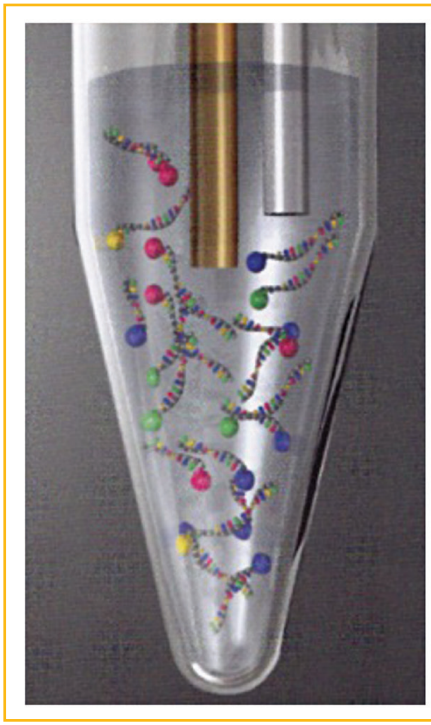
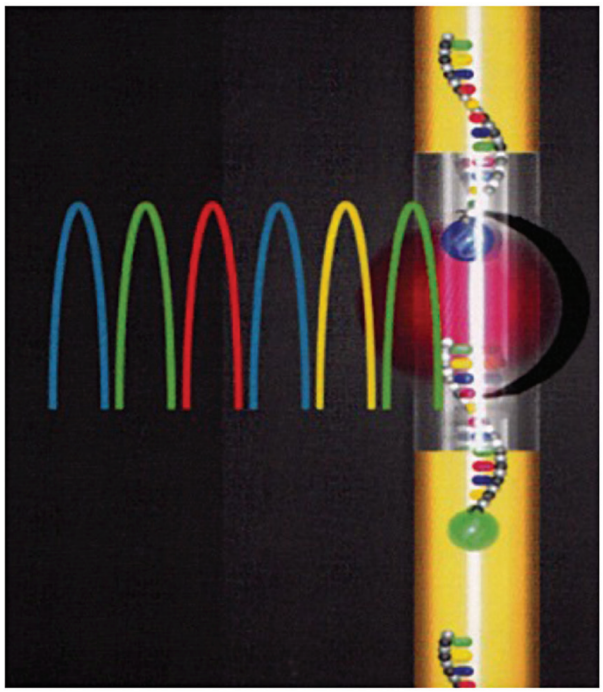
# Forensic DNA Analysis

## Current Practices and Emerging Technologies





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Jaiprakash G. Shewale

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*Dedicated to my wife Manisha J. Shewale*
*who loves me more than herself and has supported me during ups*
*and downs in my scientific career as well as in family life.*

**Jaiprakash G. Shewale**

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# Foreword

The world population is expected to reach over 9 billion people by 2050, with the majority of this growth occurring in the developing regions of the world. This unparalleled expansion in population density will challenge our existing social infrastructure as governments deal with complex and evolving issues in public health, food production and distribution, environmental protection, and public safety.

Within any society, the need to identify and convict those individuals who commit harm to others and to exonerate those who are falsely accused is fundamental to a modern criminal justice system. No field of science has contributed more to this goal than DNA forensics, and today it is difficult to imagine a system of public safety not supported by the scientific methods developed, tested, and validated for genetic identification and human individualization. The fields of forensics and criminalistics have captured the imagination of the public, as evidenced by the proliferation of books, television shows, and media attention given to the topic. However, beyond mass media appeal, the need exists to provide the most up-to-date and scientifically accurate road map for forensic DNA analysis for those engaged in performing, analyzing, and explaining this global and rapidly evolving field. It has been estimated* that by 2015, 60% of the world's population will live in countries that have either passed DNA database legislation or operate under governmental policies requiring DNA collection from individuals suspected of specific criminal acts. Today, over 40 million profiles populate forensic DNA databases around the globe.

In the early days of DNA forensics, the technology for analyzing biological crime scene evidence was based on radioactive labeling and the detection of DNA, and it could take weeks or months to acquire and analyze the samples. In the 1990s, an alternative to radioactivity was developed: enzyme reactions coupled with chemical reagents that emit light reduced the time to complete a case to a week or so. Today, using the latest technology of DNA amplification (PCR), a complete analysis can often be performed in less than a day, depending on the specifics of the case. However, there are still difficult scientific issues and unsolved needs for the forensic DNA scientist.

Criminal justice systems are challenging the limits of forensic DNA technology, as DNA test results are requested from an expanding array of identity management situations, sample types, and criminal cases. Challenges of forensic DNA investigation include the application to biological samples that yield smaller quantities of DNA, resolution of DNA mixtures (this is of particular importance as DNA testing is applied to more cases involving trace evidence, such as guns and fingerprints), and the application of next-generation sequencing methods to increase the information from crime scene evidence. These new technologies will challenge the scientific community to provide reliable, reproducible, and validated forensic protocols, while legal and ethical considerations will determine the

* Gordon Thomas Honeywell Governmental Affairs.

extent to which these technologies are employed. This volume provides insight into the latest advances as they evolve into 21st century DNA forensics.

Forensic scientists collecting DNA reference samples or performing casework and analysis are presented with an array of possible choices to determine the best operational methods and policies for their laboratories. The latest technologies for collection, storage, and extraction of forensic samples, choice of DNA analysis methods, automation, training, and a path to next-generation advances in DNA forensics are presented in this book. The editors have assembled a comprehensive overview of DNA forensics methodologies that should be of interest to researchers, students, and forensic scientists, both as practitioners and as visionaries of the future.

**Leonard Klevan, Ph.D.**
*Orinda, California*

# Preface

The first recorded application of medical knowledge to the solution of criminal cases was reported in a 1248 A.D. Chinese book, *Hsi Duan Yu* ("the washing away of wrongs"), which contains a description of how to distinguish drowning from strangulation, and which became an official textbook for coroners (http://www.forensicdna.com/Timeline020702.pdf and http://www.crimezzz.net/forensic_history/index.htm). Nearly seven centuries later, the discovery of ABO blood groups in 1902 by Karl Landsteiner helped to solve crime and paternity cases by a simple immunological technique. In less than another century, the discovery of restriction fragment length polymorphism (RFLP) in 1980 by Ray White, David Botstein, and colleagues, and the generation of individual-specific DNA "fingerprints" using multilocus variable numbers of tandem repeats (VNTR) in 1985 by Sir Alec Jeffreys laid the foundation for current DNA-based human identification methodologies. The invention of polymerase chain reaction (PCR) and multiplex capabilities further enhanced genotyping capabilities. Since then, forensic DNA analysis is probably the fastest growing method for crime investigation. This is evident from the fact that the number of countries adopting a national DNA database has more than tripled, from 16 in 1999 to 54 in 2008, according to a survey by INTERPOL. Many more countries are expected to pass legislation to establish their own national DNA databases. It is estimated that 60% of the world's population will soon live in a country with a DNA database program. The exponential growth in forensic DNA analysis can be attributed to the high power of discrimination provided by these genetic markers, acceptance of DNA results by court systems, legislation passed by government agencies, increased funding, advancements in DNA analysis technologies, and continued success in worldwide case resolution. Genotyping of biological samples is now routinely performed in human identification (HID) laboratories for applications including paternity, forensic casework, DNA databasing, the hunt for missing persons, family lineage studies, identification of human remains, mass disasters, and more. It is important to note that milestone contributions in several other areas played key roles in shaping the currently used genotyping methods in forensic DNA analysis. Some of these milestones are related to the discovery and optimization of restriction endonucleases, Southern blotting, the polymerase chain reaction, multiplex PCR, genetically engineered *Taq* polymerases, spectrally resolvable fluorescence dyes, capillary electrophoresis and automated DNA sequencers, liquid-handling robots and automated systems, and software capabilities.

Forensic DNA analysis in casework encompasses activities ranging from sample collection to testimony in court. The whole process includes multiple steps such as sample collection, sample preservation, evidence examination, body fluid identification, extraction of DNA, assessment of DNA recovered, amplification of target loci, detection of amplified products, data analysis, results interpretation, and report generation. Automation and workflow integration streamlines the entire process.

The types and quality of samples received in forensic laboratories vary to a great extent. These can be grouped on different platforms like body fluids/tissues, source, nature of substrate on which the biological sample is deposited, age, quantity of biological sample, and so forth. Needless to say, attempts have been made to develop "tailor-made" workflows, protocols, and/or genotyping systems for the different sample types. Some examples of such dedicated workflows or systems are direct amplification workflows for reference/single-source samples, miniSTRs for degraded samples, robust short tandem repeat (STR) genotyping systems for inhibited samples, differential extraction for sexual assault samples, and cell separation methods for separation of cell types, and so forth.

It is evident from the literature that STRs received a great deal of attention from forensic scientists over the course of the past two decades. Nevertheless, the potential for applications of single nucleotide polymorphism (SNP) genotyping was not forgotten. Technological challenges and the high cost of analysis have so far prohibited the utilization of these approaches. However, innovations in multiplex PCR design, microarrays, next-generation sequencing, automation, and analytical software provide a promise that new methods for SNPs that are amenable to forensic scientists for routine analysis may be developed in the near future. In recent years, attempts have been made to expand the capabilities of forensic DNA analysis, for example, obtaining genotypes from samples containing minimal quantities of DNA (trace/touch evidence), mixture resolution, obtaining genotypes for investigation tools, DNA profiling at the collection site, and so forth. The potential of next-gen sequencing in forensic investigations is just now beginning to be explored.

The topics covered in this book encompass almost all aspects of forensic DNA analysis, from sample collection at a crime scene to generation of genotypes as well as the utility of new technologies such as next-gen sequencing and sample-to-answer systems.

All chapters are either revised versions or adapted from select review articles published in *Forensic Science Review* volumes 22 (2), 24 (1), 24 (2), and 25 (1) and the images therein are used with permission from the journal's publisher.

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# Sample Collection, Sample Storage, and DNA Extraction

# I

# Forensic DNA Evidence Collection at a Crime Scene
## An Investigator's Commentary

# 1

JOSEPH BLOZIS

**Contents**



**Abstract**: The purpose of this chapter is twofold. The first is to present a law enforcement perspective of the importance of a crime scene, the value of probative evidence, and how to properly recognize, document, and collect evidence. The second purpose is to provide forensic scientists who primarily work in laboratories with insight on how law enforcement personnel process a crime scene. Among all the technological advances in the various disciplines associated with forensic science, none have been more spectacular than those in the field of DNA. The development of sophisticated and sensitive instrumentation has enabled forensic scientists to detect DNA profiles from minute samples of evidence in a much timelier manner. In forensic laboratories, safeguards and protocols associated with American Society of Crime Laboratory Directors/Laboratory Accreditation Board (ASCLD/LAB) International, Forensic Quality Services, and/or ISO/IEC 17020:1998 accreditation have been established and implemented to ensure proper case analysis. But no scientist, no instrumentation, and no laboratory could come to a successful conclusion about evidence if that evidence had been compromised or simply missed at a crime scene. Evidence collectors must be trained thoroughly to process a crime scene and to be able to distinguish between probative evidence and nonprobative evidence. I am a firm believer in the well-known

phrase "garbage in, garbage out." The evidence collector's goal is to recover sufficient DNA so that an eligible Combined DNA Index System (CODIS) profile can be generated to not only identify an offender but also, more importantly, to exonerate the innocent.

## 1.1 Introduction

As a former detective sergeant with 28 years of service, including 20 years assigned to the Forensic Investigations Division of the New York Police Department (NYPD), I believe that the most important factor pertaining to an investigation is the value of the crime scene. It is imperative that, from the outset, the crime scene be handled properly by first responders. The scene must be properly safeguarded and preserved to best maintain its integrity, since it is the crucial source of probative evidence. Proper handling of the crime scene ultimately will ensure a successful conclusion to an investigation and prosecution.

The investigation begins with the initial 911 call. It then is followed by the first responders pivotal actions at the crime scene. First responders have many responsibilities and some are described as follows. First responders must be cognizant of their own safety, including on-scene arrival. They quickly must assess and evaluate what actions must be taken. These actions may include suspect confrontation, victim aid, searching for a suspect, a complainant interview, the determination of potential witnesses and, subsequently, provisions to separate them. First responders also will request additional assistance, determine if there are multiple crime scenes, make command notifications, take copious notes, prepare detailed reports, and safeguard and preserve the scene. All of this must be done in an expeditious manner while maintaining scene integrity.

To successfully preserve a scene, the first responder or responsible party must keep unauthorized persons out, often including police personnel. From firsthand experience, many police officers, including high-ranking supervisors, have a morbid curiosity for the dead. At times, we are our own worst enemies. Once first responders deem the scene safe and victims are tended to, there is no reason to reenter the scene until arrival of the crime scene unit. After the crime scene has been established, the only official personnel permitted into the area are crime scene detectives, medical personnel, medical examiners, the district attorney, the detective supervisor, and the assigned detective. A safeguarding officer should maintain a log of all responding officers at the scene, including those who previously departed. DNA elimination samples of those individuals at the scene are as important as the unknown DNA samples recovered.

Crime scene investigators should establish a single path in and use the same path out to minimize contamination. Edmond Locard's theory of evidence transference states that whenever you enter a crime scene, you leave something in that scene, and whenever you leave the scene, you take a part of that scene out with you. Crime scene investigators should escort the personnel listed above into the scene only when the crime scene investigator deems it safe to do so and in such a way that forensic evidence is not jeopardized. Investigators, by necessity, conduct walkthroughs of the scene to make an assessment and evaluate what occurred; however, these should be conducted only when it is forensically safe to do so.

Once the scene is safeguarded and preserved, detectives and the crime scene unit are notified to respond. The assigned detective and his or her supervisor are in charge of the investigation. The crime scene unit is a support unit to the detectives and forensically

processes the scene. However, it is important that both the detectives and the evidence collectors work as a team with open communication and respect for each other's professional experience. The primary functions of a crime scene investigator are to document, process, reconstruct, and collect evidence. The following sections are a brief summary of the equipment, procedures, and materials involved in the important role of the crime scene investigator.

## 1.2 Personal Protective Equipment

Personal protective equipment (PPE) is used to both prevent contamination and protect the wearer. It consists of a full Tyvek® suit including a hood, booties, face mask, and gloves. DNA evidence becomes contaminated when DNA from another source is mixed in with DNA relevant to the case. There are potentially serious health and safety concerns associated with touching biological evidence. For these reasons, crime scene investigators and laboratory personnel should always wear PPE, use clean instruments, and avoid touching other objects (including their own bodies) when handling evidence or items used to collect evidence. To prevent sample contamination, it is imperative that crime scene investigators change their disposable gloves after the recovery of *each* sample. Contamination of a sample could jeopardize the investigation and subsequent prosecution of suspects. In addition, the investigator must always remember that allowing biological evidence to contact his or her skin may be hazardous to his or her health. PPE protects investigators from hazards such as bloodborne pathogens, prevents contamination of the evidence DNA samples with the collector's DNA, and eliminates chances of cross-contamination of samples collected at the same site.

Not only are frequent glove changes critical to preventing contamination, but equipment must also be free of any DNA contaminants. Equipment should be cleaned prior to use and after each sample is collected. To clean the equipment:

i. Dip the instruments in a 10% chlorine bleach and water solution and swish them around.
ii. Remove the instruments, dip them in a 70% ethanol and water solution, and swish them around.
iii. Rinse the instruments with plain water and allow them to air-dry.

The three-step method above is necessary to ensure that the instrument is not only free of any DNA residue from the previous sample but also safe for use with the next sample. The bleach solution sterilizes the instrument, but if left on the instrument, bleach residue will destroy any future DNA samples. The ethanol solution removes any bleach residue, and the plain water removes any ethanol. Bleach degrades quickly, so it is important to prepare a new bleach solution weekly, or more frequently if practical.

Since it is easier to change gloves than to sterilize instruments, consider collecting items such as cigarette butts and clothing with a gloved hand instead of using tweezers or forceps. Using the tweezers and forceps only when necessary saves time and effort. Equipment brought into the crime scene, such as flashlights, tripods, alternative light sources, and so forth, also can contaminate the scene by introducing trace evidence from other crime scenes. Consider using clean laboratory mats beneath any equipment transported from

another scene. Laboratory mats are laminated sheets with a plastic material on the bottom layer and an absorbent paper layer on top. This prevents any equipment contamination from being deposited on a substrate containing DNA evidence.

## 1.3 Establishing a Forensic Technical Plan and Documentation of the Crime Scene

Upon arrival at the crime scene, a crime scene investigator should confer with first responders, investigators, and victims to ascertain what has happened and what each person's role was at the scene. The investigator then should establish a singular entry-and-exit path within the scene to minimize contamination and disruption of the scene. Perform a scene walkthrough to determine a sequence of events for forensic scene processing.

Before an item with possible DNA evidence is recovered, it must be documented in place. The item must be photographed, documented in the investigator's notes, measured, sketched, and logged into each of the crime scene logs (crime scene photography, evidence log, etc.) before recovery is attempted. When documenting an item with DNA evidence:

i. *Photograph the item in place*, showing how it looked before collection (Figure 1.1).
ii. *Take written notes*, describing the condition of the evidence, what was collected, and how it was collected.
iii. *Take measurements*, showing the location of the evidence and its position relative to other objects.
iv. *Sketch the location* of the evidence in the crime scene sketchbook.

## 1.4 Recovery of Biological DNA Evidence

Investigators do not always consider DNA as a factor unless there is blood at the scene. However, an investigator can expect to find DNA evidence anywhere within a crime scene with which a suspect has had contact; this may be referred to as contact DNA, transfer DNA, or touch DNA. Almost every component of the human anatomy is a potential source for DNA. Any personal contact between individuals, or between an individual and an object, has the

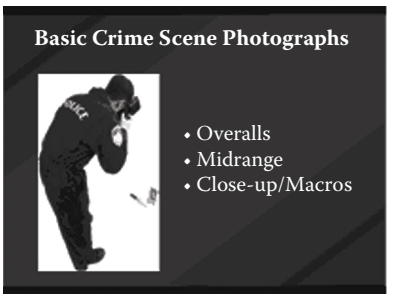


**Figure 1.1** A crime scene investigator photographically documents the scene. Photographs consist of overalls, midrange, close-ups, and/or macros. (Photograph from an unknown source; text was inserted.)

possibility of transferring DNA, even where the possibility of recovering a fingerprint itself is remote. DNA may be extracted from many kinds of biological evidence, including blood, semen, saliva, perspiration, hair (root), skin cells, bone marrow, tooth root/pulp, urine/feces (which may contain epithelial cells), and vomit (which may contain cells from the throat). DNA evidence is readily transferred from the human body and deposited nearly anywhere.

### 1.4.1 Sources of DNA Evidence

DNA deposit sources are not limited to obvious biological material, such as tissue, stains, or fluids, but also include items such as drinking containers, clothing, surface areas, and any other touched items, and items that may have "received" bodily fluids or skin cells, such as perspiration or saliva. Personal items handled regularly often contain skin cell deposits that are an excellent DNA source. If an item is touched or handled repeatedly, it is likely that skin cells have been deposited onto the item. Items in close daily contact with an individual are potentially rich sources for DNA. Items such as clothing, bedding, and eyeglasses potentially hold thousands of skin cells, hair, perspiration, and oils that have been transferred from the body. Items discarded by suspects at crime scenes, such as drink containers, gum, hats, masks, and bandanas, should be documented, collected, packaged, and sent to the laboratory for DNA analysis. Any physical evidence repeatedly or forcibly handled should be considered a prime source to recover DNA. For example, ligatures often contain skin cells and perspiration from being handled and pulled on by the assailant.

Vehicle surfaces, both interior and exterior, are also prime sources of DNA evidence. For example, a vehicle that strikes an individual and was in close proximity to the detonation of a bomb, or when the vehicle itself was used as a vehicle-borne improvised explosive device (VBIED), also called a "car bomb," is certain to contain DNA evidence. Often the vehicle's grill, fender, and undercarriage are prime locations from which to recover physical evidence with DNA. A vehicle's interior also can contain numerous sources for DNA recovery, including skin cells or perspiration deposited on the arm rests, blood or other trace evidence on the floor mats, and hair stuck to the headliner. Discarded trash in a vehicle also is an important source for DNA. Look for cigarette butts, drink containers, candy, napkins, tissues, and partially eaten food.

The point of entry or exit to/from a crime scene is another excellent place for DNA evidence. It is not uncommon for a perpetrator to be injured during a forced entry, so look for blood, tissue, and hair near windows and doors. Biological evidence recovered at the point of entry is compelling evidence in a judicial proceeding.

### 1.4.2 Touch DNA Sample Sizes

DNA technological advances of the last 25 years have reduced the size of a usable biological sample. In the 1980s, a 1–2 cm sample drop of blood had a reasonable probability of yielding a DNA profile. In the 1990s, the size was reduced to 1 cm or less. Presently, the recovery of 10 to 20 skin cells has the probability of yielding a DNA profile. In other words, DNA can be extracted from the miniscule amount of skin cells that the human body naturally sheds when it comes in contact with an object. One of the challenges investigators face when recovering touch DNA is that it is rarely visible to the human eye. The investigator must determine where touch DNA is likely to be located, swab the area, and hope that the forensic scientist has enough skin cells to obtain a CODIS-eligible DNA profile.

### 1.4.3 Probative and Nonprobative Evidence

Probative evidence is recovered from a crime scene and would provide the case investigator with probable cause to make an arrest. It is evidence that would prove or disprove an alleged fact relevant to the investigation. Nonprobative evidence is evidence recovered from a crime scene that would not provide the case investigator probable cause to make an arrest. However, it may provide the case investigator with an investigatory lead or it may be evidence that, at a later date, would prove significant to the investigation. Crime scene investigators must be trained in recognition and collection of probative evidence and the ability to differentiate probative from nonprobative evidence. Evidence collectors must be trained not to burden their crime laboratories with too much nonprobative evidence. Evidence that may yield an investigatory lead should be noted on laboratory requests. Nonprobative evidence at times may be recovered; however, it should be forwarded to an approved storage facility with proper notification to the assigned case investigator.

### 1.4.4 Alternative Light Source

An alternative light source (ALS) is one of the many commercially available pieces of equipment that will facilitate the search for DNA evidence at a scene. The ALS uses a variety of wavelengths to detect trace evidence normally invisible to the naked eye. Various wavelengths cause certain types of trace evidence to fluoresce and become visible when viewed with filtered goggles, indicating their precise location on a substrate. Examples of trace biological evidence that will fluoresce include fingerprints on both porous and nonporous surfaces, body fluids, skin damage resulting from bitemarks and bruising, bone fragments, and hair. All of these have the potential to yield a DNA sample.

### 1.4.5 Chemical Enhancements

Chemical enhancements such as BLUESTAR®, luminol, and leuco crystal violet are used at crime scenes to detect the presence of blood. BLUESTAR is a latent bloodstain reagent used to reveal bloodstains that have been washed out, wiped off, or are otherwise invisible to the naked eye. Luminol is a chemoluminescent reaction in the presence of an oxidizing agent on contact with blood; it is visible without the use of an ALS. Leuco crystal violet and hydrogen peroxide in contact with blood triggers a chemical reaction that turns the solution to a purple/violet color (Figure 1.2).

**Figure 1.2** **(See color insert.)** The luminescent reaction that occurs when BLUESTAR®, luminol, or leuco crystal violet are applied to a substrate. (Photograph from BLUESTAR Forensic Web site, http://www.bluestar-forensic.com/, accessed June 9, 2010.)

### 1.4.6 Fingerprints and DNA

Touch DNA exists where a suspect has touched a surface at the crime scene, possibly leaving a fingerprint as well as DNA. Therefore, before the scene can be processed, it must be decided whether to process for DNA, fingerprints, or both. Areas that are smooth, hard, and nonporous should be processed for fingerprints. Areas that have irregular surfaces should be processed for DNA. Before swabbing an area for DNA, make sure the area is not conducive for fingerprints, since one may be swabbing through a latent fingerprint. As a critical piece of evidence in criminal proceedings, fingerprints always should be a priority at any crime scene for several reasons:

i. Fingerprint processing is more cost-effective than DNA analysis.
ii. Databases containing fingerprints are significantly larger than DNA databases, resulting in a greater opportunity for an identification to be made.
iii. Fingerprint identifications can be made in hours, whereas DNA results can take weeks.

When evaluating a fingerprint, use a magnifying glass to determine if there is a sufficient amount of ridge detail to recover the print. When there is an insufficient amount of ridge detail or the fingerprint is clearly a smudge, consider processing it for DNA. It is possible to recover DNA from areas that have already been processed for fingerprints by swabbing the area after the fingerprint was lifted. Be mindful of contamination issues concerning the fingerprint brush: a fingerprint brush used at other crime scenes has the potential to contaminate DNA from those scenes and be transferred to surfaces at the present crime scene. Substrates, such as drinking glasses, can be processed for both DNA and fingerprints. First, process the rim area for DNA, then process the entire glass for fingerprints. Other small substrates with minute surface areas that will not provide sufficient ridge detail for comparison should be processed for DNA.

### 1.4.7 DNA Recovery Supplies

Proper supplies at the scene, and the knowledge to use them, are critical to collection of usable DNA samples. A crime scene investigator's toolkit should include basic and necessary supplies for DNA evidence recovery. The supplies normally used for processing and recovering DNA samples include:

i. Sterile cotton-tipped applicators (swabs) to collect samples. Some examples of swabs are presented in Figures 1.3, 1.4, and 1.5.
ii. Hydration to moisten the swab.
    a. Distilled water is acceptable.
    b. Sterile water is better.
    c. Sterile phosphate-buffered saline (PBS) solution is the best.
iii. Plastic pipettes for transferring the distilled water to the swabs.
iv. Paper envelopes used for packaging.


**Figure 1.3** **(See color insert.)** Swabs, distilled water, plastic pipettes, and paper envelopes are components of a basic DNA recovery kit. (Photograph by the author.)

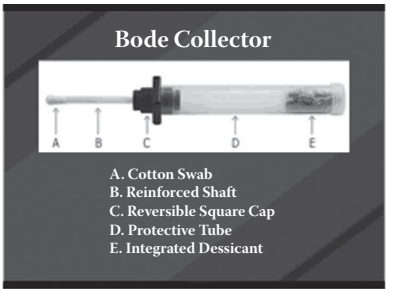


**Figure 1.4** The Bode Technology Group's SecurSwab™ Collector consists of a cotton-tipped swab, a reinforced shaft, reversible square cap, a protective tube, and an integrated desiccant. (Photograph from Bode Technology Group Web site, http://www.bodetech.com/, assessed June 6, 2012.)

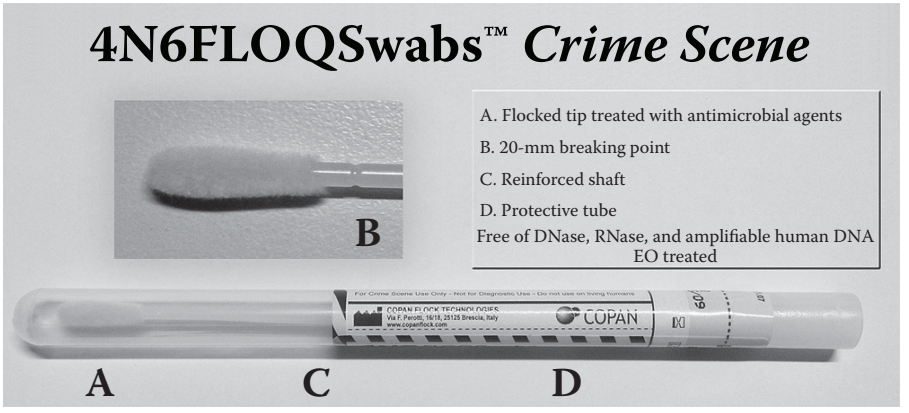


**Figure 1.5** 4N6FLOQSwabs™ *Crime Scene* swabs utilize patented flock technology to maximize DNA collection and elution efficiency. The swabs are certified as free of DNase, RNase, and amplifiable human DNA and are treated with antimicrobial agents to prevent microbial contamination. (Photograph from Copan Flock Technologies, Brescia, Italy. With permission.)

### 1.4.8 Swabbing Techniques for Touch DNA

In processing a crime scene for touch DNA, proper swabbing techniques maximize the ability to recover as much DNA as possible from particular substrates. The following procedures are a guide for proper swabbing:

i. Don personal protective equipment.
ii. Withdraw distilled water from the vial with a sterile plastic pipette.
iii. Remove a sterile cotton-tipped swab from a sealed container. Use each swab only once.
iv. Place a drop of distilled water on the swab's side. Avoid saturating the swab. Avoid dipping the swab in distilled water.
v. Swab the area. Rotate the swab so the entire swab surface is used. Avoid reusing areas of swab if possible (may redeposit samples onto substrate). Use one swab for approximately every 15 sq cm of surface area. After using a hydrated swab, it is permissible to use a dry swab when swabbing the same area. Confer with your laboratory concerning the usage of wet and dry swabs.
vi. Use additional swabs as necessary and use a unique identifier to label the swabs accordingly. When swabbing an irregular grainy surface, swab with the grain using a back-and-forth motion while rotating the swab surface to ensure that the same area of the swab is not reused. Reswab the area with a dry sterile swab. After the swabs are air-dried, it is permissible to place both the swabs into one paper enclosure.

### 1.4.9 Recovery of Biological DNA Evidence from Various Substrates

The following guidelines pertain to the recovery of wet and dried biological samples. A biological sample such as a dried droplet of blood can be recovered by using the moistened portion of a swab as described in Section V. Be cognizant that, for proper swab saturation, only a small amount of water is required for the swab to air-dry quickly and ensure that the sample will be of a proper concentration (Figures 1.6 and 1.7). Improper saturation occurs

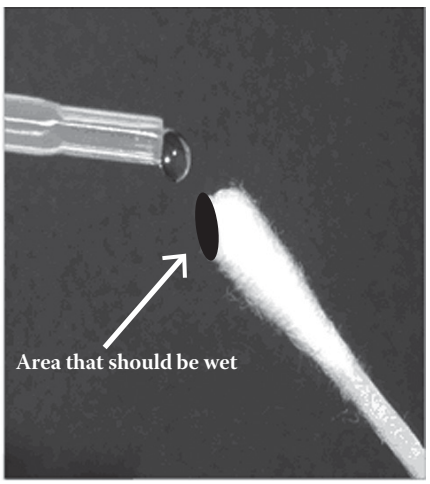


**Figure 1.6 (See color insert.)** For proper hydration and swabbing, a single drop of distilled water should be applied to the side of a cotton-tipped swab. (Photograph from the New York City Office of the Chief Medical Examiner.)

**Figure 1.7** **(See color insert.)** The proper swabbing technique for the recovery of a dried blood sample from the side handle of a refrigerator door. Note the use of personal protective equipment. (Photograph by the author.)

when too much water is applied to the swab; this causes a diluted sample concentration. Use multiple swabs when necessary to collect the entire stain. Reswab the area with a dry sterile swab and subsequently air-dry all swabs. It is permissible to package all swabs used for the same biological sample into one paper enclosure.

Biological evidence such as skin tissue, bone fragments, teeth, and nails have a high likelihood of yielding a full DNA profile. These items must be packaged in paper or cardboard and forwarded directly to the laboratory for DNA profiling.

Prior to recovering a blood sample from glass or other substrates, use a magnifying glass to examine the blood for the presence of ridge detail. Ridge detail in blood indicates a patent fingerprint, which must be processed accordingly. If at all possible, collect the entire substrate that the bloody patent print is on, package it, and forward the entire item to the laboratory. If the substrate cannot be packaged and sent to the laboratory, photograph the print using a scale, then proceed with the swabbing process.

Swabbing is not the only way to collect evidence for DNA analysis. Additional DNA collection methods include scraping dried biological evidence, cutting a swatch from the substrate that contains the unknown stain, and submitting the entire substrate to the laboratory.

Dried biological samples on substrates other than clothing can be scraped with a sterile scalpel and collected. The scrapings are then placed in filter paper, which is folded and inserted into a paper envelope. Scalpel blades must be sterilized or replaced after each DNA sample is recovered. In addition, it is much easier and safer to swab a sample than to scrape a sample and possibly cut yourself. Confer with the laboratory personnel and establish a protocol to determine which collection method is preferred. Submitting the entire substrate to the laboratory often can be advantageous. In the event that the DNA results are negative or the quantity is insufficient for a full DNA profile, the evidence is easily accessible for reprocessing. Processing a substrate at the crime scene eliminates the opportunity for a second analysis. When it is not practical to submit the entire substrate, a swatch can be cut and submitted instead. Clothing should not be processed at the crime scene. Instead, document, package, and submit the clothing to the laboratory, which will process it for stains, hair, skin cells, and so forth. Additional DNA evidence recovery procedures include:

i. Blood samples found in snow or water should be collected immediately to avoid further dilution. The largest possible quantity of these samples should be collected in a clean, suitable container, avoiding contamination as much as possible. Label the samples and submit them to the laboratory directly.
ii. The crime scene should be carefully examined for hair, since hair is difficult to detect and can be overlooked easily. Hair is a potential source of DNA evidence and should be documented, recovered, packaged, and forwarded to the laboratory. Hair should be placed in filter paper, which is then folded and inserted into a coin envelope or similar-type envelope. At the laboratory, a microscopist will examine the hair and determine whether it is suitable for DNA analysis. Tweezers often are used by evidence collectors to recover hair from a crime scene. Tweezers are not recommended in most cases because they can cause damage to the hair structure. Tweezers also must be sterilized between uses. When tweezers are a necessity, an alternative method to sterilizing the tweezers is to use disposable tweezers. However, collecting hair using a gloved hand is the fastest, easiest, and safest method. Another safe and easy collection method is the use of a gel lifter. Confer with your laboratory for proper submission standards.
iii. Burglary tools, such as pry bars and hammers, can be good sources of touch DNA. When these tools are used as weapons, they can carry not only touch DNA, but also blood, hair, and skin. Tools should be sent to the laboratory for analysis rather than processed at the scene so potential trace evidence is not missed. A laboratory has the proper lighting and equipment to process the tool for DNA, wound comparison, and the like. Communicate with your laboratory and establish protocols as to what types of evidence are to be processed in the field and what types are to be submitted directly to the laboratory.
iv. DNA can be typed from epithelial cells found in saliva. Saliva may be deposited on drink containers, cigarette butts, bottles, telephones, cell phones, envelope flaps, stamps, and bitemarks. If a drinking glass is involved, swab the rim, air-dry the swabs, and package them. Following swabbing, the glass can be processed for fingerprints. Alternatively, the entire glass can be sent to the laboratory for processing.
v. Ligatures are any items used by a suspect to tie or bind victims during the commission of a criminal act. Examples of ligatures include duct tape, rope, electric cords, belts, scarves, bandanas, and wire ties. Because these items were touched and perhaps handled roughly by the perpetrator, there is a strong possibility that skin cells were deposited in or on the ligature. Since the same likelihood exists that the ligature will contain the victim's DNA, elimination samples must be obtained from the victim and submitted to the laboratory. A ligature should be packaged in paper and submitted to the laboratory. Although a ligature is considered touch DNA evidence, it should not be swabbed at the crime scene.
vi. Paper items such as letters and envelopes should be submitted to the laboratory for analysis, although studies have shown that processing paper items for DNA has a low success rate. Processing glossy magazine covers for DNA has a slightly higher success rate, but the chances of recovering DNA still are relatively low. If saliva has been applied to the paper, such as when an envelope flap has been licked, then the likelihood of obtaining a full DNA profile increases dramatically. When a paper item is swabbed for DNA, it is possible that the swab will inadvertently destroy a latent fingerprint. Paper items chemically processed with ninhydrin often yield

fingerprints of value. Therefore, it is highly recommended that paper items be chemically processed for fingerprints.

vii. Clothing found at the crime scene must be sent to the laboratory to be processed for stains, hair, skin cells, and so forth; this includes hats and masks. A ski mask, for example, likely will contain saliva and skin cells that cannot be adequately recovered at the scene. Bloody sheets, towels, clothing, and other fabrics containing biological evidence also should be collected whole and sent to the laboratory, not processed at the scene.

viii. Cigarette butts, cigar ends, and other smoked items can contain both skin cells and saliva. They have a high success rate for DNA recovery. These items should be packaged individually in separate paper containers such as coin envelopes or similar-type envelopes and sent to the laboratory for processing. Be sure to document the location where they were recovered.

ix. Firearms can be an excellent source of DNA evidence. Most of a firearm's surface area is not conducive to recovering fingerprints due to uneven surfaces. However, the uneven surfaces make parts of the firearm very conducive to DNA recovery because such irregular surfaces tend to collect skin cells from their handlers. Prior to processing a firearm for DNA, the firearm must be rendered safe. *Safety is paramount*. Before handling the firearm, make sure you are familiar with that type of firearm and know how to unload it. Then, while wearing gloves, remove all of the cartridges from the firearm and visually inspect it to confirm no cartridges are present. Once the firearm has been rendered safe, then it should be handled minimally. DNA processing now can be performed. Firearms can be swabbed for touch DNA at the scene using a sterile hydrated swab, followed by a sterile dry swab. Prime areas to swab include grips, trigger, front sight, and slide.

In addition to swabbing the firearm for touch DNA, be sure to swab the magazine, if the firearm has one. Be sure to swab the lips and the floor plate of the magazine. (A magazine is a storage device for ammunition. It is removed from a firearm when adding cartridges. The lip area of a magazine (Figure 1.8) is the top area where cartridges are inserted; the floor plate is the bottom of the magazine.) Both discharged shells and cartridges are common firearms-related evidence that may hold the suspect's skin cells. Discharged shells generally do not yield fingerprints. Although the surface area of a cartridge is small, there is a possibility that ridge detail could be present. The head stamps, or ends, of discharged shells and cartridges can be a source of touch DNA and should be swabbed.


**Figure 1.8** **(See color insert.)** The lip area of a firearm's magazine is a potential source of DNA. (Photograph by the author.)

### 1.4.10 Types of DNA Samples

There are three basic categories of DNA samples. Crime scene samples are unknown forensic DNA samples recovered from crime scenes. An evidence collector recovers the sample, but (as with fingerprints) does not know who deposited the sample. Crime scene samples are searched in the DNA database against other crime scene samples and convicted offenders.

Elimination samples are samples from individuals such as victims, victims' family members, or any other persons who had prior legitimate access to the crime scene. Elimination samples also should include evidence collectors and laboratory personnel. Elimination samples are also referred to as "known samples," "reference samples," or "buccal swabs." Buccal swabs are elimination samples obtained by swabbing the interior of an individual's mouth. They are used for comparison purposes during DNA analysis to eliminate known DNA profiles from the unknown DNA profiles recovered from the crime scene.

Abandoned samples are DNA samples abandoned by an individual known to law enforcement. This can be as simple as an individual discarding a cigarette butt in a public domain.

### 1.4.11 Packaging DNA Evidence

DNA and other biological evidence must be allowed to air-dry before packaging. Package items separately in paper bags or cardboard boxes, making sure that the packaging is adequate to hold the item. Packaging materials that will be needed on the scene include:

i. Coin envelopes for packaging dried swabs used to collect DNA samples.
ii. Paper bags for packaging clothing or other lightweight items containing DNA evidence.
iii. Cardboard boxes for guns, knives, or any item of DNA evidence too cumbersome for a paper bag.
iv. Evidence tape for sealing the bags and boxes. Paper packaging is breathable and allows the item to dry completely. Plastic and airtight containers create conditions favorable for the growth of bacteria and mold, which are detrimental to the sample. Always package DNA in some form of paper container. The packaging process can be summarized in the following steps:
    a. Select the proper packaging (envelope, bag, or box) based on the size and weight of the item.
    b. Place the item into the package.
    c. Seal the package with evidence tape.
    d. Initial and date the taped seal.
    e. Add a unique identifier and add other pertinent case information.
    f. Prepare the chain-of-custody reports.
v. Air-drying a DNA evidence swab before packaging: swabs must be allowed to dry before packaging. The proper method is to allow the swabs to air-dry. Depending on the concentration of the sample and the temperature, drying can take anywhere from seconds to several minutes. It is recommended that the investigator not blow air on the swab to decrease drying time, as this can cause fragile DNA evidence to become detached and lost from the swab and because it can introduce contaminants from the air into the evidence. There are two suggested methods used to dry swabs collected at the crime scene. The first method is to use a block of styrofoam prenumbered with the corresponding numbers on the evidence log.

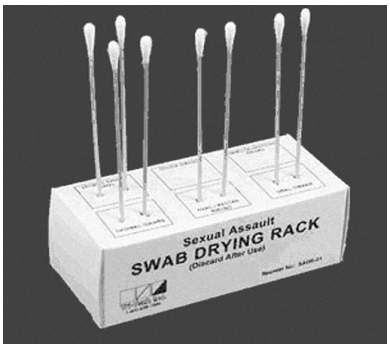


**Figure 1.9** An example of a drying rack for air-drying swabs. (Photograph from Tri-Tech Forensics Web site, http://tritechforensics.com, accessed June 6, 2012.)

> The wood-stick end of the wet swab is inserted into the styrofoam block at the proper number as it is collected. As the investigator finishes another task, he or she returns to the styrofoam block and evaluates the swab for dryness. If it is dry enough, it is packaged. If not, it should remain in the block for a longer period of time (Figure 1.9).

The second method is to tape the wood-stick end of the swab to the edge of a tabletop and let the suspended swab air-dry while other tasks are attended to. When using this method, *extreme caution must be used not to contaminate the swabs*. There are also forensic packaging containers that permit swabs to dry while packaged. The swabs are inserted directly into a tube after processing. The tubes contain desiccants to enhance the drying time. However, these tubes are expensive and some cost-conscious agencies prefer to use the air-drying methods described above. After swabs are air-dried, they should be packaged in a paper envelope. If two swabs were used on the same surface area, they may be packaged together and labeled as such. Otherwise, swabs should be packaged in separate envelopes.

### 1.4.12 Transporting and Storing DNA Evidence

DNA evidence should be transported to the laboratory expeditiously. Once crime scene processing has concluded, place all sealed individual packages of DNA evidence into a carrying box or bag. Make sure that all evidence is accounted for. Place the box into a vehicle and transport the evidence to the laboratory without delay. Be sure to keep the evidence cool and dry. Transport liquid samples in refrigerated or insulated containers. If someone other than the investigator is transporting the evidence, chain of custody must be transferred; it is transferred again when the evidence reaches the laboratory. Degradation is the breaking down of DNA into smaller fragments by chemical or physical processes. Degradation of DNA may limit its use as evidence. Factors that promote DNA degradation include ultraviolet rays (prolonged exposure); heat, humidity, and moisture; bacteria and fungi (often found in foliage and soil); and acids or chemical cleaning solutions (such as bleach).

Actions such as storing evidence in vehicle trunks, vans, office desks, direct sunlight, frost-free refrigerators, and nontemperature/humidity-controlled facilities subject the biological evidence to increased heat, humidity fluctuations, and ultraviolet rays—all factors that can accelerate degradation. Extended exposure to heat or humidity causes degradation

of biological evidence. To reduce this threat, move packaged items from the crime scene to a suitable storage facility as soon as possible. Storage of DNA evidence is the last link in the chain of custody. Whether DNA evidence is stored in the laboratory or in a storage facility, certain conditions must be adhered to in order to prevent degradation. DNA evidence should be stored in a spacious, cool, and dry environment. Although it is preferred, most DNA evidence does not require refrigeration and may be stored at ambient room temperature or cooler.

## 1.5 Conclusions

Evidence collectors assigned to law enforcement agencies around the world perform a vital investigative role. The power of the evidence they detect and collect is used to identify offenders or, more importantly, to exonerate the innocent. Within a forensic investigation, evidence collectors are part of a team whose goal it is to solve a case. Other members of the team are the forensic scientists who meticulously analyze the evidence received to reach a scientific conclusion. Agencies for both evidence collectors and forensic scientists establish protocols and training procedures to ensure that the citizens they serve remain safe and that justice prevails for all.

## Acknowledgments

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# Optimizing Storage and Handling of DNA Extracts*

# 2

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## Contents



**Abstract**: Nucleic acid sample storage is of paramount importance in forensic science as well as in epidemiological, clinical, and genetic laboratories. Millions of biological samples, including cells, viruses, and DNA/RNA, are stored every year for diagnostics, research, and forensic science. PCR has permitted the analysis of minute sample quantities. Samples such as bone, teeth, touch samples, and some sexual assault evidence may yield only low-quality and low-quantity DNA/RNA. Efficient storage of the extracted DNA/RNA is needed to ensure the stability of the sample over time for retesting of the CODIS STRs, mtDNA, YSTRs, mRNA, and other future marker-typing systems.

Amplification of some or all of these markers may fail because the biological material has been highly degraded, contains inhibitors, is too low in quantity, or is contaminated with contemporary DNA. Reduction in recovery has been observed with refrigerated liquid

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DNA extracts and also those exposed to multiple freeze-thaw cycles. Therefore, the development of optimal storage and amplification methods is critical for successful recovery of profiles from these types of samples since, in many cases, retesting is necessary.

This chapter is divided into three sections. The Introduction (Section 2.1) reviews forensic DNA storage, factors that influence DNA stability, and a brief review of molecular strategies to type nonoptimal DNA. Section 2.2 discusses the importance of DNA extract storage in forensic and nonforensic DNA databanks and the mechanisms responsible for loss during storage. Finally, Section 2.3 describes strategies and technologies being utilized to store DNA.

## 2.1 Introduction

### 2.1.1 Forensic DNA Storage Issues

Millions of biological samples, including cells, viruses, and DNA/RNA, are stored every year for diagnostics, research, and forensic science. DNA extracts from forensic evidence samples such as hair, bones, teeth, and sexual assault evidence may contain less than 100 pg of DNA (Gill 2001; Gill et al. 2000; Phipps and Petricevic 2007; Smith and Ballantyne 2007). Low DNA yields may be due to damage (Budowle et al. 2005; Coble and Butler 2005; Irwin et al. 2007) or degradation (Eichmann and Parson 2008; Hill et al. 2008; Irwin et al. 2007); small cell numbers found in low copy number (LCN) or "touch" samples (Balogh et al. 2003; Budowle et al. 2009; Gill 2001; Gill et al. 2000; Hanson and Ballantyne 2005; Irwin et al. 2007; Kita et al. 2008; Kloosterman and Kersbergen 2003); oligospermic (Sibille et al. 2002) or aspermic perpetrators (Shewale et al. 2003); or low male DNA from extended interval postcoital samples in sexual assault cases (Hall and Ballantyne 2003). Trace biological evidence (e.g., fingerprints and touch evidence) may provide low yields (Balogh et al. 2003; Kita et al. 2008; Lagoa et al. 2008; Schulz and Reichert 2002; van Oorschot and Jones 1997; Wickenheiser 2002). Biological evidence may be consumed with the result that the DNA extracts may be the only remaining genomic resource to retest and test with new technologies for retrospective and prospective testing. Optimal storage of DNA is therefore critical to retrospective (retesting) or prospective (downstream analysis with additional or new genetic markers) testing (Clabaugh et al. 2007; Larsen and Lee 2005; Lee et al. 2012).

### 2.1.2 Factors Influencing DNA Stability

Degradation is a major factor in the ability to analyze low-quantity samples such as those derived from ancient or degraded bones and teeth and those from mass disasters (Budowle et al. 2005; Budowle et al. 2009; Irwin et al. 2007; O'Rourke et al. 2000; Paabo et al. 2004). Degradation results in the reduction or loss of the structural integrity of cells and the quantity and quality of genomic DNA. Many laboratories store DNA extracts frozen in Tris-Ethylenediaminetetraacetic acid (TE) buffer. However, reduction in DNA recovery may occur with refrigerated liquid DNA extracts and those repeatedly frozen and thawed (Davis et al. 2000; Shikama 1965) or stored in certain microcentrifuge tubes (Gaillard and Strauss 1998, 2001; Larsen and Lee 2005).

Low yields or loss of DNA due to these factors may preclude or diminish the ability to test LCN crime scene samples using current STR methods; therefore, other methods such as mini-amplicon STRs (Asamura et al. 2008; Coble and Butler 2005; Hill et al. 2008; Mulero et al. 2008; Opel et al. 2006, 2007) or less discriminating mtDNA testing

(Eichman and Parson 2008; Lee et al. 2008) are typically dictated for low-quantity samples in advanced states of degradation. The quantity and quality of template DNA from many low-copy forensic samples falls below recommended thresholds (0.5 to 1.25 ng; Collins et al. 2004) and ineffective storage only exacerbates further sample loss. Poor sample quality and the presence of inhibitors may lead to incomplete genetic profiles or no profile, reducing the probative value of the results.

In addition to sample quantity and intrinsic differences in sample types resulting in differences in quality and quantity, extrinsic differences resulting from (a) the effectiveness of the extraction method utilized, (b) the type and effectiveness of preservatives and storage buffers (e.g., presence of antimicrobial agents and nuclease inhibitors in the storage matrices and buffers), (c) purity, especially regarding the amount of nuclease contamination, (d) ionic strength, (e) tube material and quality, (f) exposure to UV, (g) temperature and humidity range and duration in short- or long-term storage, and (h) exposure to multiple freeze-thaw cycles (as occurs with repeated sampling or unexpected power loss), may all lead to differences in the ability to recover and retest the samples.

### 2.1.3 Typing Strategies of Nonoptimal Samples

Modifications to existing amplification and typing protocols such as mini-amplicons, whole genome amplification (Ballantyne et al. 2007; Hanson and Ballantyne 2005), and LCN protocols (Budowle et al. 2009; Gill 2001; Gill et al. 2000; Phipps and Petricevic 2007; Smith and Ballantyne 2007) to increase the DNA signal and consequently, the analytical success rate of challenged samples, are currently being investigated (Prinz et al. 2006; Roeder et al. 2009). Other approaches have been adopted that include addition of more *Taq* polymerase and Bovine Serum Albumin (J. Wallin of California Department of Justice, personal communication) and increasing cycle number or injection time (Forster et al. 2008). Amplifying DNA with over 28 cycles is widely used (Forster et al. 2008). Nested primer amplification and increased time and voltage for electrokinetic injection of samples have also improved profiling success (Lagoa et al. 2008). Post-PCR purification to remove any ionic components that compete with PCR products during electrokinetic injection has also been used to enhance results (Lederer et al. 2002).

New PCR enhancement reagents have also recently been reported (Le et al. 2008). PCRboost™ has been reported to enhance amplification of low-quality and low-quantity samples and those containing inhibitors such as hematin and humic acid (Le et al. 2008) as well as indigo dye (Wang et al. 2010). Although these approaches have resulted in some success, they have not been universally adopted by forensic DNA laboratories due to inconsistent results (especially on highly degraded, inhibited, or low-quantity samples), high cost, and/or additional validation requirements. The mini-amplicon multiplex AmfℓSTR® Minifiler™ (Mulero et al. 2008) has greatly improved the ability to amplify degraded samples; however, it does not contain all of the CODIS core loci. In addition, new STR multiplexes are continually being developed and optimized with the goal of enhancing amplification and improving results for highly degraded, inhibited, low-quantity samples.

These new developments in typing strategies of nonoptimal samples underscore the importance of DNA storage. New methods that push the lower limit of detection expand applications to extremely low-quantity and low-quality samples. Stable DNA storage and handling over time are therefore especially important when the amount of sample is limited.

## 2.2 Importance of Sample Storage

### 2.2.1 Forensic DNA Databanks and Casework Samples

The importance of DNA storage is obvious in the global growth and expansion of forensic DNA databases and repositories. The Combined DNA Index System (CODIS) currently has 9,875,100 offender profiles and 447,300 forensic profiles (Federal Bureau of Investigation 2012 http://www.fbi.gov/about-us/lab/codis/ndis-statistics). The European Network of Forensic Science Institutes, which includes 36 countries, reports offender profiles and 9,770,475 "stains" as of December 2011 (European Network of Forensic Science Institutes 2012, http://www.enfsi.eu/sites/default/files/documents/enfsi_survey_on_dna-databases_in_europe_december_2011_0.pdf). The Armed Forces DNA Identification Laboratory (AFDIL) provides worldwide scientific consultation, research, and education services in the field of forensic DNA analysis to the Department of Defense and other agencies. AFDIL provides DNA reference specimen collection, accession, and storage of United States military and other authorized personnel and processes thousands of samples in casework each year. Forensic DNA laboratories around the world also process thousands of samples each year. All of these samples need to be properly stored and maintained. In addition to these samples, many laboratories conducting forensic DNA casework and data banking also store casework extracts as well as dilutions of the extracted DNA samples. Finally, new international forensic DNA databases, expansion of database laws to include arrestees, missing persons databases, and additional DNA samples from new property-crime casework programs collectively increase the rate of growth and expansion of the number of stored DNA extracts.

### 2.2.2 Nonforensic DNA Databanks and Biobanks

In addition to the growth and expansion of forensic DNA databanks, several other types of DNA biobanks have been established. These include clinical biobanks to assist in the development of new medicines and drugs (Roden et al. 2008). For example, the United Kingdom Biobank has set a goal to collect, store, and eventually distribute half a million samples with related medical information from 30 to 35 clinics in Great Britain (Blow 2009). Additional efforts are underway at the Vanderbilt University School of Medicine in Nashville, Tennessee, where they are planning a 250,000-person DNA study, and the Oakland, California, Kaiser Permanente DNA Biobank of 500,000 samples (Blow 2009).

Several DNA banks have been established for studying human evolution. These include the worldwide Genographic project run by Dr. Spencer Wells and the National Geographic Society (Zalloua et al. 2008). The goal of this study is to analyze historical patterns in DNA from participants around the world to better understand our human genetic roots (Zalloua et al. 2008). Biodiversity DNA databanks have also been established supporting research on global diversity in response to extinctions. One such group hosts the DNA Bank Network (Zetzsche et al. 2009) with 10,448 taxa containing 32,532 DNA samples.

### 2.2.3 Mechanisms of DNA Loss

Understanding the different mechanisms of DNA loss provides a foundation for developing the most optimal methods for efficient storage of DNA. There is a body of literature that

describes how DNA may be damaged with exposure to temperature fluctuations such as freeze-thaw cycles (Davis et al. 2000; Shikama 1965). In addition, it is well known that both water and oxygen may damage DNA through hydrolysis and oxidative damage (Bonnet et al. 2010). Many laboratories have therefore explored other options for storage, including dry state storage.

The assumption is often made that if nucleic acids are dried they are then stable for long periods of time. However, it is becoming increasingly evident that degradation can occur during storage that can irreversibly damage the samples. For example, Lindahl (1993) reviewed evidence that DNA can undergo chemical changes such as depurination, hydrolysis, and oxidation even at low moisture content. Hofreiter et al. (2001) suggest that such chemical degradation might be responsible for the difficult recovery of DNA from aged samples. Although dried DNA is stable in the short term, it is nevertheless imperative to prevent detrimental chemical changes for optimal recovery.

More recently, dry-storage DNA damage has been studied and it was found that solid-state DNA degradation is greatly affected by atmospheric water and oxygen at room temperature (Bonnet et al. 2010). DNA may be lost by aggregation. As pointed out by Bonnet et al. (2010), loss by aggregation is highly significant since laboratory plastic tubes and plate seals generally are not airtight and therefore both water and oxygen may adversely react with DNA. In this study the authors also tested the stabilizing effects of the additive trehalose. In the presence of trehalose, solid-state natural DNA, heated to 120°C, does not denature (Zhu et al. 2007). This stabilization effect of trehalose may be explained by its ability to block the negative charges on the phosphates (water replacement) or by hydrogen bonding between trehalose and DNA, which may reduce the DNA structural fluctuations (vitrification hypothesis) (Alkhamis 2008; Zhu et al. 2007).

Mechanisms for DNA damage during storage have recently been reviewed (Bonnet et al. 2010). In addition to chemical damage, loss may also occur by the co-extraction and then subsequent action of nucleases that may not have been removed in the purification procedures. This is an important consideration in crude DNA extraction procedures such as Chelex (Walsh et al. 1991) that are then stored over time. In addition, loss may also occur during any additional manipulations of the DNA via purification through additional phase separations and column purifications. Finally, dilutions of DNA and subsequent storage in distilled water may also result in loss through damage by water (Bonnet et al. 2010).

## 2.3 DNA Storage and Handling Strategies

### 2.3.1 Tube Characteristics

It has been well documented that loss of DNA may occur due to the material and quality of the tubes used to store the samples (Kline et al. 2005; Larsen and Lee 2005). Polypropylene plastic microcentrifuge tubes that are routinely utilized in forensic DNA laboratories may retain DNA (Gaillard and Strauss 1998, 2001; Larsen and Lee 2005) with the amount of adsorbed DNA as high as 5 ng/mm$^2$ of tube wall (Gaillard and Strauss 1998). In addition, different tube lots from the same manufacturer have been reported to retain variable amounts (5–95%) of DNA (Gaillard and Strauss 2001). The authors suggest the use of polyallomar tubes or introducing 0.1% detergent, Triton-X 100, to prevent the retention of DNA on polypropylene tubes (Gaillard and Strauss 2001).

Polytetrafluoroethylene (PTFE, known commercially as Teflon®) tubes have also been compared to polypropylene for DNA storage (Kline et al. 2005). The researchers conducted an interlaboratory blind quantification study and reported that recovery of the low-target DNA concentration samples (50 pg), stored in PTFE tubes was 73% versus only 56% from samples stored in polypropylene. This suggests that at this low DNA concentration, a significant proportion of the sample DNA binds to the polypropylene walls and greater DNA recovery can be achieved with storage in PTFE-coated tubes (Kline et al. 2005).

### 2.3.2 Cold Storage

Among the most common strategies for DNA preservation are cold and dry storage strategies that include: (1) 4°C refrigeration, (2) –20°C, (3) –80°C, (4) –196°C (liquid nitrogen), and (5) dry storage on a solid matrix. Protection in the "dry state" and cryopreservation at –196°C both maintain the DNA in the glassy or vitreous state. In the glassy state, DNA and other molecules lose the ability to diffuse. This results in very little movement at the molecular level. In fact, "movement of a proton (the hydrogen ion) has been estimated to be approximately one atomic diameter in 200 years"; this in turn makes any chemical reactions highly unlikely over hundreds of years (Baust 2008, p. 251). If, however, moisture is reintroduced to the "dry state" or an increase in temperature occurs above the glass transition temperature of water (nominally –135°C), chemical reactions may start again resulting in DNA instability (Baust 2008).

Storage at –20°C to –80°C may provide adequate conditions depending on the quality and quantity of DNA needed for further testing and the duration of storage. Most forensic DNA laboratories utilize –20°C to –80°C freezers for storage. Forensic DNA research efforts have focused on developing new methods of amplification and typing with low-quality and low-quantity samples due in part to the observation that current storage methods are not optimal. Neither –20°C nor –80°C conditions have been shown to provide long-term storage quality equivalent to maintenance at liquid nitrogen temperatures (Baust 2008). Unfortunately, the storage of all forensic DNA extracts at liquid nitrogen temperature is not practical with over 15 million samples in U.S. and European forensic databanks alone.

As stated by Baust, "There are few studies that provide definitive answers to the question of optimal storage conditions for DNA" (Baust 2008, p. 251). The National Institute of Standards and Technology (NIST) and the National Cancer Institute have published data that suggest that "colder is better" and NIST has shown humidity control to be an important factor in stable storage. This is consistent with the fact that cryopreservation and dry-state storage both reduce DNA chemical reactivity.

Forensic DNA scientists face additional variables in optimizing DNA storage protocols. These variables include the initial contaminants that might be co-extracted with the DNA from crime scene samples, different DNA purities and final dilution buffers utilized in DNA extraction methodologies, the integrity of storage conditions including exposure to different temperatures, humidity and light, the tube material and efficiency of the seal, and downstream sample requirements. According to Baust, "Dry matrix storage should be dry and devoid of changes in moisture content … and cold conditions should rely on stable, noncycling temperatures" (Baust 2008, p. 251). That is, when storing samples, there should be no temperature fluctuations such as those found in frost-free cycles of most

modern refrigerators and there is a need to conduct comparative tests on DNA storage methodologies on forensic DNA samples over time using different storage approaches.

### 2.3.3 Dry Storage Comparisons

*Trehalose*: Smith and Morin (2005) conducted a comparison of different storage conditions with the addition of potential preserving agents. Dilutions of known concentrations of human placental DNA and gorilla fecal DNA were stored under four conditions (4°C, –20°C, –80°C, dry at room temperature), with three additives (TE buffer, Hind III digested Lambda DNA, and trehalose). The effectiveness of the different methods was tested periodically using qPCR and PCR assay of a 757 bp fragment. The highest quantity of DNA remained in samples stored at –80°C, regardless of storage additives, and those dried at room temperature in the presence of trehalose (Smith and Morin 2005). DNA quality was best preserved in the presence of trehalose, either dried or at –80°C; significant quality loss occurred with –20°C and 4°C storage (Smith and Morin 2005). These results indicate that dry storage with an additive such as trehalose may improve recovery of low-quantity and low-quality DNA versus traditional liquid extract freezer storage.

DNA storage tests under different conditions and a literature review has been conducted by the DNA Bank Network of Germany (Zetzsche et al. 2009). This organization was established in spring 2007 and is currently funded by the German Science Foundation (DFG) and was initiated by GBIF Germany (Global Biodiversity Information Facility). DNA bank databases of all their partners are linked and are accessible via a central Web portal (DNA Bank Network 2012 http://www.dnabank-network.org/Index.php) providing DNA samples of complementary collections (microorganisms, protists, plants, algae, fungi, and animals) to support biodiversity applications. In their reviews and the results of their tests they determined that long-term storage of DNA samples in buffer should be carried out at –80°C or below. Furthermore, as expected, dried, lyophilized DNA must be stored at low relative humidity to avoid DNA aggregation (DNA Bank Network 2012). They also determined that energy and environmental costs were the main reasons to support dry storage at ambient temperature (DNA Bank Network 2012).

*FTA® Technology*: FTA cards contain chemicals that lyse cells, denature proteins, and protect nucleic acids from nucleases, oxidative, and UV damage. Others have evaluated treated filter paper for collection and storage of buccal cells. The treated filter paper technology FTA (Sigurdson et al. 2006) is used in a room-temperature storage product, offered by GenVault (Carlsbad, California). Following 7 years of storage, the researchers found only modest DNA yields and reduced recovery that was insufficient for WGA (Sigurdson et al. 2006). However, others have shown good recovery from FTA paper for forensic DNA analysis resulting in full DNA profiles following years of dry storage (Fujita and Kubo 2006; Park et al. 2008; Smith and Burgoyne 2004; Tack et al. 2007).

*SampleMatrix®/QiaSafe®*: Biomatrica Inc. has developed a proprietary technology for the dry storage of biological materials at ambient temperatures. The key component of this technology is SampleMatrix (SM, also known as QiaSafe), a synthetic chemistry storage medium that was developed based on anhydrobiosis ("life without water"), a natural protective mechanism that enables survival of some multicellular organisms in extremely dry environments (Crowe et al. 1998). Such organisms can produce high concentrations of disaccharides, particularly trehalose, a nonreducing disaccharide of glucose, to protect their cellular structures during prolonged droughts and can be revived by simple

rehydration (Crowe et al. 1998). Recent evidence suggests that trehalose can preserve intact cells *in vitro* in the dry state (Wolkers et al. 2002). Trehalose disaccharides are predicted to interact with DNA molecules through minor groove interactions based on hydrogen bonding (Figure 2.1A). Biomatrica has developed proprietary synthetic compounds that mimic the protective properties of anhydrobiotic molecules with additional improvements that are especially pertinent to protecting DNA during dry storage. SM, a much improved synthetic formulation, is predicted to form similar interactive patterns with DNA as naturally occurring anhydrobiotic molecules (Figure 2.1B).

The protective properties of SM are based on its ability to form a stabilizing structure via glass formation at a higher temperature than natural disaccharides and therefore to provide improved protective properties as compared to trehalose (Clabaugh et al. 2007).

Storage of samples at different amounts demonstrated the protective properties of SM on DNA when PCR amplicons were detected in essentially all SM-protected samples at 70°C whereas unprotected samples showed more variable results. This is especially apparent in samples containing limited amounts of DNA (≤10 ng). It is noteworthy that the 4 ng samples stored at –20°C for 24 h resulted in markedly less amplicon than an identical sample stored dry in SM at 70°C (Figure 2.1C).

Stabilization of low-concentration DNA samples in SM has also been observed over 1 year (Ahmad et al. 2009; Lee et al. 2012). For this study, purified male and female DNA was extracted from buccal swabs using DNA IQ™ (Promega, Madison, Wisconsin) followed by quantification using the Quantifiler® Human DNA Quantification Kit (Applied Biosystems, Foster City, California). DNA samples from the male and female donors were serially diluted and added in replicates into SM multiwell plates and tubes for final DNA concentrations ranging from 4 ng to 0.0625 ng in a total of 20 μL of water. Replicate DNA samples ($n = 4$) at each concentration were applied into SM multiwell plates and then dried overnight in a laminar flow hood at room temperature. Samples were maintained inside a storage cabinet with desiccant included to create a humidity-controlled environment (SM+D samples). A separate set of samples was stored inside an identical storage cabinet without desiccant to assess the effects of uncontrolled humidity on sample stability when stored in SM (SM-D samples). Identical samples were also aliquoted into empty polypropylene microcentrifuge tubes and stored frozen at –20°C as standard in-house controls (Control) for comparison. The samples were either processed immediately (0 d), or 1 d, 1 week, 2 weeks, 1.5 months, 2 months, 3 months, 6 months, and 1 year prior to recovery and analysis by quantitative PCR followed by PowerPlex®16 (Promega, Madison, Wisconsin) STR analysis. Samples stored dry in SM were rehydrated with 20 μL of water and used directly in downstream applications without further purification to remove matrix components (Ahmad et al. 2009; Lee et al. 2012).

Recovered samples were quantified to determine the yield of DNA following dry storage in SM versus frozen control reference samples and also compared to initial quantification values obtained from the original DNA stock solutions at the time of sample preparation (0 d). Based on these quantification values, the average yield of DNA recovered following dry storage in SM under controlled humidity (SM +D-Red) conditions was dramatically improved as compared to samples stored without humidity control (SM-D-Beige) and in-house control (Control-Blue) samples stored frozen for 1 year (Figure 2.2A) (Ahmad et al. 2009; Lee et al. 2012). Similar results were detected after 4 months of storage where recovery of SM-stored DNA was significantly improved versus conventional polypropylene microfuge tubes (Figure 2.2B) (Clabaugh et al. 2007).