/Criminal investigation
Requesting Party:

Table 1: Details of biological samples for analysis:

Sample	Sample Source	Sample Description	Date Submitted
2004-# A	Name	Vaginal smear on slide	
2004-# B	Name	Semen stain on underpants	
2004-# C	Name	Buccal swab from victim (reference)	
2004-# D	Name	Buccal swab from suspect (reference)	
K562	Commercial	Liquid (Positive DNA control)	

Findings:
Table 2: Result of DNA fragment analysis showing individual genotypes

Loci	Samples				
	K562	**2003#A**	**2003#B**	**2003#C**	**2003#D**
HUMCSF1P0	9, 10				
D8S306	8, 9				
HUMDHFRP2	7, 8				
HUMF13A01	4, 5				
HUMFES/FPS	10, 12				
HUMFGA	21, 24				
HUMTHO1	9.3, 9.3				
HUMTPOX	8, 9				
HUMvWA	16, 16				

DNA profiles of submitted samples

Analysis
This would be a narrative which should include (1) the state of the physical evidence and any relevant observation(s) on the handling of the sample; (2) any adjustments/corrections made on the database for issues of subpopulations or inbreeding, as well as for any existing relationship of victims and suspect, e.g. kinship; (3) the statistical analysis conducted, taking into account the assumptions presented by the prosecution and the defense; and (4) **Random Match Probability** and **Likelihood Ratio** values.

Conclusion
This contains a summary of the **results** of the DNA test.

Date reported:

I hereby certify that the results obtained are accurate and the above statement is correct.

Dr. Maria Corazon A. De Ungria
Head
DNA Analysis Laboratory
Date approved:

Document is authenticated by a signature and the DNA Analysis Laboratory dry seal

To assist judges and lawyers, the UP-NSRI DNA Analysis Laboratory coverts likelihood ratio values into phrases which describe the weight of a given DNA match based on procedures used by the UK Forensic Science Service (Evett and Weir 1998).

Converting Likelihood Ratios into Statements for the Courts	
Likelihood Ratios	Verbal Equivalent
1-10	Limited Support
10-100	Moderate Support
100-1000	Strong Support
> 1000	Very Strong Support

Before the introduction of DNA analysis, clinicians used physical-based and serological methods for identifying humans and establishing parentage. Physical-based methods include radiological, anthropological and dental examinations. These methods often provide only limited assistance, however.

Similarly, serological methods, such as ABO blood typing, are also inadequate for identification. There are only four different blood types and two of these (A and O) are carried by a large majority of the population. Consequently, in many cases even if the blood type of the suspect correctly matches that of the sample found at the crime scene, it does not provide any proof that he is the offender. Blood typing may be of extremely limited use for screening out individuals whose blood type does not match the reference sample; however, blood typing requires confirmatory tests, such as DNA analysis, and most countries will no longer accept it as evidence.

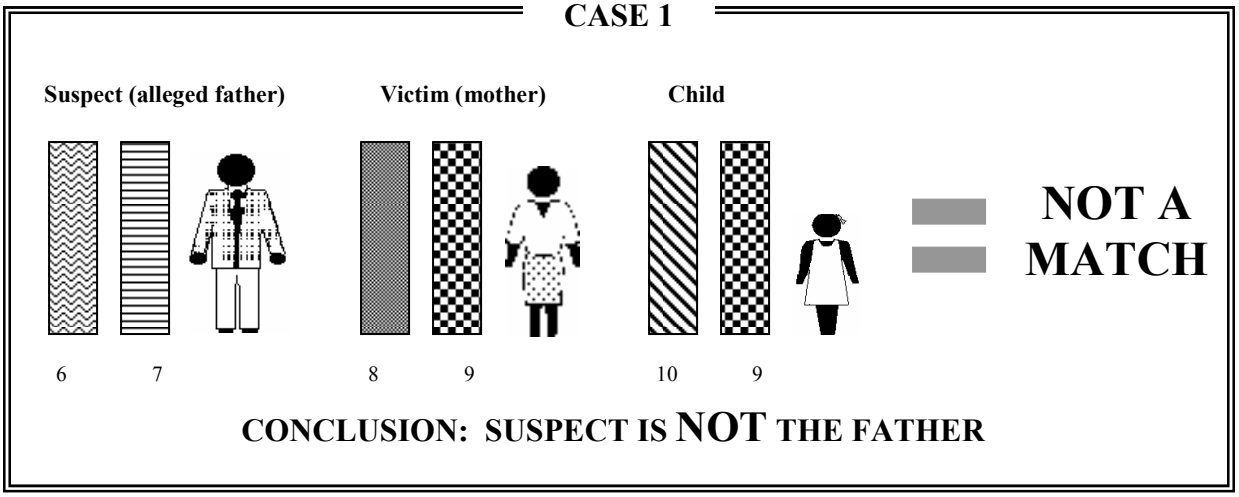
Neither physical-based methods nor serological methods can match the accuracy of properly conducted DNA analysis.

PART III: PATERNITY TESTING IN SEXUAL ABUSE CASES

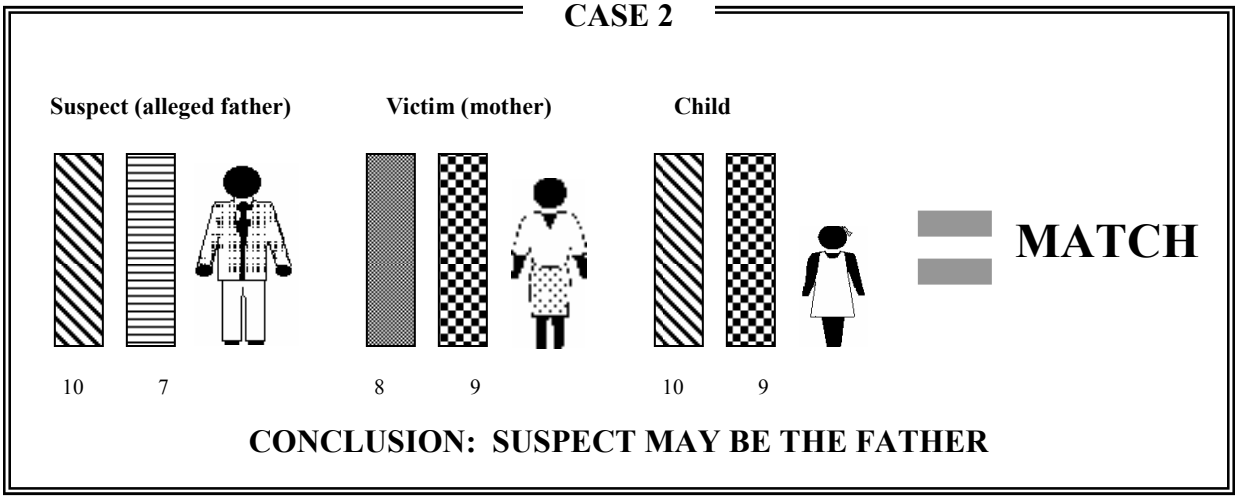
Paternity testing using DNA is also useful in child sexual abuse investigations, particularly those involving statutory rapes and incest. In cases where the victim becomes pregnant, the DNA of resulting child can be compared with that of the suspect. In cases where the victim is a minor, determining statistically that the suspect is the father demonstrates conclusively that he had sexual intercourse with the victim. Regardless of consent, the suspect is guilty of statutory rape. Further, if the suspect is also a relative of the victim, he is guilty of incest as well, which has a significantly stronger sentence.

DNA testing to prove paternity is based on the premise that the DNA of a child is a composite mixture of his parents' DNA. All DNA must come from either one parent or the other. To determine paternity, the DNA profile from an individual, such as the child of the victim, is compared with the profile of the mother (the victim) and that of the suspect. DNA profiles are generated using blood or buccal cell samples, following the same laboratory process used for criminal cases. The profiles are then analyzed to determine if there are any mismatches between the father and the child. Many labs, including those in the Philippines, require at least two mismatching locations in order to positively exclude the suspect as the father (Hou et al. 2000).

The premise is illustrated in the following two cases of paternity testing. Patterned bars and numbers represent different DNA types. In **Case 1**, the child has inherited type 9 from her mother; type 10 must therefore come from her father. Type 10 is not observed in the DNA profile of the alleged father, consequently he cannot be the father of the child.



In **Case 2**, the child likewise inherited type 9 from her mother; type 10 must therefore come from her father. Type 10 is observed in the profile of the alleged father, consequently he *may* be the child's father.



As demonstrated above, there are two possible outcomes in paternity testing:

Paternity Exclusion

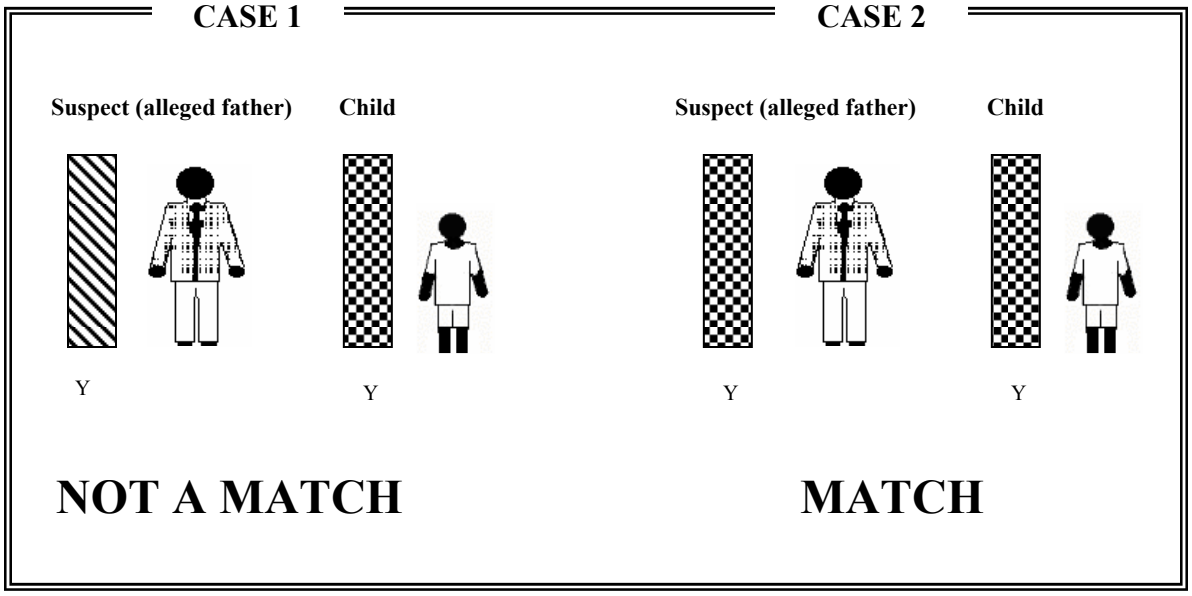
Non-matching profiles in at least 2 two DNA locations constitute conclusive proof that the suspect is not the biological father of the child in question. This outcome, however, does not necessarily exonerate the suspect from abuse charges; results support that the suspect did not impregnate the victim but say nothing regarding whether abuse occurred at other time.

Paternity Inclusion

Unlike exclusions, complete matches between the DNA profile of the child and the suspect do not necessarily establish paternity. As in analysis for criminal cases, the strength of the match must be determined statistically. Using population databases, the rarity of the profile can be determined thus measuring the likelihood of a match occurring by chance.

Analysis of parentage cases is slightly more complex when the mother and the alleged father are closely related, as in cases of incestuous paternity. In these cases, there is greater probability of the child's profile matching to that of the alleged father compared to a random man in the population due to kinship. For example, if the alleged father is also the mother's father, the resulting child may share some of his DNA simply because he is the suspect's grandchild. The introduction of Y chromosomal DNA typing in paternity cases involving male children simplifies the issue of incestuous paternity.

As the name suggests, Y chromosomal DNA typing focuses on STRs found only on the Y chromosome. The Y chromosome determines the "maleness" of an individual since only males possess a Y chromosome together with an X chromosome. Females do not have a Y chromosome but instead possess two X chromosomes. Since a male child inherits his Y chromosome entirely from his father, his DNA profile on that chromosome must be identical to that of his father.



Further, the Y chromosome profile of a father will be identical in all of his sons and his own father, as well as in anyone else paternally related to him. Consequently, when incestuous paternity is suspected in cases involving a male child, Y chromosomal DNA typing should be combined with regular STR analysis. In order to adjust for this statistically, the likelihood ratio must be modified to take into account the blood relationship between the alleged father and the child's mother.

If the baby is a girl, Y chromosomal DNA typing cannot be used; instead, analysts will use autosomal markers and adjust the statistical calculations to reflect the relation of the alleged father and the child's mother. Although STR typing using the X chromosome is available, it is not recommended for incest cases because the statistical calculations required have not yet been finalized.

On the following pages, sample paternity reports illustrate the alleged father having a high probability of paternity and, in the subsequent report, the alleged father being completely excluded.

Sample Paternity Form: Inclusion

DNA Analysis Laboratory
NATURAL SCIENCES RESEARCH INSTITUTE
University of the Philippines
Diliman, Quezon City

Case no:
Nature of DNA Analysis: Paternity Testing
Requesting Party:

Table 1: Details of biological samples for analysis:

Sample	Sample Source	Sample Description	Date Submitted
2004-# A	Name	Blood on FTA™ card	
2004-# B	Name	Blood on FTA™ card	
2004-# C	Name	Blood on FTA™ card	
2004-# D	Name	Blood on FTA™ card	
K562	Commercial	Liquid (Positive DNA control)	

Findings:
Table 2: Result of DNA fragment analysis showing individual genotypes

Loci	Samples			
	K562	**Child**	**Mother**	**Alleged Father**
HUMCSF1P0	9, 10			
D8S306	8, 9			
HUMDHFRP2	7, 8			
HUMF13A01	4, 5			
HUMFES/FPS	10, 12			
HUMFGA	21, 24			
HUMTHO1	9.3, 9.3			
HUMTPOX	8, 9			
HUMvWA	16, 16			

DNA profiles of Child, Mother and Alleged Father

Analysis
Assuming that **Mr. Alleged Father** is equally likely to be the father and not the father of the child prior to the conduct of DNA tests, there is a **Probability of Paternity** of _____% that **Mr. Alleged Father** is the biological father of **Child**. The **Likelihood Ratio** (e.g. LR=100) was calculated using the available genetic database of the Philippine population. This means that the matching DNA profile is ____ times more likely if **Mr. Alleged Father** is **Child's** father than any random man from the population.

Conclusion
Based on the results of DNA analysis, there is a _____% probability that **Mr. Alleged Father** is the biological father of **Child**.

Date reported:

I hereby certify that the results obtained are accurate and the above statement is correct.

Dr. Maria Corazon A. De Ungria
Head
DNA Analysis Laboratory
Date approved:

Document is authenticated by a signature and the DNA Analysis Laboratory dry seal

Sample Paternity Form: Exclusion

DNA Analysis Laboratory
NATURAL SCIENCES RESEARCH INSTITUTE
University of the Philippines
Diliman, Quezon City

Case no:
Nature of DNA Analysis: Paternity Testing
Requesting Party:

Table 1: Details of biological samples for analysis:

Sample	Sample Source	Sample Description	Date Submitted
2004-# A	Name	Blood on FTA™ card	
2004-# B	Name	Blood on FTA™ card	
2004-# C	Name	Blood on FTA™ card	
2004-# D	Name	Blood on FTA™ card	
K562	Commercial	Liquid (Positive DNA control)	

Findings:
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HUMCSF1P0	9, 10			
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HUMF13A01	4, 5			
HUMFES/FPS	10, 12			
HUMFGA	21, 24			
HUMTHO1	9.3, 9.3			
HUMTPOX	8, 9			
HUMvWA	16, 16			

DNA profiles of Child, Mother and Alleged Father

Analysis
It is assumed that prior to the conduct of DNA analysis there were equal chances of **Mr. Alleged Father** being or not being the father of **Child**. Conduct of DNA tests show that the DNA profiles of **Mr. Alleged Father** and **Child** do not match at the (*insert names of mismatching loci*). **Mr. Alleged Father** is therefore **excluded** from being the father of **Child**.

Conclusion
Based on the results of DNA analysis, there is no possibility that **Mr. Alleged Father** is the biological father of **Child**.

Date reported:

I hereby certify that the results obtained are accurate and the above statement is correct.

Dr. Maria Corazon A. De Ungria
Head
DNA Analysis Laboratory
Date approved:

Document is authenticated by a signature and the DNA Analysis Laboratory dry seal

PART IV: DNA EVIDENCE IN THE COURTS

Forensic DNA is powerful and compelling evidence in criminal cases. When properly collected and handled, it is an unbiased tool in the reconstruction of the sequence of events surrounding a crime. Objective evidence of this kind is especially useful in crimes of a highly sensitive nature, such as child sexual abuse. For example, children finding it difficult to name their offenders need not be subjected to repetitive interviews or extensive cross-examination since the detection of male DNA in a child's genitalia is already conclusive evidence that rape occurred.

The Supreme Court of the Philippines has already expressed its confidence in the value and admissibility of DNA in 2001. In *Tijing v. Court of Appeals* (G.R. No. 125901, March 8, 2001) the Court stated the following:

> **"Fortunately, we have now the facility and expertise in using [the] DNA test for identification and parentage testing…As the appropriate case comes, courts should not hesitate to rule on the admissibility of DNA evidence. For it was said, that courts should apply the results of science when competently obtained in aid of situations presented, since to reject it is to deny progress."**

It did not take long for the "appropriate case" to arrive. The following year, in People v. Vallejo (G.R. No. 144656), the Supreme Court used DNA evidence to affirm the decision of the trial court finding the accused guilty of rape with homicide. The National Bureau of Investigation obtained the DNA evidence from buccal swabs and hair samples taken from the accused, and vaginal swabs taken from the victim during autopsy. The NBI forensic chemist testified that the vaginal swabs from the victim contained the DNA profiles of both the accused and the victim. The Court admitted the DNA evidence as **corroborative evidence** which, together with the other evidence, indicated the guilt of the accused.

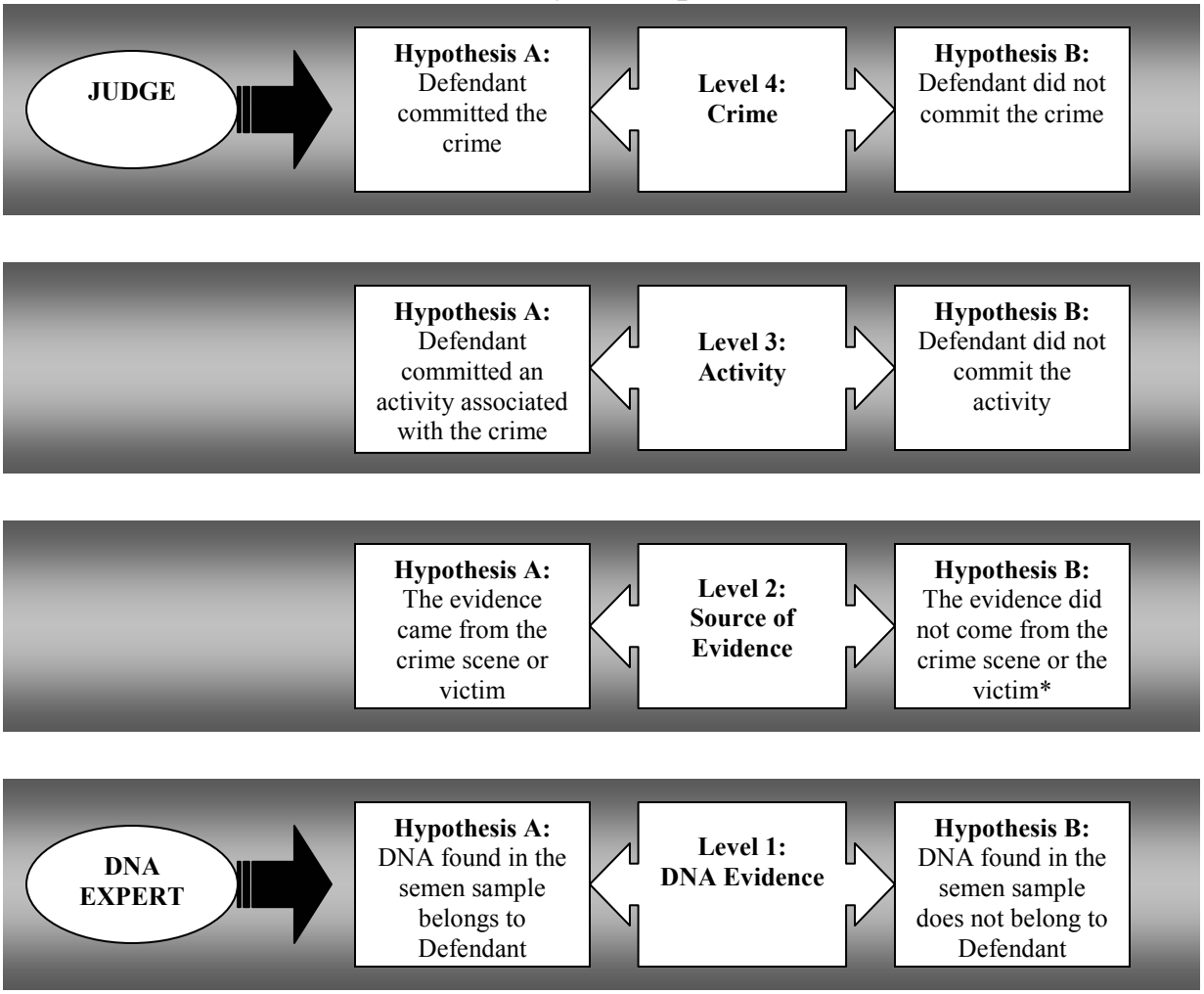
DNA as Evidence

DNA evidence *by itself* cannot establish the guilt or innocence of an individual. It cannot prove definitively that the suspect actually committed the crime. Although it is often powerful corroborative evidence, DNA is just one of several factors that will suggest the guilt or innocence of the suspect. It is the duty of the judge to weigh all of the evidence together, including DNA, and arrive at a verdict.

To better explain this concept, consider the **hierarchy of propositions**, a method for the interpretation of scientific evidence in which two competing hypotheses are evaluated in order to come to an overall conclusion. At each level, the decision-maker must decide which of the two explanations is the truth. The top level (*Level 4*) of the hierarchy represents the actual commission of the crime, the suspect's guilt or innocence. This final decision is the sole provenance of the judge; she has the obligation to determine whether all of the evidence presented, including DNA, is enough to confirm that the defendant committed the crime.

The other three levels (*Level 3*, *Level 2*, and *Level 1*) all represent evidence the judge must consider when arriving at her conclusion. While expert witnesses can testify on the particular evidence presented, they cannot offer an opinion on the overall guilt or innocence of the defendant (*Level 4*). For example, a DNA expert can only testify on the likelihood that the DNA sample tested belongs to the defendant; **she cannot testify on the defendant's guilt or innocence**.

Hierarchy of Propositions



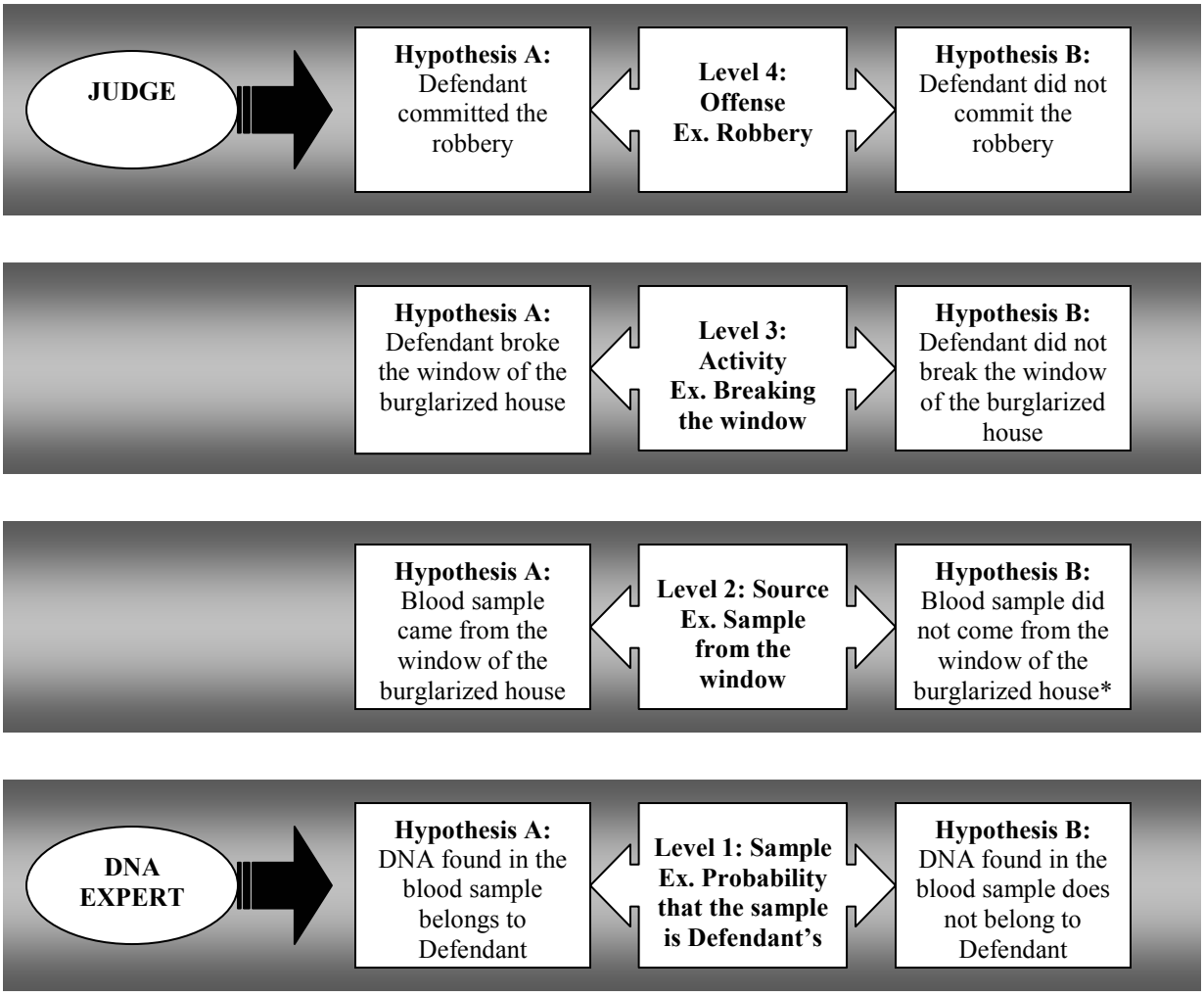
*Chain of custody was broken

The following examples further clarify the role of the judge and compare it with that of the DNA expert.

Case #1: Robbery

A house has been robbed. Police investigators collect a blood sample from the house's broken front window, believing the blood stain may belong to the burglar. The **DNA expert** will *only* testify about the DNA found within the blood sample she was given. Other expert witnesses may testify about different aspects of the case, such as the collection of the blood sample, and the chain of custody for the evidence collected at the scene. None of these experts can state definitively that the defendant is guilt of the robbery, however. It is the responsibility of the **judge** to consider all of the evidence and testimony, and reach an independent verdict.

Case #1: Robbery

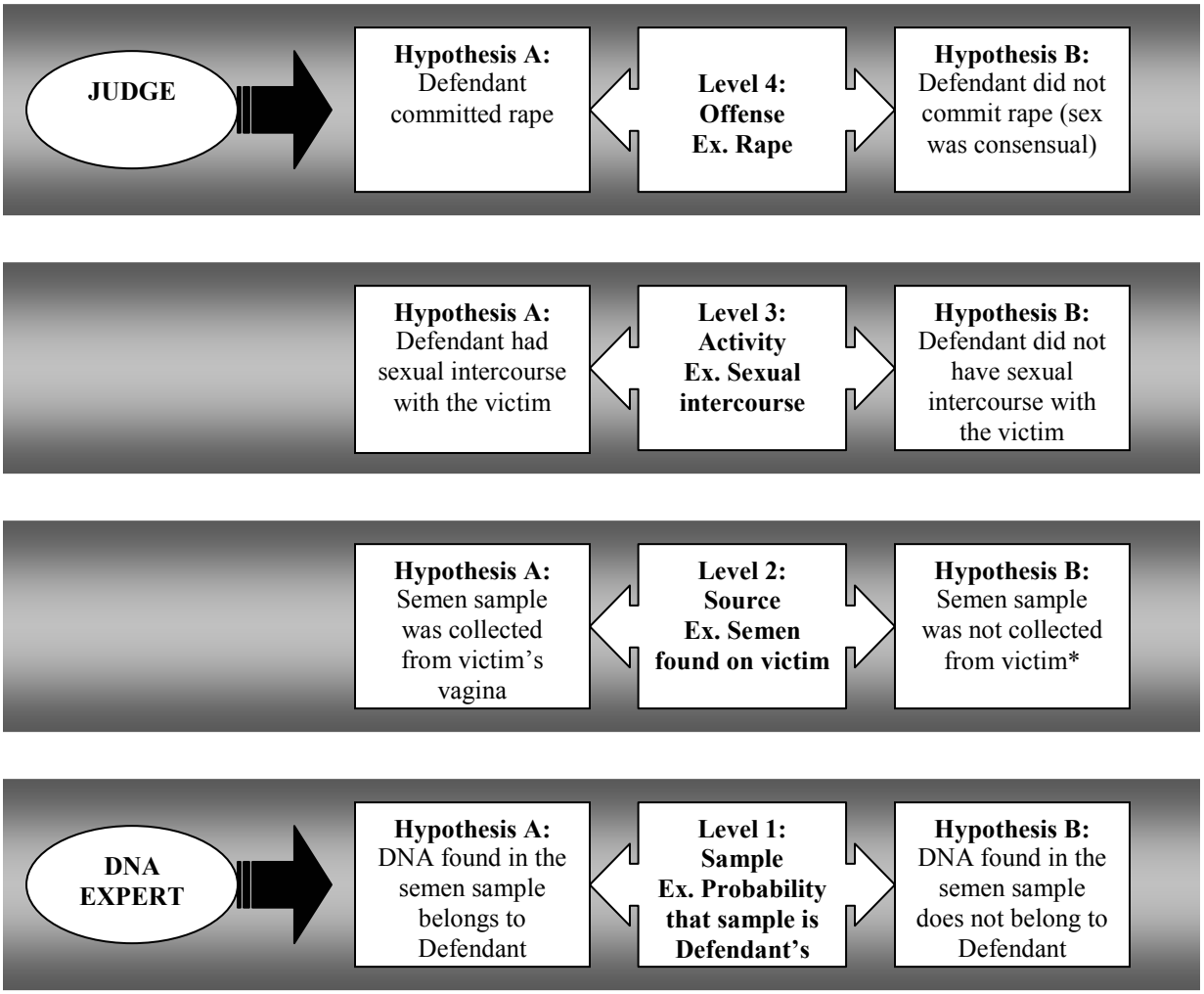


*Chain of custody was broken

Case #2: Adult Rape

A woman claims that she was raped by an acquaintance but the acquaintance insists that the sexual intercourse was consensual. The victim goes to the emergency room immediately after the incident where physicians collect traces of semen during an internal examination and send the sample to the DNA laboratory for analysis. The **DNA expert** can only testify about the DNA found within the semen sample and on the probability that it belongs to the defendant. She cannot offer any opinion on whether the defendant raped the victim; the DNA expert is not in the position to conclude whether the sexual act was consensual or not. The **judge** has the sole responsibility of concluding whether the sexual intercourse was consensual or if rape indeed occurred.

Case #2: Adult Rape

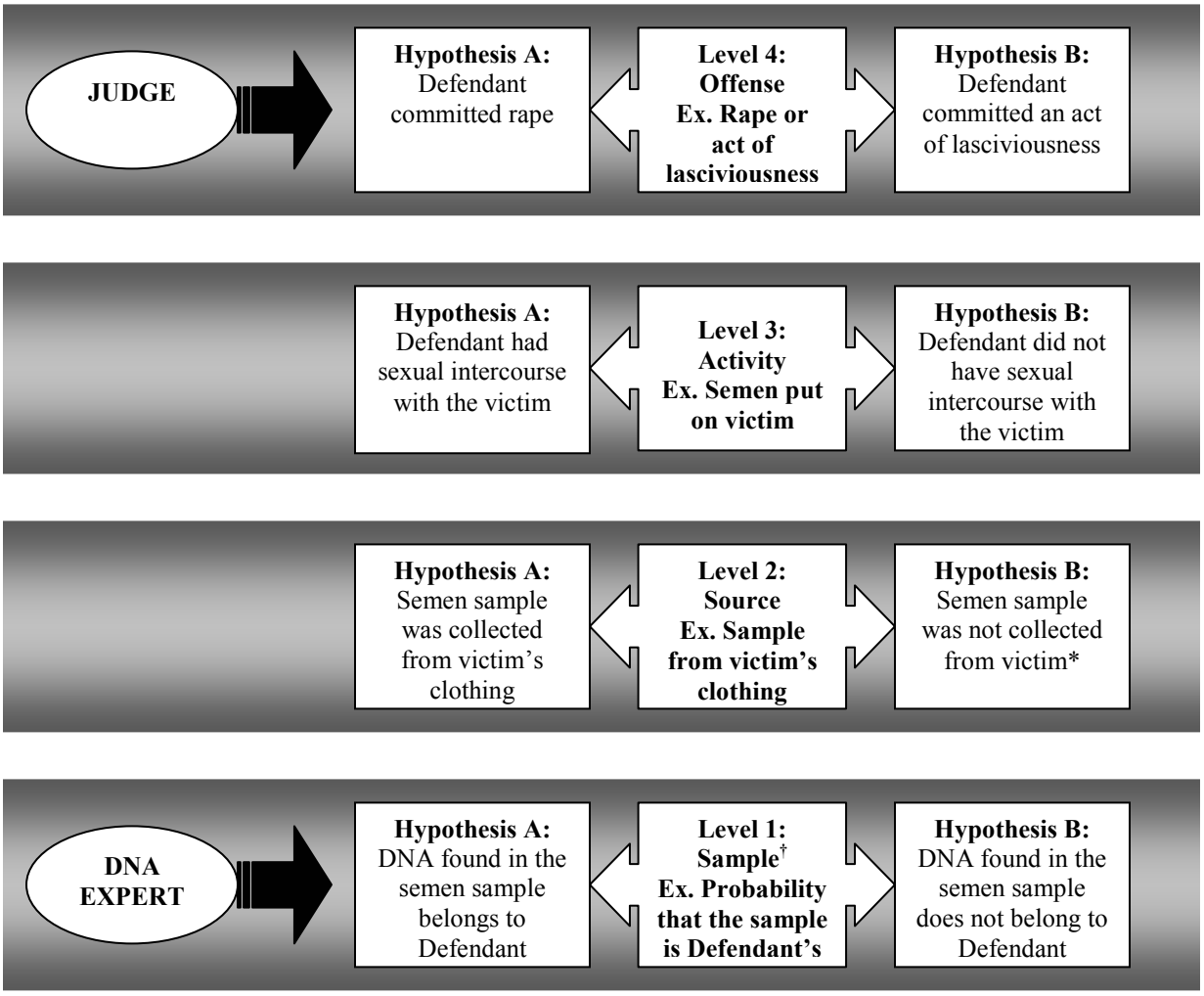


*Chain of custody was broken

Case #3: Child Rape or Lascivious Conduct?

A child has been brought to a child protection unit by her mother who claims the child was raped. The examining physician collects a semen sample from the victim's clothing and submits it to the DNA laboratory for testing. The **DNA expert** will only testify what she has discovered about the DNA found within the semen sample. She cannot state definitively whether the defendant raped the victim or committed an act of lasciviousness. The judge will evaluate all of the evidence and testimony and decide if the defendant is guilty of rape or lascivious conduct, or is innocent of the charges.

Case #3: Child Rape or Lascivious Conduct



*Chain of custody was broken

†Semen found in the child's body is already definitive evidence of child sexual abuse. The issue is what crime has been committed and by whom.

Testimony by DNA Experts

A DNA expert witness is the most appropriate individual to address questions about the DNA forensic process. Typically, the head of the DNA analysis laboratory, or a representative for the head, will testify and will likely be asked questions on the following subjects:

- Professional qualifications and expertise
- Reliability of the DNA testing process
- Reliability of the laboratory itself
- Interpretation of the results of the DNA test

Overall, the DNA expert will merely state the weight of the match (inclusion) or exclude a suspect as possible source of DNA (exclusion). For example, if the random match probability is one in ten million (**1:10,000,000**) in a Philippine population of seventy-five million, then there are approximately seven other people (aside from the suspect) who could possess the DNA profile found in the evidentiary sample. In short, the conclusion of the testimony could be as follows:

> **DNA Expert:** *"The chance that a particular individual, unrelated to the defendant will match the DNA profile from the crime sample is one in ten million. In order to assess the value of these matching DNA profiles, I have considered the following explanations:*
>
> 1. *The DNA in the crime sample originated from the defendant*
> 2. *The DNA in the crime sample originated from an unrelated person in the general population*
>
> *"Having considered these two possibilities, the evidence obtained is ten million times more likely if the crime sample originated from the defendant."*

It still remains the role of the judge to use DNA evidence together with other evidence to evaluate the guilt or innocence of a suspect. The DNA expert cannot make such a determination.

Testimony by Examining Physicians

Physicians performing the medico-legal examinations may testify only on the areas of the DNA forensic process with which they are involved and therefore have personal knowledge. Those areas are likely to relate only to the following:

- Medico-legal examination procedure
- Evidence collection during the examination
- Procedure for using the rape kit
- Physician's involvement in the chain of custody

Unless qualified as an expert on the DNA forensic process, a physician can only testify as an ordinary witness with regard to the DNA evidence. An ordinary witness, unlike an expert witness, cannot testify on matters where he or she does not have personal knowledge.

Physicians should expect that their credibility as witnesses, whether ordinary or expert, will be subject to close scrutiny by legal counsel and the court. The testifying physician must not take offense and should simply respond to questions based on what she actually did or, if she has been qualified as expert, on what she knows to be the best information available to experts in her field.

CONCLUSION

DNA forensic testing could change the face of child abuse investigation in our country. As an evidentiary method, it is reliable, valid and accepted in courts worldwide. Here in the Philippines, it has the potential to markedly enhance our ability to apprehend and convict the guilty, while exonerating those wrongly accused.

Laboratories in the Philippines have the knowledge and capability to analyze DNA evidence as successfully as those of other nations. As with any advance in forensic science, however, DNA analysis is only as strong as the professionals involved in the process. It is vital for physicians who treat child abuse patients to understand their role in the process and to perform that role responsibly and effectively. A protocol for evidence collection and preservation must be established and training should be incorporated into our medical school curriculum. Further, we must heighten awareness in the legal community of DNA's strengths and its limitations, what it can tell us about child sexual abuse and what it cannot. Finally, we must develop and put in place professional standards through a laboratory accreditation program.

Forensic DNA analysis has much to contribute in assisting law enforcement and the courts in the investigation and prosecution of child abuse offenders. LAST SENTENCE.

APPENDICES

APPENDIX A: GLOSSARY

(Adapted from the web site of the Human Genome Project of the U.S. Department of Energy and the National Institutes of Health, http://www.ornl.gov)

Base: One of four chemicals (adenine, thymine, guanine, and cytosine) that comprise the genetic code.

Base Pair: Two complementary nucleotide bases which form a rung of the DNA "ladder" structure. In base pairing, adenine always pairs with thymine, and guanine always pairs with cytosine

Chain of Custody: Documentation process in which every individual who handles a particular piece of evidence is recorded, ensuring that the evidence has not been tampered with or switched.

Chromosome: One of the threadlike "packages" of genes and other DNA in the nucleus of a cell. Humans have 23 pairs of chromosomes, 46 in all. Each parent contributes one chromosome to each pair, so children get half of their chromosomes from their mothers and half from their fathers.

Contamination: When DNA from another source gets mixed with the DNA relevant to the case.

Corroborative Evidence: Evidence that strengthens or confirms other evidence.

Crime Scene Investigation: The investigation of the location of the crime in which trained investigators search for and recover evidence of the crime for later analysis.

Degradation: When DNA evidence is damaged through improper storing and handling, such as exposure to direct sunlight, warm temperatures and moisture.

Deoxyribonucleic Acid (DNA): The chemical inside the nucleus of a cell that carries the genetic instructions for making living organisms.

DNA Database: A genetic database containing the DNA profiles from the members of a specific population. DNA analysts rely on databases to measure how often a particular DNA profile will be found in the general population.

DNA Profile: An individual's unique DNA sequence.

Exclusion: If the DNA profile from the evidence sample does not match the profile of the suspect's reference sample, the suspect is "excluded" as a source of the evidence.

Extraction: A chemical process in which DNA is separated from other cellular material for analysis.

Forensic Evidence: Evidence that is arrived at by scientific means.

Forensics: The branch of law enforcement dealing with the evaluation or interpretation of evidence using scientific or technical facts.

Gene: The functional and physical unit of heredity passed from parent to offspring. Genes are pieces of DNA, and most genes contain the information for making a specific protein.

Inclusion: When the DNA profile of the reference sample matches that of the evidence sample, the individual is "included" as a potential source of the evidentiary DNA.

Likelihood Ratio: Statistical expression of the significance of the profile match. The ratio of the probability that the DNA profile in the evidence sample came from the defendant and the probability that the DNA came from a random, unrelated person.

Mitochondrial DNA: The genetic material of the mitochondria, the organelles that generate energy for the cell. Mitochondrial DNA is inherited solely from the mother.

Mitochondrial DNA Analysis: A technique for DNA analysis that is especially useful when the DNA is highly degraded or when only small sample sizes are available.

Nucleotide: One of the structural components, or building blocks, of DNA. A nucleotide consists of a base (one of four chemicals: adenine, thymine, guanine, and cytosine) plus a molecule of sugar and one of phosphoric acid.

Nuclear DNA: The **DNA** contained in the **chromosomes** within the nucleus of eukaryotic cells.

Polymerase Chain Reaction (PCR): A fast, inexpensive technique for making an unlimited number of copies of any piece of DNA.

Principle of Exchange: Edmund Locard's theory contending that when any two objects come into contact, there is a transfer of material between them.

Random Match Probability: Statistical expression of the significance of the profile match. The probability that an innocent individual, unrelated to the suspect and chosen randomly from the population, will match the DNA profile taken from the crime sample.

Rape Kit: A sexual assault evidence kit that contains materials for collecting and preserving evidence found on a patient's body and clothing.

Reference Sample: A DNA sample of known origin whose profile is compared with that of the DNA evidence in order to determine if any association exists between the two individuals.

Reverse Dot Blot Method: A technique for analyzing DNA in which nucleotide bases in specific parts of a gene are detected using single-stranded DNA probes fixed onto a matrix.

Restriction Fragment Length Polymorphism (RFLP): A technique for DNA analysis in which DNA is cut into fragments by enzymes, sorted according to length and then radioactively tagged to reveal a unique pattern.

Sex Chromosome: One of the two chromosomes that specify an organism's genetic sex. Humans have two kinds of sex chromosomes, X and Y. Normal females possess two X chromosomes and normal males one X and one Y.

Short Tandem Repeat (STR): Short sequence of DNA (normally 2 to 5 base pairs in length) that is repeated numerous times.

STR Analysis: A technique for analyzing DNA in which individuals are distinguished from one another by the number of repeats of STRs within a particular region.

Y Chromosome DNA Typing: A technique for analyzing DNA that focuses on STRs found only on the Y chromosome.

APPENDIX B: CONTACT INFORMATION OF DNA LABORATORIES IN THE PHILIPPINES

The DNA Analysis Laboratory at the Natural Sciences Research Institute of the University of the Philippines, Diliman (UP-NSRI)

The National Bureau of Investigation, Taft Avenue (NBI)

The Philippine National Police Headquarters at Camp Crame (PNP)

St. Luke's Medical Center

APPENDIX C: SAMPLE FORMS FOR SEXUAL ASSAULT EVIDENCE COLLECTION

APPENDIX D: REFERENCES

Butler, J. M. (2001). Forensic DNA Typing: Biology and Technology Behind STR Markers. Academic Press, San Diego.

Christian, C., Lavelle, J.M., De Jong, A.R., Loiselle, J., Brenner, L., and M. Joffee. (2000). Forensic evidence findings in prepubertal victims of sexual assault. *Pediatrics* 106: 100-104.

Delfin, F., Madrid, B.J., and M.C. de Ungria. (2003). Y-STR analysis in child sexual abuse cases in the Philippines. 14th International Conference in Human Identification. Phoenix, Arizona.

De Ungria, M.C.A., Frani, A.M., Magno, M.M.F., Tabbada, K.A., Calacal, G.C., Delfin, F.C., and S.C. Halos. (2002). Evaluating DNA tests of motherless cases using a Philippine genetic database. *Transfusion* 42: 954-957.

Evett, J.W. and B.S. Weir (1998). Interpreting DNA Evidence: Statistical Genetics for Forensic Scientists. Sinauer Associates, Inc., Stinderland.

Halos, S.C., J.C. Chu, A.C.M. Ferreon, and M.M.F. Magno. (1999). Philippine population database at nine microsatellite loci for forensic and paternity applications. *Forensic Science International* 101: 27-32.

Holland, M.M., Fisher, D.L., Mitchell, L.G., Rodriguez, W.C., Canik, J.J., Merril, C.R., and V.W. Weedn. (1993). Mitochondrial DNA sequence and analysis of human skeletal remains: identification of remains from the Vietnam War. *Journal of Forensic Science* 38: 542-553.

Hou, Y., Song, Z., Wu, J., and J. Zhang. (2000). How many STR markers are enough for paternity identification? *Progressive Forensic Genetics* 8: 384-386.

Morrenssens, A., Starrs, J.E., Henderson, C.E., and F.E. Inbau. (1995). Scientific Evidence in Civil and Criminal Cases, 4th Edition. The Foundation Press, Inc., New York.

Padilla, C.D., Santos Ocampo, P.D., and Q. Kintanar. (2004). Human Genome: Perspectives and Applications. The National Academy of Science and Technology, Manila.

Tabbada, K.A., Magno, M.M.F., De Ungria, M.C.A., and S.C. Halos. (2001). Philippine population database at STR locus FGA for forensic applications. 23rd Annual Scientific Meeting of the National Academy of Science and Technology. Manila, Philippines.

Takahashi, M., Kato, Y., Manaka, K., Mukoyama, H., Miyakawa, G., and S. Kamiyama. (1995). Human cell line identification based on D1S80 and HLA DQα genotyping by PCR amplification of alleles. *Tissue Culture Research Communication* 14: 103-107.