Chapter 2
Forensic Biology: Serology and DNA

Lisa Gefrides, MS, FABC and Katie Welch, MS, FABC

Contents

L. Gefrides (✉)
DNA Analyst I Harris County Medical Examiner's Office, 1885, Old Spanish Trail,
Houston, TX 77054, USA
e-mail: lisa.gefrides@meo.hctx.net

K. Welch
DNA Analyst I Harris County Medical Examiner's Office, Forensic Biology Laboratory,
1885, Old Spanish Trail, Houston, TX 77054, USA
e-mail: skwelch17@sbcflobal.net

2.1   Introduction

In the forensic community, serology and DNA analyses are closely related. In fact, in many laboratories they are included within the same unit, collectively titled Forensic Biology. In the forensic laboratory, serology analysis refers to the screening of evidence for body fluids while DNA analysis refers to the efforts to individualize body fluids to a specific person. In most cases, body fluid identification is performed on evidentiary items before DNA analysis is attempted. Depending on the qualifications of laboratory personnel, analysts can be trained to perform either serology or DNA analysis or can be trained in both disciplines. While serology procedures have been employed for most of the twentieth century and the techniques have essentially remained unchanged, DNA has emerged in the forensic realm within the last two decades and its applications and technology are continuously developing.

2.2   Types of Evidence Examined

The types of evidence submitted to crime laboratories for serology/DNA analysis are those items on which body fluids are thought to be present. A large majority of DNA/serology cases involve sexual assaults. Evidence from these types of cases commonly includes sexual assault kits, complainant clothing, bedding, and, sometimes, suspect clothing. Other common case submissions include potential blood evidence from homicides, aggravated assaults, and burglaries. Items commonly submitted for blood testing include swabbings from crime scenes, clothing, weapons, or any number of other items that may possess bloodstains. If an item is small, it can be submitted to

the laboratory in its entirety. For larger items, stains can either be collected on a sterile cotton swab or a cutting from the item can be taken for submission.

It is also possible to collect items that have been in contact with an individual's mouth, such as cigarette butts, drinking cans, cups, bottles, gum, candy, toothbrushes, or ski masks. These items usually provide enough DNA for a profile to be established. Objects that have been touched or handled, such as a steering wheel, gun, phone, or even a fingerprint, may also contain biological evidence, which can be collected for analysis but may not always produce a DNA profile. Generally, these pieces of evidence do not contain a substantial amount of biological material and are processed for DNA without going through any type of serological screening to maximize the amount of sample available for DNA testing.

While the majority of cases processed for DNA testing involve violent crimes, more and more law enforcement agencies are tapping into the potential of DNA to assist in solving property crimes. Biological evidence is often left at burglary and theft scenes by the perpetrator. For example, a burglar may be injured breaking a window and leave blood at the crime scene, which can then be processed for DNA. In addition, DNA can be obtained from fingerprints or clothing left behind by a suspect. Historically, evidence from property crimes was not collected or processed by the laboratory due to the significant backlogs of violent crime cases, which take precedence. In recent years, the increased sensitivity of DNA testing and the decrease of violent crime backlogs has prompted some jurisdictions to focus efforts on using DNA to help solve these property crimes. Results of a recent study conducted by the National Institute of Justice indicate that twice as many property crime suspects were identified and arrested when DNA evidence was collected as when it was not [1].

Cases involving kinship determination do not require serology screening and can be sent immediately for DNA analysis. Most often, DNA profile comparisons to determine kinship are used for cases of criminal paternity, child abandonment, or remains identification. All of these cases rely on the comparison of known DNA profiles from individuals to determine whether two people are related, instead of the comparison of evidence to a known profile to determine the source of the biological fluid on a piece of evidence.

Reference samples from known individuals are used for kinship determination and also for comparison with evidentiary samples. Many types of reference samples are available to the forensic biologist. Typically, blood or saliva is collected from a living individual to serve as a reference sample. Blood is collected intravenously and stored in a purple- or lavender-top blood tube, which contains EDTA, an additive to prevent DNA from becoming degraded. The blood is then placed onto a filter paper card, dried, and stored. Blood samples dried in this manner are stable for many years even at room temperature. Saliva samples can be collected either by chewing sterile gauze, depositing saliva onto a collection card, or epithelial cells can be collected by swabbing the inside of a person's cheeks (buccal [buck' ul] swabs). Pulled hairs can also be used as a reference sample but are not as abundant a source of DNA and so are not preferred. Reference samples can also be collected from deceased individuals in the form of blood, tissue samples, or bone samples, depending on the state of decomposition of the remains.

2.3   Planning the Examination

The real challenge in evidence screening is determining which items of evidence should be processed and the most effective way in which to process them. In general, probative samples are those in which a transfer of body fluids, and therefore DNA, has occurred. Generally, a suspect's body fluid on a complainant's body or clothing, or a complainant's body fluid present on clothing or items belonging to a suspect are the objects that hold the most evidentiary, or probative, value. For some cases, the most logical course of evidence examination is rather obvious. For example, in most cases of sexual assault, the identification of semen is central to supporting a claim of sexual assault. Furthermore, semen found on swabs in a sexual assault kit may have more probative value than semen found on clothing or bedding because, along with demonstrating the presence of semen on the complainant, semen can only survive inside a victim for a finite amount of time, whereas semen stains on clothing or bedding can have a much longer duration depending on whether the evidence is washed. For these cases, a determination can be readily made for the type of testing to perform and the most efficient order in which to process the items. Other cases are less obvious. If a sexual assault is oral, digital, or utilizing a foreign object, then it is useful to determine the details associated with the alleged assault to process the evidence most effectively. In these sexual assault cases, examining an item for the presence of semen may have no evidentiary value. All cases may be affected by any post-assault activity by the victim such as washing, wiping, eating, drinking, etc. The time between the assault and the examination can be a critical factor in the successful identification of body fluids because the longer the time span, the more evidence that may be lost. In any forensic case, the order of analysis for each test should be planned in advance to lessen the chance of losing evidence for the next test.

Homicide cases are more time-consuming to process than other types of cases because the victim cannot verbally relate any details of the assault. Homicides generally involve many items of evidence that must be analyzed because a determination cannot always be made regarding which evidence has the most value. Thorough crime scene investigation is essential to ensure that probative items in a case are collected and submitted to the laboratory. In cases such as these, communication with law enforcement is necessary to convey important case details to ensure that evidentiary items are processed in the most logical manner.

When evidence is submitted, a determination must be made as to whether that evidence must go through serology screening or whether the evidence can be sent directly for DNA analysis. Generally, all evidence goes through serology screening first. However, cases involving samples with trace amounts of DNA may not benefit from serology screening. Paternity and remains identification cases also do not require any type of serology screening because only reference samples are processed.

Criminal paternity cases involve a sexual assault in which conception occurs. For these sexual assault cases, serology analysis is rarely performed. Instead, DNA analysis can be performed on the conceptus (living or aborted) and the alleged

father to establish or disprove parentage (paternity testing). While it is not necessary to have a reference sample from the mother/complainant for paternity testing, having the DNA profiles of the offspring and both parents facilitates the DNA interpretation. Maternity/paternity testing can also be used in cases of child abandonment to establish whether a suspected individual is a parent by comparing the profiles obtained from the child and alleged parent(s).

Comparing DNA profiles to test for kinship is also useful for remains identification and missing person cases. DNA can be collected from the blood or tissue of a decedent or from skeletal remains and compared with the blood or saliva from a potential relative. In cases where there is a suspected identity for a set of remains, the profile of the deceased can be directly compared with a family member for confirmation. In some cases, investigators may have no idea of the identity of the deceased individual. Unidentified remains can be analyzed and DNA profiles placed into a database of missing persons. Relatives of individuals who are missing can also be placed into this database to be searched against unidentified remains with the hope of establishing a relationship. Depending on the type of DNA analysis performed, kinship can be established from immediate family members to aunts, uncles, cousins, or possibly even more distant relatives.

2.4 Evidence Processing, Note Taking, and Report Writing

Most of the evidence processing and note taking occurs during serology analysis because this is usually the first time evidence is opened in the laboratory. Serologists are responsible for documenting the type, quantity, and packaging of the evidence received. In addition, a description of the evidence with notes and diagrams or pictures regarding the types of stains present and their location on each item is placed into the case file. Serologists also take detailed notes of their testing and outcomes, and this documentation is referenced during an analyst's testimony during criminal proceedings. Thorough and precise note taking is essential because there may be a substantial amount of time between the completion of case analysis and an analyst's testimony in court. It is also important in circumstances in which a different analyst must interpret the case notes.

Reports are written copies of an analyst's findings and should be an accurate representation of the results as the analyst would testify during criminal proceedings. Results should be conservatively stated and should take into account guidelines established by the forensic community and accrediting agencies. Reporting statements should also take into account the individuals who will be receiving the results. Police officers, attorneys for both parties, and jurors may find the scientific principles behind serology and DNA analyses difficult to interpret. For this reason, reporting statements should be clearly written and in laymen's terms whenever possible.

The paperwork associated with an individual case, such as the analyst's case notes, photographs, data, results, and reports are maintained in a case file. Historically,

case files contain hard copies of all documents associated with a case. Electronic Laboratory Information Management Systems (LIMS) may also be used to store case information. Electronic LIMS systems provide for the electronic input and storage of case data so that paper copies do not need to be maintained except for court purposes. Most LIMS systems can also track the chain of custody of evidence items through a system of barcodes. Each evidence item is assigned a separate barcode and each evidence transfer to a person or a storage location can be tracked by scanning the barcode thus replacing manual written copies of chain of custody on paper. Electronic systems are helping laboratories maintain paperless case files in order to ease overcrowded file rooms. Analysts have the option of printing hard copies of any document to take to court or to provide to attorneys upon request.

2.5 Serology Testing

Serology methods are relatively simple and straightforward. Forensic serology is not to be confused with conventional serology, which deals solely with serum and its properties. Instead, forensic serology involves the identification of different types of body fluids. The identification of biological fluids during serology analysis is accomplished through presumptive and confirmatory testing. Presumptive testing refers to testing that is sensitive, fairly specific to the body fluid in question, and can be performed quickly. It allows an analyst to narrow down the number of items or areas of an item to focus on for further testing. Presumptive testing can only indicate that a body fluid *might* be present on an item. It is not considered specific enough to state that a particular body fluid is unequivocally present on an item because other substances may also produce a positive test result, known as a "false positive." The limitations and types of false-positive reactions will be discussed for each particular presumptive test in the following sections.

Confirmatory testing is specific to the body fluid in question and sometimes also to a particular species. Confirmatory testing is still sensitive, but the time required for the testing can be much longer than that required for presumptive testing. In some instances, DNA analysis can be considered a type of confirmatory test because it is species, although not body fluid, specific for human DNA. Confirmatory testing is discussed in more detail in Sects. 2.5.4 and 2.5.5.

2.5.1 Identification of Semen

The identification of semen is important in many cases of alleged sexual assault. Semen is a body fluid produced by male individuals for fertilization. For forensic purposes, the composition of semen can be simplified into two components: seminal fluid and spermatozoa. Seminal fluid is a protein-rich body fluid originating primarily from the prostate and seminal vesicles.

Spermatozoa, commonly referred to as "sperm," are the male gametes, or sex cells, produced in the testis. Not all men produce spermatozoa. In men who have had a vasectomy, certain birth defects, or as the result of some diseases, seminal fluid will either not contain spermatozoa or contain very few. Therefore, it is useful to be able to forensically test for the presence of both seminal fluid and spermatozoa.

2.5.2   Acid Phosphatase Screening

The most commonly used presumptive test for the detection of seminal fluid relies on the identification of an enzyme known as acid phosphatase (AP). The acid phosphatase that is present in seminal fluid originates in the prostate. Body fluids such as blood, saliva, urine, vaginal secretions, and other fluids also contain acid phosphatase. However, the amount of AP in seminal fluid is greater than that found in other tissues. This property makes AP important for the screening of seminal fluid. The detection of acid phosphatase is only considered a presumptive identification of seminal fluid because other body fluids might also give a positive reaction; therefore, a positive test result indicates the possible presence of seminal fluid but its actual presence must be confirmed by other testing methods.

Acid phosphatase is identified in most forensic laboratories by using the Brentamine spot test, which commonly employs the chemicals -napthyl phosphate and diazo blue dye in a buffered solution. When these chemicals are placed onto an item where seminal fluid is present, the tested area quickly changes to a purple color. It is not advisable to test evidentiary items directly because of the possibility of contamination and also because the chemicals used in AP detection may interfere with subsequent analysis. Instead, a small portion of the stain is either cut from the item or some of the stain is transferred to sterile filter paper or a sterile cotton swab for testing. The speed and intensity of the color change reaction can be used to determine if the stain in question is seminal fluid. A rapid color change with intense coloration strongly indicates that a stain is seminal fluid. A slow and weak color change may indicate either a small amount of seminal fluid or the presence of a different body fluid containing acid phosphatase. Because the test for acid phosphatase is very sensitive, a lack of color change may indicate that no seminal fluid is present; however, it may also indicate that the level of AP is below the detection limit of the test. There have been instances in which spermatozoa were found from a stain negative for AP. For this reason, both negative and positive results must be interpreted with caution. Further testing may be required to confirm the presence or absence of semen.

2.5.3   Alternate Light Source or Ultraviolet (UV) Light

It would be time consuming, costly, and tedious to test large items in their entirety for the presence of AP using the Brentamine test. Instead, large items are visually examined and stained areas are identified and tested. Unfortunately, not all semen

stains are visible to the naked eye, depending on the amount of semen deposited and the fabric on which the deposition was made. To enable the laboratory analyst to identify these non-visible stained areas, a method utilizing an alternate light source is applied to pre-screen evidence in an effort to identify discrete areas for AP testing. Many body fluids fluoresce when excited with light at 450-nm wavelength. Semen stains have the tendency to fluoresce more intensely than most other body fluids. In this way, fluorescing areas of an item can be identified and tested for AP. Alternate light screening works well on light-colored fabrics but dark-colored and coarse fabrics are notoriously difficult to examine under visible or alternate light.

2.5.4   Microscopic Identification of Spermatozoa

Items that have tested presumptively positive for seminal fluid using the AP test can be confirmed either by microscopic detection of spermatozoa or chemical detection of a semen-specific protein (Sect. 2.5.2). Positive swabs or a small cutting from a positive stain can be smeared onto a microscope slide and then stained for visualization. Two common stains used for visualization of spermatozoa are nuclear fast red (red stain) and picroindigocarmine (green stain) and are sometimes referred to as the Christmas tree stain because of the red–green color combination. Once stained, epithelial cells (a group of cells such as skin cells and cells that line body orifices) and spermatozoa have a specific appearance (refer to Fig. 2.1).
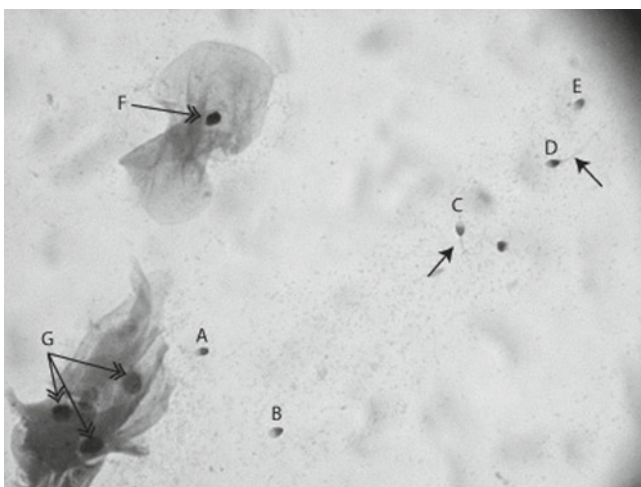
Fig. 2.1 A laboratory analyst can identify spermatozoa (A–E) to confirm the presence of semen on an item. The presence of spermatozoa (C–D) with tails (*arrows*) indicates that the semen may be of relatively recent deposition. Nuclear DNA resides in the spermatozoa heads (A–E) and the nuclei of the epithelial cells (F and G). Leica DM LS2 Microscope at 400×

The nucleus of an epithelial cell will turn red while the cytoplasm takes on a light green or blue appearance. The heads of spermatozoa will turn red with a lighter or white tip while the tail, if present, will turn blue-green.

Microscopically identifying spermatozoa is an absolute indicator that semen is present on an item. It is also useful because the relative quantity of spermatozoa and epithelial cells can be assessed. This determination becomes important during subsequent DNA analysis because spermatozoa contain male DNA while most epithelial cells in a male–female sexual assault will contain female DNA from the complainant. The drawbacks to using microscopy for spermatozoa identification are that it can be more time consuming than the protein confirmation method described below and that it is not necessarily specific to human spermatozoa. Automatic sperm detection is one way to decrease the amount of time analysts spend searching for sperm under the microscope. Automated imaging systems scan a microscope slide using specific search algorithms and recognize sperm cells based on their morphology. At this time, the use of such systems still requires human confirmation that the recognized cells are actually sperm cells.

2.5.5   *Protein Confirmation of Semen*

Not all cases where seminal fluid is identified can be confirmed with microscopy. If the semen belongs to a male who is vasectomized, or a male with a congenital or other defect of the male reproductive system, spermatozoa may not be present in the semen. In cases such as these, it is useful to have another method to confirm the presence of seminal fluid. It is possible to test for the presence of a protein specific to semen known as prostate-specific antigen (PSA), also referred to as p30 in forensic terminology.

Several different methods can be used to confirm the presence of p30 on an item. Traditional p30 detection tests utilize electrophoretic or diffusion methods such as crossover electrophoresis and Ouchterlony double diffusion, or ELISA. Commercial test kits for p30 are also available and have become prevalent in forensic laboratories because of their sensitivity and ease of use. All of these methods require a small cutting of an AP-positive stain to be incubated in water or saline until rehydrated. Afterwards, the liquid is separated from the cutting by centrifugation so that the stain will be retained in a liquid form instead of dried to the cutting. At this point, a portion of the extracted stain can be used to test for the presence of p30.

All of the methods that detect p30 rely on the formation of an antibody–antigen complex. If semen is present in a stain, the binding of p30 to the test antibody produces a visual result. The lack of a result in any of the tests would indicate that the stain does not contain semen or that not enough is present to facilitate a visible reaction. This test can be used alone to confirm semen, or it can be used in conjunction with the microscopic method described in the previous section.

2.6   Identification of Blood

The identification of blood is important in many cases submitted to the crime laboratory for analysis. Blood identification is central to many homicide investigations and is also useful in cases involving aggravated assault, sexual assault, and burglary. The evaluation of blood evidence can be crucial to substantiate a complainant's or suspect's account of alleged events. The presence of blood on evidentiary items can be critical in establishing guilt or innocence during criminal proceedings. The analysis of blood evidence can be important not only in establishing which individual might have been bleeding, but also in the manner in which blood was deposited. Blood spatter interpretation can be valuable in determining how blood was deposited on an item or at a scene, thus making it useful in crime scene reconstruction (Chapter 4). All of these factors can be taken into account during the investigation and prosecution of a crime and may corroborate or refute an individual's account of an assault.

2.6.1   Presumptive Testing for Blood

The presumptive identification of blood relies on the peroxidase activity of the heme group in hemoglobin. Phenolphthalein (PH), tetramethylbenzidine (TMB), leucomalachite green (LMG), and other indicators work by oxidation of the test sample in the presence of hemoglobin to produce a color change reaction. Phenolphthalein is the most commonly used presumptive test for blood and may be used by itself or in concert with other presumptive tests. A positive phenolphthalein result is indicated by a bright pink color that appears typically within ten to fifteen seconds after the test chemicals are added. This test is very sensitive and positive results can be obtained from stains that are barely visible or invisible to the naked eye. One drawback to this presumptive test is the number of substances that can produce false-positive results. Rust, copper and metal salts, salt-treated lumber, potatoes, and horseradish may all cause a positive result with PH. Usually, if one of these substances is present, the reaction time is slower and the color change takes longer to appear. Some laboratories use PH together with TMB in a double presumptive test. TMB, which works in the same manner as PH, turns a blue-green color in the presence of blood. Although TMB is more specific than PH, meaning fewer false-positive results are indicated, it is less sensitive than PH and does not work as well on highly diluted blood stains.

In any case where blood is suspected, the analyst must first determine what areas of an item of evidence may possibly contain blood. While the color change presumptive tests are good indicators for the presence of blood, they are not practical for testing whole items on which no stains are visible. Porous materials that have been stained with blood may absorb some of the blood even if the object has been washed and appears clean. For this reason, the luminol and fluorescein tests are used to indicate nonvisible blood stains. Luminol is a chemical presumptive test

that, instead of producing a color change reaction, causes stained areas to emit light which must be observed under 'black' light. Fluorescein also causes a light reaction but the fluorescence must be observed using an alternate light source. Either luminol or fluorescein can be sprayed onto large surfaces such as walls or floors and the positive areas marked for further testing. Both tests are very sensitive and will indicate bloodstains that may not be visible. Positive areas should be marked and photographed immediately because the light reaction is not permanent and will fade. One disadvantage to these tests is that both can have false-positive reactions. Luminol and fluorescein will react with the same false positives as PH and also with bleach and other cleaning fluids, which may interfere with blood detection on surfaces that have been cleaned. For this reason, fluorescein- or luminol-positive areas should be retested with one of the color change presumptive tests. Another problem with the light-based tests is that they are typically used on very faint stains. Spraying a chemical onto an already weak stain may dilute the stain even further, which could then lessen the chances of obtaining DNA from the sample.

2.6.2   Species Testing of Blood

Species testing of blood is typically accomplished through an antigen–antibody reaction. The Ouchterlony method works by diffusion, where an extract of the suspected blood stain and an antibody are placed opposite each other in a gel medium. As they migrate toward each other, the blood antigens and antibodies attach together to form a precipitate band that is visualized in the gel. Commercially available kits work in the same manner; however, in these, the stain extract is placed on a test card and the result is indicated by a visible band in the test area. The kits are less time consuming than the Ouchterlony test, but some may show cross reactivity with other species besides human and upper primate blood. Ouchterlony can be more widely applied because it can be used to determine whether a stain may have come from a variety of different species, as long as an antiserum has been made to that species. In a typical forensic case, it is only necessary to determine whether a stain is of probable human origin, unless there are indications that animal blood may be present on a sample.

2.6.3   ABO Blood Typing

Prior to the advent of DNA analysis for forensic science, other methods were developed for the comparison of biological fluid stains to individuals. The most common of these is ABO blood group typing. ABO blood typing identifies specific antigens present on the surface of blood cells. Within the population, individuals may have different forms of these antigens producing what is commonly referred to as a person's "blood type." Comparing the blood type obtained from an evidence stain to that of a known individual allows for the determination of whether the individual

could have contributed to the stain. A proportion of individuals known as "secretors" produce similar substances in other body fluids in addition to blood, which enables ABO typing to be performed on all body fluids in such individuals. The main drawback to ABO blood typing is that there are relatively few different ABO blood types throughout the population, making it difficult to individualize crime stains. Nearly 40% of the population has blood type A and another 40% type O. In addition to being much less informative than DNA analysis, ABO typing requires a fairly large amount of sample for accurate testing, much more than is required for current DNA testing procedures. With the development of faster and more accurate DNA methods, most forensic laboratories have given up ABO testing.

2.6.4   Blood Spatter Interpretation

Blood spatter interpretation can be a useful tool during the investigation of a crime. Interpreting bloodstain patterns can yield information on the manner that a bloodstain was deposited. The distance from the impact origin, the object that may have been responsible for the impact, the direction of the impact, the number of impacts (shots, blows, etc.), or the movement of an individual after injury may be determined by studying blood deposition [2]. All of this information can help investigators establish events that may have occurred at a crime scene and also whether an individual's account of an offense can be corroborated.

2.7   Identification of Saliva

The detection of saliva can be a useful tool in many types of criminal cases, although saliva testing is not requested as often as testing for semen or blood. While presumptive tests are available that can be used to indicate saliva, they have many limitations. Of the forensic laboratories that perform presumptive testing for saliva, the detection of amylase, an enzyme found at high levels in saliva, is currently the most widely utilized method.

Amylase is found in a variety of body fluids, saliva, blood, urine, sweat, tears, semen, breast milk, feces, and vaginal secretions [3, 4], but is more concentrated in saliva than in other body fluids. It should be noted that amylase is also found in plants and in some bacteria. In the body, amylase functions to break down starch into smaller molecules. A number of presumptive tests for amylase are available. While some of the presumptive tests are very sensitive for the presence of amylase, none can actually confirm the presence of salivary amylase [5]; as a result, many laboratories forego screening of evidence for saliva when the amount of test sample is limited. Instead, depending on the circumstances surrounding a case, some laboratories opt to save these samples for DNA testing.

Alternate light can also be used to pre-screen clothing and other evidence to identify possible saliva stains. Like seminal fluid, saliva will fluoresce when excited with alternate light. In these instances, it is important for a laboratory analyst to

know the details of an alleged assault so that a determination can be made on the most likely area where saliva might be present. Because many body fluids and other substances can fluoresce, this screening method only identifies areas for further examination using presumptive, confirmatory, or DNA testing.

DNA testing can be thought of as a type of confirmatory test for the presence of saliva and other body fluids on an item because DNA testing is specific to human DNA. However, DNA analysis without initial presumptive or confirmatory testing only indicates that human DNA is present on an item, not from which body fluid the DNA came. Nevertheless, on items that are suspected of having been in contact with a person's mouth (drink containers, bitemark swabbings, cigarettes, envelopes, toothbrushes, partially ingested food), it is logical to expect that saliva might be present and that any DNA obtained from these items might be from that saliva.

2.8 DNA Testing

After evidentiary items have been screened and positive samples identified, DNA analysis can begin. DNA, or deoxyribonucleic acid, is the inherited cellular material that is the blueprint for human development. DNA molecules are found in almost every cell in a person's body, inside each cell's nucleus where it is packaged into 23 pairs of chromosomes. One chromosome from each pair is contributed by an individual's mother and the other by an individual's father. Each person's DNA is unique, except in the case of identical twins. Identical twins will have exactly the same DNA sequence. Fraternal twins' DNA, on the other hand, will not be any more similar than that of regular siblings. Another property of DNA that is important to forensic analysis is that a person's DNA is the same in every cell in that person's body throughout life. While there are rare instances related to cancer, aging, and other cellular events when this statement might not be true, these occurrences rarely affect forensic examinations.

Although each person's DNA is unique, most of the sequence of the DNA molecule is the same for all individuals. Forensic DNA analysis is interested in the small percentage of DNA sequence that is different between people. Because forensic DNA analysis attempts to individualize DNA to a specific person, it would not be useful to look at segments of DNA that are the same across the population. Instead, polymorphic, or highly variable, regions of DNA are targeted for analysis. The various methods by which this is accomplished are discussed in Sects. 2.8.3–2.8.9.

2.8.1 DNA Extraction

The first step in any forensic DNA analysis is the purification of DNA from an item, also called a substrate, on which the DNA is deposited. This process is commonly referred to as DNA extraction. There are a wide variety of DNA extraction techniques, all of which function to (a) separate the cells containing DNA away from the substrate on which they are embedded, (b) lyse, or break open, the cells to release

DNA and other cellular material, and (c) separate the DNA from these cellular components and any inhibitors that might be present in a sample. (Inhibitors are chemicals or other compounds in a sample that might interfere with subsequent DNA analysis.) The goal of DNA extraction is to yield purified DNA in an aqueous, or liquid, solution that can be used in other applications.

Some of the methods used for DNA extraction are better at purifying DNA, increasing maximum DNA yield, decreasing processing times, or a combination of these, depending on which method is used. Different extraction techniques may work better for different types of samples. It is the forensic laboratory's responsibility to find the best DNA extraction technique for each sample type. New techniques are being developed all the time in attempt to make DNA extraction more streamlined with a higher DNA yield (quantity).

Regardless of which type of DNA extraction is being performed, or which type of chemicals are used, all DNA extractions attempted in forensic laboratories must be processed concurrently with an extraction negative control, also known as a reagent blank. A reagent blank is a sample that goes through the extraction process without a substrate being added to it. Its purpose is to monitor for contamination. In this sense, "contamination" refers to the presence of foreign DNA in a sample. Ideally, reagent blanks should never give any DNA result. If DNA is detected in a reagent blank, it can either mean that DNA contamination is present in the chemicals or plastic consumables used for the extraction process or that an event occurred during the extraction process to introduce foreign DNA into the extracts. If this happens, the DNA extraction for all the samples processed with that reagent blank should be repeated from the beginning unless the laboratory can show that the contamination event was isolated to the blank sample only. It is very important that reagent blanks are treated just like every other sample in the reaction process so that they can monitor for contamination most effectively.

In forensic casework, it is not good laboratory practice to consume an entire sample during DNA extraction. Typically, only half of a sample should be processed for each extraction in order to leave enough specimen for retesting. Retesting is important in several instances. First, if the original extraction becomes compromised, either by contamination of the extraction reagents or another event, or the results are inconclusive, then the extraction may need to be repeated by the laboratory. For items for which no DNA profile is obtained, saving a portion of the sample can be important so it can be processed in the future when new technology becomes available. Finally, a portion of each sample should be saved so that the evidence can be retested by another laboratory to confirm findings if requested by the defense.

2.8.2   Differential DNA Extraction

One distinctive type of DNA extraction used in forensic laboratories is commonly referred to as "differential extraction." Semen-positive sexual assault samples are usually swabs from a sexual assault kit, sometimes referred to as intimate samples,

or cuttings from a complainant's clothing or bedding. These types of samples generally involve a mixture of DNA from the perpetrator, in the form of spermatozoa, and the complainant, in the form of epithelial cells. Because these samples contain DNA from more than one source, it is useful to attempt to separate DNA derived from spermatozoa from all other sources of DNA.

Differential extraction relies on the distinction in the physical properties of spermatozoa from other, usually epithelial, cells. Spermatozoa are more robust than other cell types when it comes to the process of DNA extraction. They can withstand higher incubation temperatures for longer periods and remain intact. Using this property, it is possible to perform a two-step incubation in attempt to separate epithelial DNA from spermatozoa DNA. In the first step, the entire sample is incubated for a short period of time under less stringent conditions. Afterwards, the sample is centrifuged to separate lysed epithelial cells, which remain in the aqueous solution, from unlysed spermatozoa, which pellet at the bottom of the tube. The aqueous solution, now called the non-sperm or epithelial fraction, can then be removed to another tube for further processing while the spermatozoa pellet can be resuspended and digested under more stringent conditions to release the spermatozoas' DNA.

Differential extractions are by no means exact. In a perfect world, differential extraction results in a pure non-sperm or epithelial cell fraction and a pure sperm cell fraction. Ideally, during subsequent DNA analysis, the non-sperm cell fraction would yield a single DNA profile consistent with the complainant, while the sperm cell fraction would yield a single DNA profile consistent with a male perpetrator. While this sometimes happens, it is not always the case. One of the variables that influences the success of a differential extraction is the amount of spermatozoa in relation to other cells. This distinction is usually made during the microscopic examination of spermatozoa either during semen confirmation or by preparing a slide from the differential extraction products themselves (see Fig. 2.1). This determination can produce valuable information on the best way to process the sample during DNA extraction. Large quantities of the complainant's epithelial DNA may not all be lysed in the first incubation step and the unlysed cells may pellet with the spermatozoa to introduce complainant DNA into the sperm cell fraction. Another factor that may influence a differential extraction is the presence of perpetrator DNA in a form other than spermatozoa. It is possible for a perpetrator's blood cells or epithelial cells (from skin, saliva, or seminal fluid) to be present in a sample. These cells would become a part of the epithelial cell fraction and may cause the perpetrator's profile to be observed in the non-sperm cell fraction.

Even though differential extraction is not always precise, it is still worth performing because it allows at least a partial separation of spermatozoa from other cells. Researchers are currently working on other methods to more reliably separate spermatozoa from other cells. Some of these techniques involve passing the DNA through a filter to separate spermatozoa from other cells based on cell size (epithelial cells are large in relation to spermatozoa), or binding the spermatozoa to a membrane using antibodies. Also, a new method of DNA analysis has been developed in the last few years that is specific to male DNA, called Y-STR analysis (see Sect. 2.8.7)

that can give additional information on samples with small amounts of male DNA or samples that are a mixture of male and female DNA.

2.8.3   DNA Quantification

After evidentiary samples and comparison reference samples have been extracted, the amount of DNA in each sample must be measured in a process known as DNA quantification. It is important for subsequent steps to determine the amount of DNA in each sample. The DNA analysis techniques currently being used in most laboratories, such as short tandem repeat (STR) analysis, mitochondrial DNA (mtDNA) sequencing, and Y-STR, all require very precise amounts of DNA for processing.

The most popular technique available in forensic laboratories for DNA quantification involves a process known as real-time polymerase chain reaction (PCR). The polymerase chain reaction (PCR) is a way to amplify (copy) specific regions on the DNA strand. Real-time PCR is a way to monitor the amplification process as it occurs. Commercially available kits use real-time PCR to quantify the *amplifiable* human DNA in a sample [6–8]. The probes used in the real-time procedure are human specific, but some may also react to upper-primate DNA if present in a sample. Except in cases where a biological fluid of an upper-primate may be present in a sample, these quantification methods can be considered a reliable estimate of human DNA in a sample.

Real-time PCR, when applied to DNA quantification, is useful because instead of only determining the amount of DNA in a sample, this method can also predict how the DNA will respond during subsequent PCR analysis conditions. In this way, the real-time PCR technique identifies potential inhibitors of the PCR reaction (substances in the DNA extract that prevent amplification). Newer quantification kits offer the ability to determine the total amount of human DNA and male-specific DNA in a sample at the same time. Quantifying the amount of male specific DNA in a sample is useful for Y-STR analysis (see Sect. 2.8.7).

2.8.4   Restriction Fragment Length Polymorphism (RFLP) and Early PCR-Based Methods

In the mid to late 1980s, the technique known as Restriction Fragment Length Polymorphism (RFLP) was introduced to forensic science and became the first assay used for forensic DNA analysis. The DNA molecule contains sequences known as variable number of tandem repeat (VNTR) sequences, which are pieces of DNA whose sequence repeats over and over a different number of times in different individuals. These repeating sequences, each up to several hundred bases in length, can be cut out of the DNA strand using restriction enzymes and then isolated and their size determined using the Southern blotting technique. The VNTR

sequences were shown to be highly variable in the population and revolutionized DNA typing in forensic science. RFLP was eventually supplanted by STR analysis as the leading DNA technology in forensic crime laboratories because RFLP requires a large amount of sample to obtain enough DNA for detection and is not suitable for processing very small or degraded samples. It is also very time consuming to perform. A typical case from start to finish could take approximately 8 weeks to complete. In contrast, current STR-DNA analysis can be completed in days.

The PCR-based methods for DNA typing were developed to handle the many crime scene stains that are of limited DNA quantity. PCR is a process that amplifies, or copies, specific regions on the DNA strand to produce enough DNA to examine. This process is accomplished by combining the sample DNA with the enzyme Taq polymerase and human-specific DNA primers, which are short segments of DNA that indicate which area of the DNA should be copied, in a buffered chemical solution. Each sample, including reagent blanks, is placed into a separate tube for analysis. In addition, for each set of amplification reactions, a positive and negative amplification control is processed. A positive control is a DNA sample for which a profile is already established. A negative control is set up just like any other sample but does not contain DNA; instead, the water or buffer used for sample dilutions is added to the amplification reaction. Once all of the samples and controls are prepared, they are placed into a machine known as a thermal cycler. The thermal cycler facilitates PCR by incubating the samples in repetitive cycles of denaturation (unwinding and separating the DNA strands at high temperature), annealing (laying down primers in the target region at the primer specific temperature), and elongation (addition of bases to create a copy at the enzyme-specific temperature). Each cycle increases the amount of target DNA inside the sample tube. The amplification process generally continues for 25 to 40 amplification cycles depending on the manufacturer's established procedure and internal laboratory validation.

The AmpliType HLA DQ Forensic DNA and the AmpliType7 PM PCR Amplification and Typing Kits (formerly supplied by Perkin-Elmer, Foster City, CA) used sequence-based polymorphisms at specific DNA locations instead of the size-based polymorphisms used in RFLP analysis. These PCR-based methods were preferable to RFLP because DNA from very limited sources could be amplified to detectable levels and the time required for processing was greatly reduced. The biggest disadvantage to these PCR-based methods was that the sequential differences at DQ and the polymarker loci showed less variability in populations than the repeating sequences in RFLP, making statistical analysis less discriminating.

2.8.5   Short Tandem Repeat (STR) Analysis

The most widespread method of DNA analysis currently used in crime laboratories is Short Tandem Repeat (STR) analysis. STRs are repetitive sequences of DNA, usually 2–5 base pairs in length. Forensic STR analysis determines the number of

tetranucleotide (four base) or pentanucleotide (five base) repeats at specific locations (loci) on the DNA strand. The numbers of repeats observed at these locations are compiled into what is known as a DNA profile. Profiles from evidence can be compared with profiles from known individuals and conclusions can be drawn regarding whether specific individuals may have contributed to the DNA on evidentiary items.

The STR procedure is similar to RFLP in that it examines repetitive units on the DNA strand, although the repeat units in STR are significantly smaller in size than the VNTR units analyzed in RFLP. STR analysis is also a PCR-based procedure, making it much more sensitive than RFLP. STR analysis has become popular in crime laboratories because of its sensitivity, reduced processing time, and increased statistical discrimination over previous forensic DNA methods. Although each location examined for STR shows less variability than those examined in RFLP typing, the increased number of DNA sites examined during the STR procedure makes it more discerning than RFLP.

After DNA samples have been extracted and quantified, a small amount, approximately 1ng of DNA, is used for the PCR portion of the STR procedure. The PCR procedure is as described in Sect. 2.8.4, except that during each cycle of amplification a fluorescent tag is attached to each new copy of DNA. After the DNA is amplified, the amount and size of the DNA must be determined. This process is accomplished by detecting the fluorescently labeled tags attached to the amplified DNA. First, the DNA is separated by size using electrical current. The amplified DNA is applied either to a polyacrylamide slab gel (gel media sandwiched between two large glass plates) or to a polymer-filled capillary (gel-like medium contained in a long, thin glass capillary). When electrical current is applied to either the slab gel or capillary, the shorter DNA fragments migrate through the gel medium faster than the longer fragments. In this way, DNA fragments can be resolved down to a one base difference in size. After the amplified DNA has been separated by size, each fragment is detected by its fluorescent label. Detection is accomplished either by using the electrophoretic instrument's laser and CCD camera or by using a flatbed scanner with fluorescent detection capability. After the fluorescence has been read, computer software converts the fluorescent information into a format that can be analyzed.

STR systems detect DNA at several different locations on the DNA strand. At each of these locations (loci) a person will have up to two different fragment sizes (alleles) (see Fig. 2.2). Because DNA is packaged into pairs of chromosomes, the occurrence of two alleles is caused when the fragment size at one locus on one chromosome differs from the fragment size of that same locus on the other chromosome (heterozygous). If the sizes of the detected fragments are the same on both chromosomes, then a person will only have one allele at that locus (homozygous).

Once fragment sizes are determined for all of the loci under examination, then a DNA profile can be generated. A DNA profile is a listing of all observed allele sizes at each locus (see Tables 2.1 and 2.2). The DNA profile of an evidentiary sample can then be compared with the DNA profile of a known reference sample (complainant, suspect, witness, or relative). If the evidentiary sample is from a single
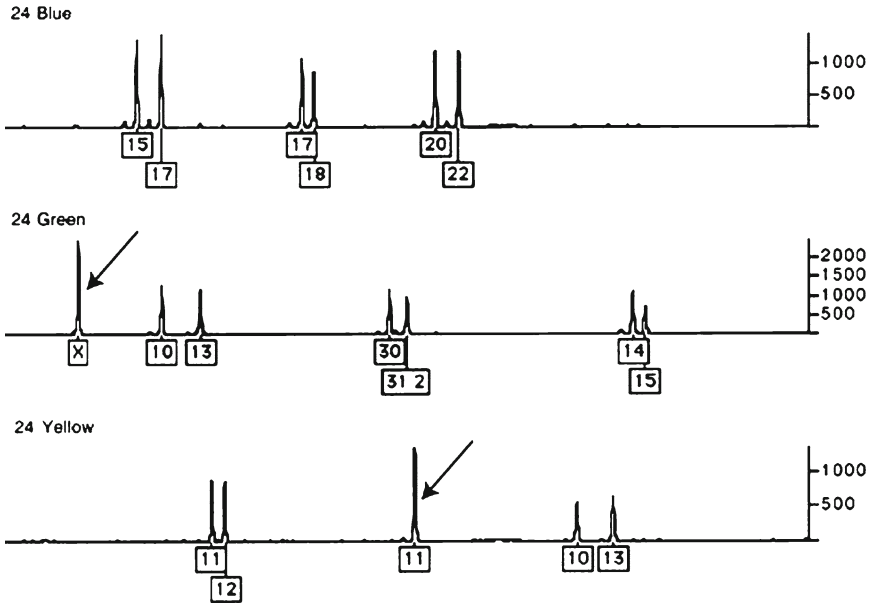
Fig. 2.2 A DNA profile from a single female individual. Loci where only one peak is present are homozygous (arrows). Loci where two peaks are present are heterozygous. The presence of an "X" allele without a "Y" allele indicates that this profile is from a female individual. The numbers indicated below the peaks are the number of repetitive fragments observed at each locus. These numbers are listed as a person's DNA profile

Table 2.1 CODIS case data

Locus	White styrofoam cup	Plastic drinking straw
D3S1358	16	15, 16
VWA	15, 16	15, 16, 18
FGA	22, 23	20, 25
Amelogenin	X, Y	X, Y
D8S1179	11, 12	11, 14, 15
D21S11	27, 28	28, 29
D18S51	14, 20	16, 19
D5S818	11, 12	11
D13S317	12, 13	11, 13
D7S820	8, 11	8, 11
D16S539	10, 13	9, 12
THO1	6, 9	7, 9
TPOX	8, 10	10, 11
CSF1PO	10, 12	8, 11

source and the DNA profiles are the same between an evidence sample and a reference sample, then that individual cannot be excluded as the individual to whom the body fluid belongs.

Table 2.2 Aggravated sexual assault data

Locus	C	S1	S2	V-SP	A-SP
D3S1358	15, 18	16	15	15	15
VWA	18	15, 16	16, 18	16, 18	16, 18
FGA	24, 25	22, 23	20, 25	20, 25	20, 24, 25
Amelogenin	X	X, Y	X, Y	X, Y	X, Y
D8S1179	12, 13	11, 12	14, 15	14, 15	12, 13, 14, 15
D21S11	29, 33.2	27, 28	28, 29	28, 29	28, 29, 33.2
D18S51	15	14, 20	16, 19	16, 19	15, 16, 19
D5S818	10, 13	11, 12	11	11	10, 11, 13
D13S317	11, 14	12, 13	11, 13	11, 13	11, 13, 14
D7S820	8, 10	8, 11	8, 11	8, 11	8, 10, 11
D16S539	11	10, 13	9, 12	9, 12	9, 11, 12
THO1	9.3	6, 9	7, 9	7, 9	7, 9, 9.3
TPOX	8	8, 10	10, 11	10, 11	8, 10, 11
CSF1PO	10, 11	10, 12	8, 11	8, 11	8, 10, 11

C complainant; *S1* suspect 1; *S2* suspect 2; *V* vaginal; *A* anal; *SP* sperm fraction

In forensic science, reporting statements for profiles that are the same between a piece of evidence and an individual rarely use the word "match." Because only representative areas of the DNA molecule are tested in STR analysis, the possibility still exists that if other locations on the DNA strand were tested, the results might be different between the evidence's and individual's DNA profiles. Statistics can be calculated to determine how common the DNA profile of the evidence is in a given population. There are databases for the frequency of alleles at each locus in different populations. The statistical rarity of a profile will be influenced by the number of loci tested and the rarity of the observed alleles at those loci [9]. If the statistics generated for a certain profile meet a specified threshold, then some laboratories may make a reporting statement indicating that a certain individual is the source of an evidentiary stain or body fluid [10].

More than two alleles observed at one or more loci is indicative of a mixture of DNA from more than one individual (Fig. 2.3). Mixtures can arise if body fluids from more than one person are present on a sample, or when more than one individual contributes the same type of body fluid to a stain. Mixtures are observed frequently on sexual assault evidence because the DNA of the complainant in the form of skin cells, sweat, or vaginal secretions and DNA from semen may be present on the same item. Likewise, during homicides or assaults it is possible for more than one person to deposit blood on an item. A profile with more than two alleles at only one locus must be interpreted with caution. In rare instances, it is possible for an individual to have more than two alleles at a single locus, but this exception is identified when a profile is established from an individual's known saliva or blood. When this tri-allelic pattern does occur, it is typically only observed at one locus, where a true mixture will be observed at two or more loci.

Mixture profiles can be difficult to interpret. If there are only two contributors to a DNA mixture and all of their alleles are present at equal intensity with no
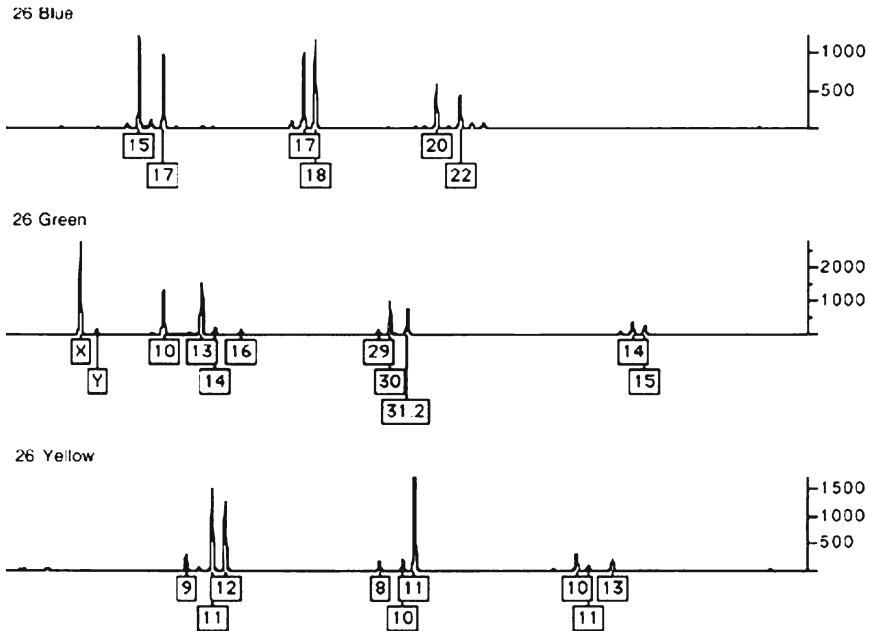
Fig. 2.3 A mixture of DNA from the individuals from Figs. 2.2 and 2.4. The taller peaks at each locus are consistent with the female individual from Fig. 2.2, indicating that more of her DNA is present in the mixture. Not all of the alleles from the male individual from Fig. 2.4 are observed in this profile above the threshold. When not all of the alleles from an individual are evident in a mixture, it makes interpreting the mixture difficult

additional alleles observed, then the interpretation is fairly straightforward. However, if a mixture is from more than two individuals, or if the amounts of DNA from different individuals vary in intensity so that not all of the alleles are observed (Fig. 2.3), mixture interpretation becomes more difficult. In any event, it is not possible to determine with absolute certainty that a specific individual is present in a mixture; instead, it is only possible to conclude that a specific individual might be a contributor. Because mixtures are a combination of alleles from more than one person, it is possible to have more than one combination of profiles that would explain the mixture on an item. As a result, it is difficult to state that a person is represented in a mixture absolutely. Using allelic frequencies, mixture statistics can be calculated to determine the number of unrelated individuals in the population who might be expected to contribute to a certain mixture profile.

While it is helpful to have a reference sample from a developed suspect to be used for DNA comparison, it is not always necessary. The advent of the CODIS database (see Sect. 2.9) makes it possible to process cases without a known suspect. If a presumed perpetrator profile is developed, the profile can be entered into the CODIS database and compared with evidentiary samples from other cases and with convicted offender samples. In some cases, even mixture profiles can be entered into the CODIS database. Success in using the CODIS database to help

resolve crimes has produced an incentive to work old, unprocessed, or cold cases. Cases that were not processed for DNA because the technology was not available at the time, or the available technology was not sensitive enough, or a suspect sample was not available for comparison, can now be worked in an attempt to identify a suspect using the CODIS database.

2.8.6 Mitochondrial DNA (mtDNA) Sequencing

What many people do not realize is that there is more than one type of DNA in a cell. All of the discussion to this point in this chapter has been in reference to "nuclear DNA," which is specific to an individual (refer to Sect. 2.8). The second and less commonly discussed type of DNA in a cell is mitochondrial DNA (mtDNA). Mitochondria are very small organelles found outside the cell nucleus within a cell's cytoplasm. Each mitochondrion has its own DNA. MtDNA is only inherited maternally; therefore, mtDNA is not unique to any one person. Each individual will share the same mtDNA sequence with their mother, siblings, and other maternal relatives. Because of the shared mtDNA profile between maternal family members, mtDNA is not as discriminating as nuclear DNA analysis. However, mtDNA has several properties that make it useful in forensic science.

MtDNA is circular in shape in contrast to nuclear DNA, which has a long, linear configuration that is packaged into chromosomes. MtDNA's circular shape enables it to be more stable over time because it is less susceptible to degradation. There are also many more copies of mtDNA per cell than nuclear DNA, making mtDNA testing much more sensitive than nuclear DNA testing. This stability and additional sensitivity allows mtDNA to be utilized in cases involving skeletonized remains or old biological samples that are not able to yield a nuclear DNA profile. MtDNA is also useful in cases of mass disaster, where remains may be subjected to harsh conditions such as salt water, charring, or other elemental conditions that degrade DNA. As a consequence of the maternal inheritance of mtDNA, mtDNA analysis also allows for the comparison of remains to more distantly related individuals than nuclear comparisons allow. This aspect is helpful in cases where no immediate family members are available to supply a reference sample.

One of the most famous remains identification cases in which mtDNA sequencing was utilized involved the identification of the Romanov family [11]. STR analysis established that the remains from a mass grave in Ekaterinburg, Russia were of a family unit (both parents and three daughters) and four unrelated individuals. Because the Romanov family disappeared in 1918, no immediate living relatives were available to confirm the identity of the remains by STR testing. Instead, Tsarina Alexandra was identified through a mtDNA sequencing comparison between her remains and that of Prince Philip, Duke of Edinburg, a maternal grand-nephew. Tsar Nicolas II was identified using an mtDNA comparison with two separate individuals: his sister's great granddaughter and his maternal grandmother's great-great grandson.

In addition to remains identification cases, mtDNA analysis is routinely used to compare DNA derived from single hairs with known reference samples. Hairs with intact roots can yield enough nuclear DNA for STR analysis, but hairs without available roots will typically be unable to produce any analyzable nuclear DNA. Even very small cut hairs are capable of generating an mtDNA profile for comparison. Because mtDNA analysis is time consuming and not as statistically discriminating as nuclear analyses, mtDNA analysis is usually only performed on hair or other evidence when there is no other physical evidence available in an investigation.

Unlike STR analysis, which looks at repetitive segments of DNA, mtDNA analysis actually compares the DNA sequence between individuals. DNA sequencing breaks down the DNA fragment by order into its respective bases (A, C, T, or G). To facilitate interpretation, the sequence is then compared with a reference sequence and any difference from the reference sequence is noted. This annotation becomes a mtDNA profile or haplotype. MtDNA haplotypes can be compared between evidence and reference samples and conclusions can be drawn as to whether a certain individual may have contributed to the mtDNA on an item. If the profiles are consistent between a reference sample and an evidentiary sample, statistics can be generated to indicate how many times that mtDNA haplotype has been observed in a given population.

More recently, a method using sequence specific oligonucleotide (SSO) probes, similar to DQ and Polymarker for nuclear DNA (Sect. 2.8.4), has been developed in attempt to circumvent the need for actual sequence determination, thereby decreasing the time necessary for mtDNA analysis [12]. These probes have been used in some instances, but may require sequencing for confirmation [13]. The SSO probe approach to mtDNA analysis may be helpful to laboratories who would like to begin mtDNA analysis but do not want to have to purchase costly equipment. One drawback is that SSO probes do not provide as much genetic information as actual sequencing of the mtDNA molecule.

Like STR analysis, reagent blanks and amplification positive and negative controls must be processed through sequencing to determine if there is any underlying DNA present in any of the chemicals used during analysis. Because mtDNA amplification is so sensitive, evidentiary samples are usually processed individually, each with their own reagent blank to closely monitor for contamination. Due to the necessity of processing evidentiary items singly and because of the time involved in the sequencing analysis, mtDNA casework takes many times longer to complete than STR casework. Generally, laboratories that perform mtDNA sequencing can only process one or two mtDNA cases per analyst per month [14].

MtDNA analysis for criminal cases can only be performed when a reference sample is available for comparison. Unlike nuclear DNA analysis, a database does not exist for the comparison of unknown mtDNA profiles from criminal cases. MtDNA profiles are not unique to individuals, so any database match would not necessarily aid an investigation. On the other hand, a mtDNA database does exist for searching profiles obtained from unidentified remains against relatives of missing persons. Databases similar to a missing person database are also useful in mass disaster identifications and identifying remains from mass graves, human right violations, or war.

2.8.7   Y-Chromosome STR Analysis

Because many sexual assault cases involve the DNA typing of a semen donor and most case samples are a mixture of complainant and semen donor sources, a technology focusing on the Y-chromosome, which is only present in males, has been developed. Y-STRs are useful in forensic testing because they are specific to the Y chromosome, and therefore to male DNA. Y-STR analysis is able to simplify interpretation in cases where there is a mixture of male and female DNA by focusing on the male portion of DNA only. Y-STR is also applicable to cases where there is a mixture of more than two people. In complex mixtures such as these, Y-STR analysis can provide information on how many male donors have contributed to a sample. Y-STR is also instructive in cases where semen is present on a sample but no sperm type is detected because the ratio of complainant to sperm DNA is too large. In cases where the complainant is female, the complainants contribution to the DNA in a sample is ignored and a Y-STR profile can be identified for the semen donor. Using Y-STR analysis on a sample may also preclude the need for performing a differential extraction in cases of male–female sexual assaults because separation of male and female DNA becomes unnecessary.

While Y-STR is practical for many sexual assault cases, it is not without limitations. Similar to mtDNA, the Y-chromosome is inherited uniparentally, meaning it is passed from father to son. Therefore, male relatives will have the same Y profile as other male members of their family. For this reason, Y-STR testing is not as statistically discriminating as nuclear DNA. Consequently, Y-STR analysis is usually employed as an extension of nuclear DNA testing to provide additional information and will not replace traditional nuclear DNA testing. Like mtDNA testing, there is not a searchable database for comparing unknown Y-STR profiles from crime scenes to known individuals. For Y-STR casework to be useful, a reference sample must be available for comparison.

Y-STR analysis is essentially the same as STR analysis. The only real difference is that the primers for Y-STR analysis are specific to male, human DNA instead of only being human specific. Male-specific quantification kits have been developed to determine the amount of male DNA in a sample prior to amplification. Testing with these kits can be performed either separately or in conjunction with autosomal DNA quantification (see Sect. 2.8.3).

2.8.8   Single-Nucleotide Polymorphism (SNP) Analysis

Single-nucleotide polymorphisms (SNPs) are scattered throughout the genome. A SNP is one base pair of DNA that is variable between people. SNP technology for forensic science applications is currently under development for nuclear DNA, mtDNA, and Y-chromosome testing. It has already been used to help identify victims of the World Trade Center collapse in New York.

Research into SNP technology is aimed at the identification of meaningful markers and grouping as many probes as possible for these informative sites onto a microchip or similar technology so that many sites can be screened at once with very low quantities of DNA. This new technology should be better able to produce a profile from degraded DNA because the probes utilized are very short sequences. Each SNP site on its own is less informative than a single STR locus, so more SNPs will have to be processed to achieve the level of discrimination of current STR analysis. However, using microchip technology, hundreds or more SNPs can be analyzed at the same time, offering a much higher level of discrimination than current STR analysis. The more SNP sites assayed, the more discriminating the testing ability to be able to individualize evidentiary stains.

2.8.9 Mini-STR

Mini-STRs are a recent addition to the repertoire of DNA tests that a forensic scientist can use. Mini-STRs test some of the same DNA locations that are analyzed in the traditional STR method described in Sect. 2.8.5, but the size of the amplified DNA segment is shorter in the mini-STR method [15]. Another difference between traditional and mini-STRs is that mini-STRs were developed to be more sensitive than the traditional method. Mini-STRs use more PCR cycles in order to amplify more DNA for analysis. These extra cycles make the mini-STR test more sensitive. Because the amplified DNA fragments are also shorter, it is possible to detect DNA from degraded samples. Currently, mini-STRs are not a replacement to traditional STR testing because they do not allow all of the standard STR loci to be tested and are therefore available as a supplemental testing method only.

2.8.10 Low Copy Number (LCN) DNA Testing

In general, low copy number DNA testing refers to the testing of samples containing less than 100pg of DNA. Laboratories may employ several different techniques to enhance the DNA signal to a detectable level for samples in this range. One method is to use additional PCR cycles over the standard cycle number to boost the amount of detectable DNA [16]. Some laboratories may also use mini-STRs to analyze samples containing such small amounts of DNA (refer to Sect. 2.8.9). Another technique is to use a post-PCR purification procedure to boost DNA signal during capillary electrophoresis [17].

The additional sensitivity that LCN testing offers is not without its drawbacks. LCN samples have such a low abundance of DNA that the profiles from these samples can be difficult to reproduce because of sampling effects. Furthermore, as the sensitivity of the testing increases, so do the chances of detecting low-level

contamination that may be present below the detection limit of standard DNA testing procedures. To combat these issues, some laboratories test replicate samples and only analyze DNA alleles that are observed in the majority of replicates tested (Fig. 2.4).

2.9 Combined DNA Index System (CODIS) Database

The Combined DNA Index System, or CODIS, is a database of DNA profiles maintained at the local, state, and national level. Its purpose is to help aid criminal investigations by linking perpetrators to biological evidence. In order for a laboratory to participate in the CODIS system within the United States, the laboratory must follow the Quality Assurance Standards for Forensic DNA Testing Laboratories [18,19] or the Quality Assurance Standards for Convicted Offender DNA Databasing Laboratories. Locally, laboratories participating in CODIS may enter DNA profiles obtained from forensic evidence. These profiles are then uploaded to a designated state laboratory and searched against forensic DNA profiles from other cases within the state and also against the DNA profiles of convicted offenders from that state. In this way, the investigation of crimes for which there is no known
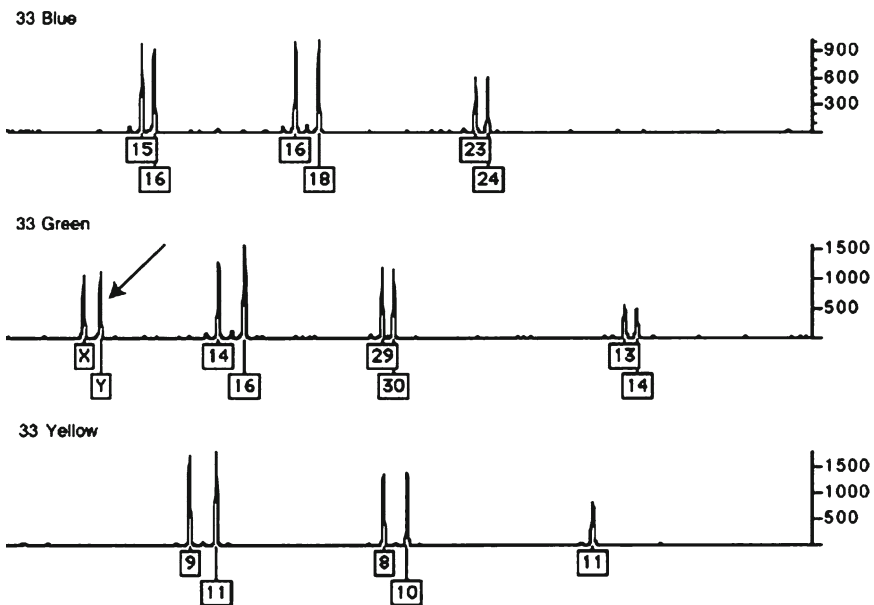

Fig. 2.4 The DNA profile of a male individual. The presence of an "X" allele and a "Y" allele at the amelogenin locus (*arrow*) indicates that this profile is from a male. Each locus has no more than two alleles, each with similar height, indicating that the source of this profile is from a single individual. This individual contributed to the mixture profile from Fig. 2.3

suspect may be aided by linking the biological evidence to either another case perpetrated by the same individual or to an individual who has been previously convicted of a felony. Each state participating in CODIS can upload their forensic unknown and convicted offender profiles to the national CODIS database, administered by the Federal Bureau of Investigation (FBI). Searches at the national level can link cases and offenders from across the country. In 2008, the national database contained more than six million convicted offender profiles and more than 200,000 evidence profiles from all fifty states.

Internationally, there are approximately thirty countries outside the United States that have their own CODIS databases, including countries in Europe, Asia, and North and South America. Because many countries use some of the same loci for DNA analysis, it is possible for profiles to be sent to these countries to be searched against their CODIS databases if warranted by the investigation.

2.10   DNA in Criminal Proceedings

Typically, DNA evidence does not stand alone in a criminal investigation. DNA evidence must be used in conjunction with the other case evidence to provide a whole scenario to allow jurors to make the best decision on the alleged course of events. The most important thing to remember when dealing with DNA during criminal proceedings is that the presence of an individual's DNA on an item does not prove their guilt; conversely, a lack of DNA does not necessarily prove their innocence. DNA evidence, in and of itself, is only as probative as the piece of evidence on which the DNA is found. DNA from semen in a sexual assault case that is consistent with the defendant's DNA would only have evidentiary value if the defendant denies knowing or having sexual contact with the victim. This same DNA would not be probative if both parties admit that a sexual encounter occurred but the question is one of consent. If the DNA profile of a semen sample in a case of sexual assault does not match the defendant, it could either mean that he was not the perpetrator, or that he did not ejaculate, and the semen may belong to a sexual partner of the victim. Likewise, if semen is not present on samples from a sexual assault case, it does not necessarily mean that a sexual assault did not occur.

2.11   Sample Processing

Forensic biology laboratories use different strategies to get as many cases processed each year as possible. This is important because governmental crime laboratories have backlogs sometimes reaching thousands of cases awaiting DNA analysis. Each lab must consider available personnel and laboratory equipment and determine how to allocate resources effectively. Federal grant funding has also been available to help laboratories equip, staff, train, or outsource DNA casework in attempts to reduce the number of cases waiting for DNA testing.

2.11.1 Case Batching

Batching, or processing more than one case at a time for DNA analysis, is one of the ways that laboratories increase their sample throughput. Traditionally, DNA laboratories have practiced case ownership where one analyst works one or multiple cases at a time from start to finish. Private DNA laboratories and some of the larger governmental labs process cases in batches using an assembly line approach. In this way, multiple people participate in sample processing in each case and a larger number of samples can be processed simultaneously. Other methods utilized by laboratories to increase case throughput include limiting the number of samples that are tested per case in order to be able to perform DNA testing on as many cases as possible and restricting the types of cases that the laboratory will accept for processing to personal crimes (homicide, sexual assault, assault) instead of property crimes (burglary and theft).

2.11.2 Laboratory Automation

Laboratory automation means using robotic systems to process case samples. The 1, 4, 16, and 96 capillary genetic analyzers used for fluorescent detection that DNA labs have been using for the past decade was the first step to be automated in the DNA process. These instruments replaced the need for laboratory personnel to pour and load polyacrylamide gels. This invention saved the analysts time, improved sample quality, and was also a safety improvement.

More recently, forensic biology laboratories are beginning to automate all aspects of DNA processing. There are a variety of robotic systems that can be automated for DNA extraction, quantification, and amplification. There are even automated systems for data analysis, but these are only beginning to be used for reference samples in databasing laboratories. Automation frees personnel to focus on laboratory processes that cannot be automated, such as serology screening, profile interpretation, and expert testimony. Automation can also be a quality improvement measure because samples are processed by machine and therefore theoretically free of human error.

2.11.3 Outsourcing

The National Institute of Justice has provided millions of dollars to laboratories for capacity enhancement and backlog reduction. This grant funding has allowed laboratories to increase the number of cases that they can process by buying new equipment and improving laboratory technology. Grant funding has also allowed laboratories to send unworked cases to private laboratories for analysis. This has been an effective means of reducing the number of backlogged cases awaiting DNA analysis.

2.12   Quality Assurance

Forensic testing must be reliable. To achieve this end, forensic laboratories are required to have comprehensive quality assurance and quality control programs. For forensic biology laboratories that participate in CODIS, the minimum required standards were originally developed by the DNA Advisory Board and issued by the FBI Director: the Quality Assurance Standards for Forensic DNA Testing Laboratories (July 1998) and/or the Quality Assurance Standards for Convicted Offender DNA Databasing Laboratories (April 1999). These standards have been revised and were reissued in 2008 as the Quality Assurance Standards for Forensic DNA Testing Laboratories and the Quality Assurance Standards for DNA Databasing Laboratories. The newer versions of the standards will take effect on July 1, 2009. These standards contain requirements pertaining to quality assurance, organization and management, personnel, facilities, evidence control, validation, analytical procedures, equipment calibration and maintenance, reports, review, proficiency testing, corrective action, audits, safety, and subcontracting. Laboratories that participate in CODIS, which includes nearly all public forensic laboratories in the nation, must be audited against these standards every year.

2.13   Laboratory Accreditation

There are two accrediting bodies for forensic crime laboratories that operate in the United States: Forensic Quality Services (FQS) and the American Society of Crime Laboratory Directors Laboratory Accreditation Board (ASCLD/LAB). Both agencies offer accreditation under International Organization of Standardization (ISO) and use the Quality Assurance Standards during their inspection. As of November 2008, 350 crime laboratories were accredited by ASCLD/LAB, including 179 state laboratories, 114 local agency laboratories, 22 federal laboratories, 11 international (non-US) laboratories, and 24 private laboratories. Of these, only 71 crime labs are accredited under ISO (http://www.ascld-lab.org/legacy/aslablegacylaboratories.html). An additional 53 crime laboratories are accredited under ISO through FQS (http://www.forquality.org/fqs_I_Labs.htm). As of this writing, only four states - New York, Texas, Oklahoma and Missouri - require their crime labs to be accredited; however, federal rules require that CODIS-participating laboratories must be accredited.

2.14   Educational Requirements for Forensic Biology Personnel

To work at an accredited crime laboratory, or one that participates in CODIS, a minimum of a Bachelor's degree is required. For personnel who interpret DNA profiles, their degree must be in the field of Biology, Chemistry, Forensic Science,

or a related area. These individuals must also have coursework covering Genetics, Biochemistry, and Molecular Biology and have at least some training in Statistics or Population Genetics. Individuals serving as DNA Technical Leaders must have a minimum of a Master's degree or possess a degree requirement waiver from the American Society of Crime Laboratory Directors Laboratory Accreditation Board (ASCLD/LAB).

2.15   Proficiency Testing

Part of the quality assurance program requires analysts performing casework to undergo proficiency testing. For DNA analysts, two external proficiency tests are required each year the analyst is performing casework. An external proficiency test is one that is administered by a separate agency. The analysts that are taking the test may know that they are being tested, but no one at that agency will know what the corresponding results of the test should be until the results are all turned in and graded and the test results released. Proficiency testing is a quality assurance measure used to monitor performance and identify areas in which improvement may be needed.

2.16   Certification

Certification is a voluntary process of peer review by which a practitioner is recognized for attaining the professional qualifications necessary to practice in one or more disciplines of criminalistics (http://www.criminalistics.com/cert_ovw.cf). Currently, certification is not required for forensic biologists. The only certifying agency for forensic biology is the American Board of Criminalistics. They offer certification as a comprehensive criminalist (general certification in forensic science) or in molecular biology, which is the specialty area for forensic biologists. It is estimated that in 2008, only 10% of forensic biologists were certified by the American Board of Criminalistics.

2.17   Case Studies

2.17.1   Case Study: Probable Saliva for CODIS

In June of 2002, a woman's car was reported stolen. The car was later recovered and a white styrofoam cup, a straw, and white plastic spoon were collected from the interior of the vehicle and submitted to the laboratory for analysis. Serology testing was not performed on these items to save as much sample as possible for DNA

testing. Instead, swabbings were collected from areas that were suspected to contain saliva, the lip of the cup, the entire straw, and the spoon, and all were extracted and processed for DNA.

A DNA profile was obtained from the white cup that was consistent with a single male individual (Table 2.1). A DNA profile was also obtained from the straw consisting of a mixture of DNA from more than one person; the major portion of the DNA was different from the male type from the white cup (Table 2.1). A DNA profile was not obtained from the white plastic spoon. Because no suspects had been developed for this case, the profiles from the white cup and straw were both submitted to the CODIS database.

2.17.2   Case Study: Aggravated Sexual Assault

Also in June of 2002, a woman was abducted and sexually assaulted by two men. A sexual assault kit was collected at a local hospital and submitted to the laboratory for analysis. Serology testing was performed on the items from the kit and seminal fluid (AP-positive reaction) was detected on the vaginal and anal swabs. Spermatozoa were visualized on the vaginal and anal smears (microscope slides). These items were saved for DNA analysis.

A differential DNA extraction was performed on the vaginal and anal swabs and the profiles that were obtained were compared with the profiles obtained from the complainant and two suspects (Table 2.2). The sperm fraction of the vaginal swabs was consistent with one of the two suspects identified in the case (suspect 2). The sperm fraction of the anal swabs was consistent with a mixture of DNA from more than one individual; both the complainant and the same suspect (suspect 2) were included as possible contributors to the mixture.

Because of the DNA evidence, other evidence from the case, and extenuating circumstances, both suspects pled to a considerable jail sentence. The profile from the sperm fraction of the vaginal swabs was entered into the CODIS database, as was the profile from the other suspect (suspect 1). These profiles later produced a high stringency match at the local level with the profile from the straw (to the sperm fraction of the vaginal swabs) and the styrofoam cup (to suspect 1) from the case above (Sect. 2.17.1). Since both suspects were already serving an extended sentence, the investigation into the stolen car case was closed.

Recommended Readings

American Society of Crime Laboratory Directors-Laboratory Accreditation Board (ASCLD-LAB), ASCLD-LAB Accreditation Manual, 1999.

Baechtel FS. The identification and individualization of semen stains. In: Saferstein R, ed. The Forensic Science Handbook, Vol. 2. Englewood Cliffs, NJ: Prentice Hall, 1988.

Butler, JM. Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers (2nd Edition). New York, NY: Elsevier Academic, 2005.

Federal Bureau of Investigation, *Quality Assurance Standards for Convicted Offender DNA Databasing Laboratories*, (1999).

Federal Bureau of Investigation, *Quality Assurance Standards for DNA Databasing Laboratories*, (2008).

Federal Bureau of Investigation, *Quality Assurance Standards for Forensic DNA Testing Laboratories*, (1998).

Federal Bureau of Investigation, *Quality Assurance Standards for Forensic DNA Testing Laboratories*, (2008).

Gaensslen, RE, ed. Sourcebook in Forensic Serology, Immunology, and Biochemistry. Washington: National Institute of Justice, 1983.

National Research Council. The Evaluation of Forensic DNA Evidence. Washington: National Academy, 1996.

Technical Working Group on DNA Analysis Methods. Guidelines for a Quality Assurance Program for DNA Analysis. Crime Laboratory Digest, 1995; 22(2): 21–43.

Websites

http://www.cstl.nist.gov/biotech/strbase/
http://www.ascld-lab.org/
http://ystr.charite.de/
http://www.mitomap.org/
http://usystrdatabase.org/
http://www.criminalistics.com/

2.18   Glossary

Acid phosphatase	An enzyme present in high concentrations in seminal fluid.
AP	Acid phosphatase.
Allele	Alternate forms of DNA that occur at any one locus.
Amylase	An enzyme highly concentrated in saliva.
CODIS	Combined DNA Index System.
CODIS database	A repository of DNA profiles from forensic evidence and convicted offenders.
Conceptus	Child or offspring; in criminal paternity testing, may be tissue from an aborted fetus.
Confirmatory testing	In serology analysis, testing that confirms the presence of a body fluid on an item.
Contamination	The presence of foreign DNA in a sample.
Criminal paternity testing	A comparison of the DNA profiles of a conceptus, an alleged father, and a mother/complainant (if available) to establish or disprove parentage.
Cytoplasm	The portion of a cell outside the nucleus.

Differential extraction	A DNA extraction that attempts to separate spermatozoa DNA from all other DNA in a sample.
DNA	Deoxyribonucleic acid.
DNA profile	A listing of all observed alleles at each locus.
Electrophoresis	A method to separate proteins or DNA, usually by size, using an electrical current.
Epithelial cells	Cells lining the skin surface and body orifices.
False positive	In serology analysis, a substance other than the specific body fluid in question that may produce a positive reaction during presumptive testing.
Heterozygous	Having two alleles at one locus.
Homozygous	Having a single allele at one locus.
Inhibitors	Chemicals or other compounds in a sample that interfere with DNA analysis.
Loci	Plural of "locus."
Locus	A location on the DNA strand.
Low copy number (LCN) DNA	Samples containing less than 100pg of DNA.
Lyse	In DNA extraction, to break open cells in order to release their components.
mtDNA	mitochondrial DNA.
Mitochondrial DNA	DNA that is contained within mitochondria, found outside a cell's nucleus, in a cell's cytoplasm.
Mini-STR	STR loci where the primers have been redesigned closer to the loci to produce smaller sizes of amplified DNA fragments.
Mixture	In DNA analysis, the presence of more than one individual's DNA on a sample.
Negative control	In DNA analysis, PCR (amplification) reagents without the addition of sample DNA to monitor for contamination of the amplification reagents.
Nuclear DNA	DNA that is found inside a cell's nucleus; unique to an individual.
Nucleus	A portion of a cell that contains (nuclear) DNA.
p30	Also known as PSA (prostate-specific antigen), a protein found in seminal fluid.
Paternity testing	Comparing DNA profiles from a child/offspring and an alleged father to establish or disprove paternity.
PH	Phenolphthalein.
Phenolphthalein (PH)	A chemical used for the presumptive testing of blood.
Presumptive testing	In serology analysis, testing that indicates that a body fluid might be present on an item.
Probative	Referring to items that have evidentiary value, or that are substantiating, especially when presented at court.

PCR	Polymerase chain reaction.
Polymorphic regions	Sections of the DNA strand that are highly variable between individuals.
Positive control	In DNA analysis, a sample for which a DNA profile is established.
Primers	Short segments of DNA that are used to target portions of the DNA strand for amplification by PCR.
Reagent blank	All of the reagents in the extraction process without any sample added; used to detct contamination of the extraction reagents.
Reagents	Chemicals used in laboratory processes.
Remains identification or body identification	Comparing DNA profiles from a body or set of remains to a family member to determine whether the two are related.
RFLP	Restriction fragment length polymorphism.
Semen	A body fluid containing seminal fluid and spermatozoa produced by male individuals for fertilization.
Seminal fluid	A protein-rich body fluid originating primarily from the prostate and seminal vesicles.
Sequencing	A process that breaks down the DNA strand by order into its respective bases (A, C, T, or G).
SNP	Single-nucleotide polymorphism.
Spermatozoa	Male sex cells produced in the testis, also known as sperm.
STR	Short tandem repeats.
Tetramethylbenzidine (TMB)	A chemical used for the presumptive testing of blood.
TMB	Tetramethylbenzidine.
UV	Ultraviolet.
VNTR	Variable number of tandem repeats.
Y-STR	Short tandem repeats on the Y chromosome.

2.19   Questions

1. In the forensic laboratory, what is "serology analysis"?
2. Why is the planning of analysis the biggest challenge to the forensic DNA analyst?
3. What are the advantages of an electronic LIMS system over a paper system?
4. What is the CODIS database?
5. Define the terms "presumptive test" and "confirmatory test."
6. What is the function of DNA extraction?
7. Why is differential extraction important?

8. What are the benefits of STR analysis over RFLP analysis?
9. In what ways is mitochondrial DNA analysis different than nuclear DNA analysis?
10. What types of sample processing steps can laboratories use to increase productivity in forensic DNA testing?

2.20   About the Authors

Lisa Gefrides has a Master of Science degree in Genetics from Texas A&M University. She is currently the Compliance/R&D Manager for Forensic Biology at the Harris County Medical Examiner's Office in Houston, Texas. She has been a DNA Analyst since 2000 and is certified in Molecular Biology by the American Board of Criminalistics.

Katherine Welch has a Master of Science degree in Forensic Science from Virginia Commonwealth University. She is currently the Serology/CODIS manager for Forensic Biology at the Harris County Medical Examiner's Office in Houston, Texas. She has been a DNA Analyst since 1997 and is certified in Molecular Biology by the American Board of Criminalistics.

References

1. Ritter N, DNA solves property crimes (but are we ready for that?). NIJ Journal 2008; 261.
2. James SH. Bloodstain pattern interpretation. In: Eckert WG and James SH, eds. Interpretation of Bloodstain Evidence at Crime Scenes. New York: Elsevier Science, 1989: 11–67.
3. Gaensslen, RE, ed. Sourcebook in Forensic Serology, Immunology, and Biochemistry. Washington: National Institute of Justice, 1983.
4. Quarino L, Hess J, Shenouda M, Ristenbatt RR, Gold J, Shaler RC. Differentiation of a-amylase from various sources: an approach using selective inhibitors. FSS 1993; 33(2): 87–94.
5. Pang B, Cheung B. Applicability of two commercially available kits for forensic identification of saliva stains. J Forensic Sci 2008; 53(5): 1117–1122.
6. Benjamin E, Krenke B, Ekenberg S, Frackman S, Hoffmann K, Sprecher CJ, Storts DR. Development of a Novel, Fluorescent,Two-Primer Approach to Quantitative PCR. Profiles in DNA 2005; 8(1): 3–5.
7. Green RL, Roinestad IC, Boland C, Hennessy LK. Developmental validation of the quantifiler real-time PCR kits for the quantification of human nuclear DNA samples. J Forensic Sci 2005; 50(4): 809–825.
8. Nicklas JA, Buel E. Simultaneous determination of total human and male DNA using a duplex real-time PCR assay. J Forensic Sci 2006; 51(5): 1005–15.
9. National Research Council. The Evaluation of Forensic DNA Evidence. Washington: National Academy, 1996.
10. Budowle B, Chakraborty R, Carmody G, Monson KL. Source attribution of a forensic DNA profile. Forensic Sci Communications 2000; 2(3): www.fbi.gov/hq/lab/fsc/backissu/july2000/source.htm.
11. Gill P, Ivanov PL, Kimpton C, et al. Identification of the remains of the Romanov family by DNA analysis. Nature Genetics 1994; 6: 130–135.
12. Reynolds R, Walker K, Valaro J, et al. Detection of sequence variation in the HVII region of the human mitochondrial genome in 689 individuals using immobilized sequence-specific oligonucleotide probes. J Forensic Sci 2000; 45(6): 1210–31.

13. Gabriel MN, Calloway CD, Reynolds FL, Primorac D. Identification of human remains by immobilized sequence-specific oligonucleotide probe analysis of mtDNA hypervariable regions I and II. Croat Med J 2003; 44(3): 293–8.
14. Melton T, Nelson K. Forensic mitochondrial DNA analysis: two years of commercial case-work experience in the United States. Croat Med J 2001; 42(3): 298–303.
15. Hill CR, Kline MC, Mulero JJ, Lagace RE, Chang CW, Hennessy LK, Butler JM. Concordance study between the AmpFlSTR MiniFiler PCR Amplification Kit and conventional STR typing kits. J. Forensic Sci 2007; 52(4): 870–873.
16. Schiffner LA, Bajda EJ, Prinz M, Sebestyen J, Shaler R, and Caragine TA. Optimization of a simple, automatable extraction method to recover sufficient DNA from low copy number DNA samples for generation of short tandem repeat profiles. Croat. Med J 2005; 46(4): 578–586.
17. Smith PJ, Ballantyne J. Simplified low-copy-number DNA analysis by post-PCR purification. J Forensic Sci 2007; 52(4): 820–829.
18. Federal Bureau of Investigation, Quality Assurance Standards for Forensic DNA Testing Laboratories, (1998).
19. Federal Bureau of Investigation, Quality Assurance Standards for Convicted Offender DNA Databasing Laboratories, (1999).