EXAMINATION OF FACTORS THAT AFFECT THE RECOVERY AND ANALYSIS OF DNA FROM SPENT CARTRIDGE CASINGS

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ABSTRACT

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Crimes involving firearms are extremely common, and it is therefore important that law enforcement be able to identify the individual who fired a weapon. Previous researchers have shown that DNA recovered from spent casings can be used to identify the loader of a firearm, however success has been highly variable, largely due to the wide variety of factors that have the potential to influence DNA on spent casings. The goal of this research was to test several such factors, including loading/firing order, pre-processing spent casings for fingerprints, cartridge caliber, swabbing strategy, and analysis technique. Forty caliber cartridges loaded by volunteers were fired and the casings were collected, two-thirds of which were fumed with cyanoacrylate to examine the influence of fuming on DNA recovery and analysis. Volunteers also loaded 0.45 and 0.22 caliber cartridges, which were swabbed individually or cumulatively in sets of three. DNA was extracted, quantified, and STRs were amplified using a MiniFiler™ and/or Fusion amplification kit. The HV1 and HV2 regions of mtDNA were also amplified. Cyanoacrylate fuming was found to have a negative effect on DNA recovery and analysis. Significantly more DNA was recovered from 0.45 caliber casings than from 0.22 caliber casings. Cumulative swabbing yielded more DNA and handler alleles than individual swabbing, although it also resulted in a higher number of mixtures. Fusion outperformed MiniFiler™ as an STR amplification kit, and mtDNA was successfully sequenced for all casings tested. Loading order was the only factor that did not have a significant effect, and as such all other variables should be strongly considered when DNA analysis from spent casings is undertaken.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Hundreds of thousands of crimes are committed using firearms every year in the United States (National Institute of Justice, 2013). From 1993 to 2011, approximately 70% of all homicides and 6 – 9% of all non-fatal violent crimes were committed using a firearm, the vast majority of which involved a handgun (Planty and Truman, 2013). Because firearm violence is so prevalent, it is crucial that law enforcement be able to identify the person who loaded and fired a weapon during the commission of a crime.

There are several methods for identifying the shooter when the weapon used in a criminal act is recovered from a scene. Fingerprints may be lifted from the firearm, although they are not commonly recovered (Saferstein, 2005). It is also possible to use serial numbers to help identify the shooter or owner of a gun. Every firearm is required by law to have a serial number, which is associated with the name of the owner when it is purchased (Saferstein, 2005), however criminals will commonly attempt to remove the serial number in an effort to make identifying the weapon more difficult. Forensic examiners can restore obliterated serial numbers through a variety of methods, including the use of magnetic particles and acid etching, although this is not always successful (Maiden, 2009). An additional challenge in using a serial number is that many firearms involved in crimes are not obtained legally, and therefore are not properly registered. A survey of state prison inmates revealed that only about 10% of firearms used to commit a crime were purchased from a legal source, while 40% were illegally obtained and 37% were acquired from family or friends (Planty and Truman, 2013). If the weapon is not recovered from a crime scene, or if the serial number cannot be used to identify the owner of the weapon, spent cartridge casings collected from the scene may provide valuable information that could be used to link an individual to both the crime and weapon.

Cartridge Casings: Class and Individual Characteristics

Though often referred to as a "bullet," firearm cartridges are actually composed of four elements: the projectile, the primer, the gunpowder, and the casing. The projectile for most pistol ammunition is a single bullet, while in shotgun ammunition the cartridge, or shell, contains a number of projectiles called shot, or a single slug. The primer is the component of the cartridge that is struck to generate a spark, which ignites the gunpowder and causes the gun to fire. Black powder was originally used in firearms, but modern weapons use smokeless powder (Saferstein, 2005). The casing is the metal container that surrounds the projectile, primer, and powder. Casings are commonly made of brass, although nickel, aluminum, and steel are also used (Saferstein, 2005). When a pistol or rifle is fired, the casing is ejected. It might be easy for a criminal to flee a crime scene with the firearm in hand, but locating and collecting cartridge casings after their ejection may be difficult and time consuming, so casings are often left behind and collected by crime scene investigators as evidence.

There are several class and individualizing features of cartridges that are used to associate them with a gun. One class level trait is the caliber of the ammunition, or the measurement of its width. For example, a 0.22 caliber bullet has a diameter of approximately 0.22 in, and the width of the casing may be the same or slightly wider. The caliber of the ammunition correlates with the caliber of the weapon designed to fire it, which helps firearms examiners identify the type of gun used to commit a crime. Other class characteristics of casings that can associate them with a particular make and model include the type of cartridge (rimfire or centerfire), type of rim (rimmed, semirimmed, rimless, belted, or rebated), shape and location of the firing pin impression, and presence and location of extractor and ejector marks, which are examined using a low-power microscope (Saferstein, 2005).

Individual characteristics are then examined to connect a casing to a particular firearm, rather than a class of firearms. For example, marks on the casing such as firing pin impressions, breech face marks, and ejector marks can all be used to associate the casing with a particular gun (e.g. Sarıbey et al., 2009). A firearm examiner first test fires the suspected firearm to obtain known cartridge casings for comparison, and the known and unknown samples are then evaluated using a comparison microscope. However, while this method is useful for associating a cartridge casing with a gun, it cannot identify the person who fired it.

Fingerprints on Spent Cartridge Casings

Fingerprints are placed on the casing surface when a cartridge is loaded into a magazine, which can be very useful as no two individuals are thought to share the same fingerprints. Such prints are used to identify the individual who loaded the weapon, if they are recovered. This can provide valuable evidence in a criminal investigation, although the person who loaded a firearm is not always the one who fires it. Given (1976) first examined the effect that firing had on the recovery of identifiable fingerprints from cartridge casings. Six volunteers handled sets of brass and nickel-plated 0.38 caliber cartridges, half of which were fired using a Smith & Wesson model 19, .357 Combat Magnum. The time between the handling and firing of cartridges varied, as did the time between firing and recovery of prints, which was attempted using black fingerprint powder. Time did not cause substantial degradation of the fingerprints, though it was proposed that the evaporation of water over time resulted in a decrease in the adherence of powder to the prints. Degradation of fingerprints as a result of firing was primarily due to blowback of hot gasses along surfaces of the casing not tightly sealed against the chamber wall. Another factor that influenced fingerprint detail was friction between the casing and gun barrel.

When the gunpowder ignites, internal pressure causes the casing to expand. Fingerprints were most commonly recovered from near the head of the casing (by the rim), possibly because it is where the metal of the casing is the thickest and therefore experiences less expansion and friction. Additionally, it was noted that while prints were recovered from nickel cartridges, they were recovered more successfully from brass cartridges, of which nearly all yielded an identifiable print.

Bentsen et al. (1995) furthered Given's work by testing a variety of methods to enhance latent fingerprints on spent casings. Fingerprints were rolled onto 0.38 caliber cartridges, which were fired after 1 hour using a 0.38 Webley revolver. Eleven methods were used to attempt to visualize fingerprints on the cartridge casings, including those that react both with the fingerprint and the substrate. The casings were evaluated for fingerprints, which were classified as identifiable if eight or more ridge characteristics were present. Of the 11 methods tested, vacuum cyanoacrylate (with fluorescent staining) and selenious acid treatments were most effective. However, the authors were not able to replicate the success of Given (1976), and noted that of the 104 murder/attempted murder cases in which the vacuum cyanoacrylate-fluorescent staining method was used in Northern Ireland between the years 1992 and 1993, only two yielded useable prints; one resulted from handling by investigators and the other was not identified.

The lack of success in obtaining useable fingerprints from spent cartridge casings was further demonstrated by Spear et al. (2005). Forty eight fingerprints, characterized as bloody, oily, or sweaty, were intentionally placed on cartridges, half of which were fired. Bloody prints were processed using amido black, while sweaty and oily prints were visualized using cyanoacrylate fuming followed by rhodamine 6G dye. Five useable prints were obtained from the unfired cartridges, of which two were bloody and three were oily. Only one bloody print was

recovered from the spent casings. Although half of the fingerprints that were recovered were bloody, the authors acknowledged that this type of print is not frequently encountered on casings submitted as evidence. Excluding bloody prints, only 3 out of 32 (9%) cartridges displayed useable prints, all of which were unfired. It was also noted that casings that did display a print were all of the larger caliber sizes used in the study (0.45 or 9 mm as opposed to 0.22).

Cyanoacrylate Fuming: Effect on DNA Recovery and Analysis

Cyanoacrylate fuming is a common method used to visualize latent fingerprints. First, cyanoacrylate is heated to form a gaseous vapor. When cyanoacrylate monomers interact with a fingerprint, they polymerize to form a white residue of poly(ethyl cyanoacrylate) (Dadmun, 2010). The speed of this reaction can vary greatly, and depends largely on the concentration of cyanoacrylate and the humidity of the air in the fuming chamber. Von Wurmb et al. (2001) examined the effect of cyanoacrylate fuming on polymerase chain reaction (PCR) efficiency. Blood was placed on glass slides in 5, 10, and 50 μL aliquots, while saliva was placed in 2, 5, 10 and 50 μL aliquots. All stains were allowed to dry overnight. Half of the slides for each body fluid were fumed with cyanoacrylate for 1 hr, while the remaining were left untreated. Samples were divided into two groups and extracted using either the Chelex method (Walsh et al., 1991) or an Invisorb Forensic Kit. Pure cyanoacrylate was also extracted and added to known amounts of control DNA to determine if it had an inhibitory effect. Short tandem repeats (STRs) were amplified using an AmpFLSTR® Profiler Plus® kit. The results showed cyanoacrylate had a negative effect on PCR efficiency. Fumed blood and saliva samples had reduced amounts of PCR product, though the Invisorb kit exhibited a smaller effect, as did control DNA mixed with cyanoacrylate extract, indicating it had an inhibitory effect on PCR.

The effect of cyanoacrylate fuming on the recovery of touch DNA from pipe bombs was examined by Gicale (2011). Twenty four volunteers each assembled two pipe bombs, one of which was fumed with cyanoacrylate for 15 min after deflagration. DNA was isolated using a double swab technique, in which the first swab was wetted with digestion buffer, followed by organic extraction, quantified using a Quantifiler® Human DNA Quantification Kit, and amplified using MiniFiler™. Slightly more DNA was recovered from fumed pipe bombs than from non-fumed bombs (averaging 29 and 19 pg, respectively), though the difference was not significant. Complete "consensus profiles[1]" were produced from 29% of fumed and 17% of non-fumed pipe bombs.

Touch DNA: Identification of an Individual

In the early years of forensic DNA analysis, large quantities of biological material were required to obtain a result. Consequently, most DNA profiling was performed on body fluids such as blood, semen, and saliva for which an ample amount of sample was available for testing. The amount of DNA needed to produce a profile has decreased greatly as technology has improved, and crime laboratories have been receiving more and more requests for the analysis of touch samples (Minor, 2013), or samples resulting from the transfer of cells through touch.

DNA is present in all nucleated cells of the body, including skin. Each human cell contains approximately 7 pg of DNA (Tiersch et al., 1989). Full STR profiles have been produced from 100 pg or less of DNA (e.g. Oostdik et al., 2014), corresponding to fewer than 20 cells. Several authors have stated that humans shed approximately 400,000 epithelial cells per day, so skin can be a valuable source of DNA in forensic cases (e.g. Wickenheiser, 2002;

[1] Six profiles were produced from each pipe bomb; only alleles that were "most consistent" among the profiles were included in the consensus profile.

Schiffner et al., 2005; Jenny, 2010). In addition to transferring shed epithelial cells onto touched objects, hands are also able to act as vectors for other cell types. Rubbing of the face, eyes, mouth, and nose deposits additional cells on the fingers, which can then be conveyed to another surface through touch.

The first published success in obtaining genetic information from touch samples was by van Oorschot and Jones (1997). They demonstrated that genetic profiles could be produced from swabbing handled objects including briefcase handles, pens, and car keys. These types of samples quickly became popular submissions to forensic laboratories, and DNA evidence was obtained from weapons such as knives, screwdrivers, and ligatures, as well as from door pulls, door bells, and adhesive tape involved in crimes (reviewed by Wickenheiser, 2002). The success of touch sample analysis, however, has remained highly variable. Researchers have shown that the amount of DNA transferred through physical contact depends on many variables, including the individual handler, the surface being handled, and on environmental conditions (Phipps and Petricevic, 2007; Daly et al., 2010). For example, rough, porous surfaces are more likely to yield DNA than smooth, non-porous ones (Daly et al., 2010). Surprisingly, the amount of time spent handling the substrate has not been found to affect the amount of DNA deposited, and full profiles have been reported from a contact time of 1 s from paper (Balogh et al., 2003a) and 5 s from fabrics (Linacre et al., 2010).

Several modifications to the procedures used by forensic scientists have been suggested to increase the success of DNA analysis from low template samples. The quantity of DNA collected via swabbing can be raised through the use of detergent-based solutions (Thomasma and Foran, 2013), and pre-treatment of centrifugal filtration devices has been shown to decrease DNA loss during extraction (Doran and Foran, 2014). Following extraction, the amplification of

STR alleles has been improved by increasing the number of PCR cycles (Gill, 2001) and reducing PCR reaction volumes (Gaines et al., 2002). Detection of alleles can be enhanced through post-PCR clean up and increased injection times (Smith and Ballantyne, 2007; Westen et al., 2009), which allows for the production of more complete profiles.

Despite these advances, challenges in processing low copy number samples remain, several of which were discussed by Budowle et al. (2009). Stochastic sampling, in which alleles are randomly sampled or amplified, may result in heterozygote peak imbalance and/or drop out of one or both alleles at a locus. Stutter peaks, which are generally less than 20% of the associated allele peak height in high template samples (e.g. Leclair et al., 2004), can be as tall as their parent allele, and in some instances might exceed the true peak's height. Contamination and drop-in can also have an intensity as strong as true alleles in low template samples, making interpretation difficult and unreliable. The most common method for overcoming these challenges is to perform replicate analysis, in which two or more aliquots of the sample are amplified separately (Budowle et al., 2009). A consensus profile can then be generated, including only alleles that are present in multiple profiles.

DNA from Spent Cartridge Casings

The analysis of touch DNA is becoming increasingly successful, but samples obtained from spent cartridge casings present additional challenges. DNA is deposited onto the surface of the casing during the loading process. However, when the cartridge is fired it is subjected to extremely high temperatures (the barrel of the gun may reach 1,200 °C when fired [Lawton, 2001]), pressure, and mechanical stress (U.S. Army Materiel Command, 1965), which likely have a strong degradative effect on DNA. Additionally, the metal composition of the casing and

the gunshot residue expelled during firing might inhibit PCR. Consequently, authors have stated that crime laboratories do not often attempt to recover DNA from spent casings (Horsman-Hall et al., 2009).

The feasibility of recovering DNA profiles from spent cartridge casings has been the focus of multiple studies over the past several years. Horsman-Hall et al. (2009) analyzed the effect that firing had on the recovery of DNA from spent casings. A single donor, said to leave behind substantial DNA in touch samples (although how this was determined was not described), handled ten cartridges. Five were loaded into a rifle by a gloved firearms examiner and were fired, while the remaining five were tested unfired. No magazine was used. DNA was recovered using a double swab technique, in which the first swab was wetted with 40 μL water, and was extracted using either an organic procedure (followed by Microcon® purification) or DNA IQ™ with one of three digestion buffers (proteinase K + 20% sarkosyl, DNA IQ™ Lysis Buffer, or proteinase K + SDS). DNA was quantified using a Plexor® HY System, and STRs were amplified using MiniFiler™, Identifiler®, and PowerPlex® 16 BIO kits. Organic extraction yielded significantly less DNA than the three DNA IQ™ methods. There was no significant difference between the DNA yields of the fired and unfired casings, which produced an average MiniFiler™ profile of 81 ± 20% and 85 ± 12%, respectively, indicating that firing did not affect DNA profiling. MiniFiler™ produced a significantly greater number of alleles than either PowerPlex® 16 BIO or Identifiler®.

Previous research at Michigan State University has shown that not only is the recovery and analysis of DNA from spent cartridge casings feasible (Orlando, 2011), but the optimization of recovery methods greatly increases its success. Mottar (2014) compared swabbing and soaking as means of recovering DNA, as well as three extraction methods. In total, five

recovery/extraction methods were compared: double swab/organic extraction, soak/organic extraction, double swab/QIAamp® DNA Investigator extraction, soak/QIAamp® DNA Investigator extraction, and single swab/Fingerprint DNA Finder® Kit extraction. Prior to comparing the five methods, pre-digestion treatments, soaking vessel, soak time, shaking during soak or digestion, and digestion time were optimized. Organic extractions were coupled with Amicon® filtration columns pretreated with yeast RNA (Doran and Foran, 2014). Volunteers loaded 0.40 caliber Smith & Wesson brass cartridges into the magazine of a firearm. The cartridges were fired and the casings were collected and assigned a recovery method. DNA was quantified using an *Alu* based rtPCR assay (Nicklas and Buel, 2005) and STRs were amplified using PowerPlex® Fusion. Organic extraction yielded significantly more DNA and STR alleles than either kit tested, and double swabbing proved superior to soaking. Overall, double swabbing followed by organic extraction was shown to be the most optimal method for recovering DNA from spent cartridge casings. This likely differed from the findings of Horsman-Hall et al. (2009) because Mottar (2014) pre-treated filtration columns with yeast RNA, decreasing DNA loss during organic extraction.

Mottar (2014) used the double swabbing technique developed by Sweet et al. (1997) when comparing swabbing and soaking, in which a surface is swabbed first with a wet swab and second by a dry one. All casings were swabbed individually, meaning one pair of swabs was used for each casing. However, some crime laboratories swab all casings from a crime scene which appear to have been fired from the same gun using the same pair of swabs (MSP, personal communication). This saves time and effort, as only a single pair of swabs has to be processed, instead of many. This cumulative swabbing approach has the potential to create mixtures if DNA from a different source is present on each casing, though, which has not previously been tested.

Loading and Firing Order

One variable that has the potential to influence the amount of DNA recovered from spent cartridge casings is the order in which the cartridges are loaded and subsequently fired. It is possible that most of the loose cells on an individual's fingers are deposited on the first cartridges loaded, with the number of cells decreasing with each subsequent cartridge. Conversely, the last cartridge loaded requires more force to load into the magazine, which might result in the transfer of a greater number of cells. Additionally, the temperature of the gun when it is fired may alter the amount of DNA that is present on a spent casing. The temperature inside of a gun will increase as more cartridges are fired, thus the first loaded (last fired) cartridge is exposed to the most heat, potentially having a degradative effect on DNA.

STR Analysis: Human Identification

The use of PCR to amplify DNA prior to analysis greatly reduces the amount of cellular material needed to produce a result. While previous techniques, such as restriction fragment length polymorphism analysis, required both high quantity (50 ng or more) and high quality (at least 12 kb) DNA, techniques employing PCR can amplify fragments less than 100 bp from as little as a single cell (Findlay et al., 1997). STR analysis is a PCR-based technique that is commonly performed in forensic biology (reviewed by Jobling and Gill, 2004). An STR is a short repeated sequence, which for forensic purposes typically has a repeat unit of four bases. The number of repeat units in a DNA strand is variable among individuals, which makes them useful targets for identity testing. A forensic scientist will analyze multiple STR loci, which combined have extremely high discrimination power among individuals.

Today, there is a wide variety of STR kits commercially available. Typical kits target amplicons between 100 and 450 bp (e.g. AmpFLSTR® Identifiler®, PowerPlex® 16). However, moving PCR primers closer to the STR region to reduce amplicon size allows for more successful amplification from degraded samples (Wiegand and Kleiber, 2001). As a result, new "miniplex" STR kits were introduced to the market, such as the AmpFℓSTR® MiniFiler™ PCR Amplification Kit, which targets nine loci all smaller than 300 bp and is advertised as being useful for degraded and challenging samples. Other "megaplex" kits have also been developed, such as the Promega PowerPlex® Fusion System that amplifies 24 loci, 14 of which are smaller than 300 bp, and Promega claims it is highly sensitive and inhibitor-tolerant, working well with low template samples.

MtDNA Analysis: Human Identification

Human mitochondrial DNA (mtDNA) is a circular genome of approximately 16,569 bp. Most of the genome comprises 37 essential genes, and is therefore not highly polymorphic (and consequently not useful for identity testing) (Anderson et al., 1981; Holland and Parsons, 1999). There is, however, a 1,122 bp non-coding "control" region which includes two hypervariable regions, HV1 and HV2. These regions are 341 and 267 bp in size, and are commonly targeted by forensic scientists (reviewed by Holland and Parsons, 1999).

MtDNA analysis is of great value to forensic science because mtDNA is often still recoverable after nuclear DNA has degraded. While nuclear DNA is present in only two copies, there are hundreds of mtDNA copies per cell (Robin and Wong, 1988), making it more likely that a profile can be obtained from low template samples. Multiple characteristics of mtDNA also protect it from degradation (Foran, 2006). It is possible that the circular nature of mtDNA

prevents exonucleases from digesting it. Additionally, mtDNA is located in the mitochondria of the cell, rather than in the nucleus, and is protected by the mitochondria themselves. Due to these factors, mtDNA profiling has been highly successful when working with ancient and degraded samples (e.g. O'Rourke et al., 2000). There are, however, limitations to the use of mtDNA analysis. MtDNA it is maternally inherited, so it is not unique to the individual and therefore cannot be used for positive identification. This maternal inheritance can be useful, though, when a reference sample for an individual is not available but a sample can be obtained from a maternal relative.

Metchikian (2013) previously studied the feasibility of mtDNA analysis from spent cartridges casings at Michigan State University. DNA extracts were used from a separate study (Orlando, 2011) in which volunteers loaded cartridges into a magazine, the cartridges were fired, the casings were collected, and DNA was extracted. HV1 was amplified and sequenced as two pieces (HV1a and HV1b), and the first half of HV2 was analyzed. Haplotypes were obtained from all casings, about two thirds of which were consistent with the handler (although most were mixed profiles), indicating that mtDNA analysis can potentially be used to identify the loader of a firearm.

Goals of This Study

Despite improvements in the recovery of DNA from spent cartridge casings, STR profiling success remains highly variable (Horsman-Hall et al., 2009; Branch, 2010; Mottar, 2014). Therefore, it is important to understand the many factors that may affect both the quantity and quality of genetic information produced from spent casings. The purpose of this research was to examine several such variables to determine what, if any, effect they have on DNA

recovery and analysis. Two different collections of casings were used for this study. The goal of the first collection was to examine the effect of cyanoacrylate fuming on the recovery and analysis of DNA from spent casings, in order to determine if it is advisable to fume casings prior to processing, and whether fuming casings immediately upon collection is superior to transporting them back to the laboratory prior to fuming. Collection 1 was also used to compare two commercial STR kits (AmpFℓSTR® MiniFiler™ and PowerPlex® Fusion). The goals of the second collection included determining if cartridge caliber has an effect on recoverable DNA from spent casings and examining if swabbing multiple casings with a single pair of swabs is advantageous or disadvantageous. Finally, the second collection was used to compare the success of mtDNA sequencing with STR profiling. The influence of cartridge loading/firing order was examined using both collections. All of these variables were examined in an attempt to improve the success of identifying the loader of a firearm using DNA from spent casings.

METHODS

Cartridge Casing Collection

Cartridges were fired and casings collected on two occasions. American Eagle® Federal Premium Ammunition and Blazer® Brass 0.40 S&W ammunition was used during Collection 1, while Federal® American Eagle® 0.45 Auto center fire pistol cartridges and Federal Premium® Champion Target 0.22 LR rim fire cartridges were used during Collection 2. One to four cartridges from each box of ammunition were randomly selected and DNA was extracted and quantified to determine if background DNA was present. The resulting quantities were low, so ammunition was not cleaned prior to handling.

Cartridges were placed in paper bags in sets of 21 for Collection 1. Researchers wore lab coats, sleeves, gloves, face masks, and hair nets during this process, and cartridges were transferred using hemostats which had been UV irradiated for 5 min on each side (approximately 5 J/cm^2) in a Spectrolinker XL-1500 UV Crosslinker and rinsed with 70% ethanol. Each volunteer at the Michigan State Police (MSP) Lansing Forensic Laboratory shooting range loaded one set of cartridges into two magazines of a 0.40 caliber handgun (12 in one magazine and 9 in the other). The volunteers did not wear gloves during the loading process. The magazines were loaded into the firearm by a MSP firearms examiner wearing gloves and a lab coat, and fired. The gun was fired through a denim microscope cover with a hole cut in the corner so that the casings could be collected without falling to the floor. Casings were collected in sets of three using hemostats, and were placed in new paper bags which were assigned different treatments (Appendix A). Fifty percent bleach was placed on a Kimwipe (Kimberly-Clark Corporation, Irving, TX) and used to wipe the hemostats before cartridges loaded by each new volunteer were fired.

Prior to Collection 2, cartridges of each caliber were divided into sets of 12 in separate paper bags. Researchers wore the same personal protective equipment as in Collection 1, and hemostats used to transfer the cartridges were UV irradiated for 5 min on each side (approximately 5 J/cm^2 total) in a Spectrolinker XL-1500 UV Crosslinker (Spectronics Corporation) and rinsed with 70% ethanol. Volunteers loaded sets of cartridges into two magazines for each caliber. Half of the volunteers loaded the 0.45 caliber cartridges first and half loaded the 0.22 caliber cartridges first. The magazines containing the 0.45 caliber cartridges were loaded into a 0.45 caliber handgun and the magazines containing the 0.22 caliber cartridges were loaded into a 0.22 caliber rifle. The weapons were fired through a pop-up mesh laundry hamper so that casings could easily be collected in sets of three. Casings were transferred to paper bags using hemostats (which were wiped with 50% bleach before cartridges loaded by each new volunteer were fired), and were assigned different swabbing methods (Appendix A).

Each volunteer provided two buccal swabs, which were stored in 12 x 75 mm polypropylene culture tubes (Fisher Scientific, Waltham, MA). Volunteers were randomly assigned both a letter and a number, so as to keep the samples de-identified. Bags of spent casings were labeled with the volunteer number, while buccal swabs were labeled with the volunteer letter. Buccal swabs and casings were stored at -20 °C. The use of human volunteers was approved by the MSU Institutional Review Board (IRB 12-770).

Cyanoacrylate Fuming of Collection 1 Casings

Three casings from each volunteer in Collection 1 were taken to the MSP fingerprint unit and fumed using MSP's standard protocol. The first step of this protocol was to raise the humidity inside the chamber. This was followed by a fuming step and a ventilation step. The

entire process took 1 – 1.5 hr. Another three casings from each volunteer were returned to the MSU Forensic Biology Laboratory to be fumed. The MSU fuming chamber consisted of an electric candle warmer (Rimports USA LLC, Provo, UT) in the center of a 24 x 16 x 13 in, 15-gallon plastic storage container (Incredible Plastics, Warren, Ohio). A beaker containing 200 mL of water was placed on the candle warmer. Casings were positioned in the chamber on weigh paper surrounding the candle warmer and the container was closed. After 15 min, a tea-light foil container holding approximately 4 mL of cyanoacrylate was added to the candle warmer, and the casings were fumed for 20 min. The cyanoacrylate was removed from the container, which was left slightly open to ventilate for 10 min before the casings were removed and placed back in their corresponding paper bags. Sets of 15 – 18 casings were placed in the fuming chamber at a time, and this process was repeated three times. The casings were stored at -20 °C.

The remaining casings were not fumed, and were used for a separate study. All data from non-fumed casings used in this research were taken from Collection 2 of Mottar (2014).

DNA Isolation and Digestion

Cotton swabs (860-PPC, Puritan Medical Products, Guilford, ME) and 1.5 mL microcentrifuge tubes were autoclaved prior to use. Microcentrifuge tubes and other supplies (e.g. scissors, hemostats, pipettes/pipette tips, reagents, etc.) used in all pre-amplification procedures were UV irradiated for 10 min (approximately 5 J/cm^2), or 5 min on each side, in a Spectrolinker XL-1500 UV Crosslinker. All water was purified through a Milli-Q® Water Purification System (Millipore Corporation, Billerica, Massachusetts) and filtered using a 0.22 µm Millex®-GS syringe driven filter unit (Millipore Corporation). A lab coat, face mask, sleeves, and two pairs of gloves were worn whenever handling casings or pre-amplified DNA.

DNA Isolation and Digestion: Collection 1

DNA was recovered from each casing using a double swabbing method (Sweet et al., 1997), in which the first swab was wetted with 150 µL of digestion buffer (0.1% SDS, 20 mM Tris [pH 7.5], 50 mM EDTA) and the second swab was dry. Casings were held using hemostats during swabbing. The cotton tip of each swab was cut from the shaft using a pair of scissors and both were placed in a single 1.5 mL microcentrifuge tube containing 400 µL digestion buffer and 5 µL proteinase K (20 mg/mL). A reagent blank was created by pipetting 150 µL digestion buffer onto a swab and placing it and a second dry swab directly into a 1.5 mL tube containing the same reagents. The tube was vortexed for 10 s and placed in an incubator at 55 °C, shaking at 900 rpm, for 1 h. Hemostats and scissors were cleaned with 70% ethanol between each casing.

DNA Isolation and Digestion: Collection 2

DNA was isolated as described above for the individually swabbed casings. Three casings were used for each cumulatively swabbed sample, and each casing was held by a separate pair of hemostats. The first wetted swab was used to swab the outside surface of each of the three casings (from a single paper bag), followed by a dry swab. Both swabs were placed in a single tube and digested as above.

DNA Isolation and Digestion: Buccal Swabs

Buccal swabs were placed in 1.5 mL tubes containing 500 µL digestion buffer and 5 µL proteinase K. The tubes were vortexed for 10 s and incubated at 55 °C for 1 hr.

Organic DNA Extraction

Amicon® Ultra-0.5 mL, 30 kDa filtration columns (Millipore Corporation) were pre-treated using yeast (*Saccharomyces cerevisiae*) RNA. One microliter of 10 µg/µL RNA (Alfa Aesar, Ward Hill, MA) and 499 µL low TE (10 mM Tris [pH 7.5], 0.1 mM EDTA) were applied to the columns, which were centrifuged at 14,000 g for 10 min (Doran and Foran, 2014).

The lysate from each swab was collected using a spin basket (Fitzco, Spring Park, MN) at 20,000 g for 4 min. The flow through was combined with the remaining solution from the digestion tube. Five hundred microliters of phenol was added to the digestion tube, which was vortexed for 10 s and centrifuged at maximum speed for 5 min. The aqueous layer was removed and transferred to a new tube containing 500 µL chloroform, which was vortexed and centrifuged at maximum speed for 5 min. The aqueous layer was removed and transferred to a pre-treated Amicon® column. The column was centrifuged at 14,000 g for 10 min, and the flow through was discarded. Three hundred microliters TE (10 mM Tris [pH 7.5], 1 mM EDTA) was added, and the column was centrifuged at 14,000 g for 10 min. This step was repeated two more times using 300 µL low TE, after which the column was inverted into a clean tube and centrifuged at 1,000 g for 3 min. The volume recovered was measured and DNA extracts were stored at -20 °C.

DNA Quantitation Using Real-Time PCR

Quantitation standards were created at concentrations of 2000, 200, 20, 2, 0.2, and 0.02 pg/µL by diluting Standard Reference Material® 2372 Human DNA Quantitation Standard Component A (genomic DNA from a single male donor; 57 ng/µL; National Institute of Standards and Technology, Gaithersburg, MD) in low TE with 20 µg/mL glycogen. *Alu* and IPC

primer and probe sequences can be found in Table 1. Both forward and reverse *Alu* primers were filtered through Microcon YM-100 membranes (Millipore Corporation) prior to use.

Table 1. Primer, probe, and IPC template sequences for real-time PCR. HEX and 6FAM are fluorescent dyes attached to the 5′ end of the probes. BHQ1 and IABkFQ (Iowa Black® FQ) are quenchers attached to the 3′ end of the probes. ZEN is an internal quencher. The Alu primers and probe were designed by Nicklas and Buel (2005). The IPC primers, probe, and template were designed by Lindquist et al. (2011).

Primer Name	Sequence
Alu F	5′-GAG ATC GAG ACC ATC CCG GCT AAA-3′
Alu R	5′-CTC AGC CTC CCA AGT AGC TG-3′
IPC F	5′-AAG CGT GAT ATT GCT CTT TCG TAT AG-3′
IPC R	5′-ACA TAG CGA CAG ATT ACA ACA TTA GTA TTG-3′
Alu Probe	5′-HEX-GGG CGT AGT GGC GGG-BHQ1-3′
IPC Probe	5′-6FAM-TAC CAT GGC-ZEN-AAT GCT-IABkFQ-3′
IPC Template	5′-AAG CGT GAT ATT GCT CTT TCG TAT AGT TAC CAT GGC AAT GCT TAG AAC AAT ACT AAT GTT GTA ATC TGT CGC TAT GT-3′

Real-time PCR reactions were set up in 15 µL volumes, consisting of 7.5 µL iQ Supermix™ (Bio-Rad Laboratories, Hercules, CA), 1 µL *Alu* forward primer (7.5 µM), 1 µL *Alu* reverse primer (13.5 µM), 1 µL *Alu* probe (3.75 µM), 0.75 µL IPC forward primer (20 µM), 0.75 µL IPC reverse primer (20 µM), 0.25 µL IPC probe (15 µM), 1 µL IPC template DNA (1:1 billion dilution of 100 µM stock), 0.125 µL Syzygy Taq DNA polymerase (5 U/µL; Empirical Bioscience, Grand Rapids, MI), 0.625 µL water, and 1 µL DNA extract. DNA standards were run in duplicate and a negative control was included in each assay. Reactions were set up in 0.2 mL optically clear flat-capped PCR strips (USA Scientific®, Ocala, FL). PCR amplification was performed on an iCycler™ Thermal Cycler (Bio-Rad Laboratories). The cycling parameters were 3 min at 95 °C, followed by 50 cycles of 15 s at 95 °C and 1 min at 60 °C. Fluorescence was detected using an iQ™5 Multicolor Real-Time PCR Detection System (Bio-Rad

Laboratories), and data were analyzed using iQ™5 Optical System Software. The measured concentrations were multiplied by the DNA extract volumes to calculate DNA yield.

STR Analysis of Spent Casing DNA

PowerPlex® Fusion: STR Amplification

STRs were amplified using a PowerPlex® Fusion System (Promega, Madison, WI) and an Applied Biosystems 2720 Thermal Cycler (Life Technologies, Carlsbad, CA). Six microliters of DNA extract from each casing was added to 2 µL 5X Master Mix and 2 µL 5X Primer Pair Mix in a PCR tube. DNA extracts from buccal swabs were diluted 1:300 with water, and 1 µL was added to 5 µL water, 2 µL 5X Master Mix, and 2 µL 5X Primer Pair Mix. Amplification was conducted using an initial denaturation step of 96 °C for 1 min, 30 cycles of 94 °C for 10 s, 59 °C for 1 min, and 72 °C for 30s, and a final 10 min 60 °C extension.

Amplified DNA was denatured at 95 °C for 3 min and placed on ice for 3 min. One microliter was added to 10 µL Hi-Di™ Formamide (Life Technologies) and 1 µL CC5 Internal Lane Standard 500 (Promega). DNA was electrophoresed on an AB3500 Genetic Analyzer (Life Technologies). Capillary electrophoresis was performed using the parameters: oven temperature 60 °C; pre-run voltage 15 kV; pre-run time 180 s; injection voltage 1.2 kV; injection time 24 s; run voltage 15 kV; run time 1500 s; capillary length 50 cm.

Allele calls were made using GeneMapper® v4.1 software (Life Technologies) at a threshold value of 100 relative fluorescence units (RFUs) and were verified using OSIRIS v2.2 (Goor et al., 2011). Alleles were compared to the reference profiles and were classified as consistent or not consistent with the handler. Percent profiles were calculated by dividing the number of consistent alleles by the total number of possible alleles for that individual.

MiniFiler™: STR Amplification

Forty of the DNA extracts from the fumed casings in Collection 1 with the highest DNA yields were amplified using an AmpFℓSTR® MiniFiler™ PCR Amplification Kit (Life Technologies). PCR reactions were set up in a final volume of 10 µL, consisting of 4 µL AmpFlSTR® MiniFiler™ Master Mix, 1 µL AmpFlSTR® MiniFiler™ Primer Set, and 5 µL DNA. PCR cycling conditions were 11 min at 95 °C followed by 30 cycles of 20 s at 94 °C, 2 min at 59 °C, and 1 min at 72 °C, and a final extension step of 45 min at 60 °C.

One microliter of amplified DNA was added to 9 µL Hi-Di™ Formamide and 0.3 µL GeneScan™ 500 LIZ® Size Standard (Life Technologies). Capillary electrophoresis was performed using the parameters: oven temperature 60 °C; pre-run voltage 15 kV; pre-run time 180 s; injection voltage 1.6 kV; injection time 8 s; run voltage 19.5 kV; run time 1330 s; capillary length 50 cm. Allele calls were made as above.

MtDNA Sequencing of Spent Casings

Mitochondrial DNA was analyzed from 96 extracts from Collection 2. Samples were divided into three groups based on DNA quantitation (high, medium, and low), and eight of each type (individually swabbed 0.45 caliber casings, cumulatively swabbed 0.45 caliber casings, individually swabbed 0.22 caliber casings, and cumulatively swabbed 0.22 caliber casings) were selected for mtDNA sequencing from each group. HV1 and HV2 were amplified and sequenced, using the primers in Table 2.

Table 2. Primers used to amplify and sequence mtDNA from casings and reference samples. All samples were amplified with F15989, R16410, F15, and R499. F16190 and R285 were used when sequences failed or were not suitable for analysis.

Primer Name	Region	Sequence
F15989	HV1	5′-CCC AAA GCT AAG ATT CTA AT-3′
R16410	HV1	5′-GAG GAT GGT GGT CAA GGG AC-3′
F16190	HV1	5′-CCC CAT GCT TAC AAG CAA GT-3′
F15	HV2	5′-CAC CCT ATT AAC CAC TCA CG-3′
R499	HV2	5′-CGG GGG TTG TAT TGA TGA GAT T-3′
R285	HV2	5′-GTT ATG ATG TCT GTG TGG AA-3′

PCR was conducted in 30 µL reactions, consisting of 3 µL GeneAmp 10X PCR Buffer II (Life Technologies), 3 of µL 25 mM $MgCl_2$ (Life Technologies), 3 µL of 2 mM deoxynucleoside 5'-triphosphates, 3 µL 4 mg/mL bovine serum albumin (BSA; Fisher Scientific), 3 µL of 20 µM forward primer, 3 µL of 20 µM reverse primer, 11 µL water, 1 unit AmpliTaq Gold® polymerase (Life Technologies), and 1 µL template DNA. Cycling parameters were 10 min at 94 °C, 38 cycles of 30 s at 94 °C, 30 s at 60 °C, and 30 s at 72 °C, and a final extension of 5 min at 72 °C.

Five microliters of PCR product was electrophoresed on a 1% agarose gel. Post PCR clean-up was performed using Diffinity RapidTips® (Diffinity Genomics, Inc., West Henrietta, NY). PCR products were aspirated through a RapidTip approximately 15 times, and were transferred to a new tube.

Sequencing reactions included 2.5 µL BigDye® Terminator v3.1 Cycle Sequencing master mix (1.82 µL of BDX64 BigDye® enhancing buffer [MCLAB, San Francisco, CA], 0.68 µL of BigDye® Terminator v3.1 Ready Reaction Mix [Life Technologies]), 1 µL 20 µM forward or reverse primer, 1 – 3 µL amplified DNA, and water to a final volume of 10 µL. Cycling parameters were 3 min at 96 °C followed by 30 cycles of 10 s at 96 °C, 5 s at 50 °C, and 2 min at 60 °C.

Sequencing reactions were added to 2.5 µL stop solution (1 µL of 3 M sodium acetate, 1 µL of 100 mM EDTA, and 0.5 µL of 20 mg/mL glycogen) in a 1.5 mL tube. Thirty-five microliters cold 95% ethanol was added to each sequencing reaction, which was vortexed for 10 s and centrifuged at maximum speed for 10 min. The supernatant was removed, and the pellet washed with 180 µL cold 70% ethanol. The samples were centrifuged at maximum speed for 5 min, and the supernatant removed. The 70% wash step was repeated two more times, and DNAs were vacuum dried for 10 min. Ten microliters of Hi-Di™ Formamide was added and was vortexed for 10 s.

DNAs were electrophoresed on an AB3500 Genetic Analyzer using the parameters: oven temperature 60 °C; injection time 8 s; injection voltage 1.6 kV; run time 1400 s; run voltage 19.5 kV; capillary length 50 cm. Sequences were aligned and analyzed using BioEdit v7.2 software (Hall, 1999), and compared to the Cambridge Reference Sequence (Anderson et al., 1981). Polymorphisms were identified and compared to volunteer reference sequences, and profiles were classified as consistent, inconsistent, mixed-consistent, or mixed-inconsistent (Table 3). Mixtures were identified when two peaks were detected at the same position in both the forward and reverse sequences.

Table 3. Description of categories used in mtDNA analysis.

Category	Description
Consistent	Profile was consistent with the handler
Inconsistent	Profile was not consistent with the handler
Mixed-Consistent	Profile consisted of a mixture of individuals, including the handler
Mixed-Inconsistent	Profile consisted of a mixture of individuals, not including the handler

Amplification of MtDNA from Reagent Blanks

HV2 was amplified from 21 reagent blanks, selected at random. PCR was conducted in 10 µL reactions, consisting of 1 µL GeneAmp 10X PCR Buffer II (Life Technologies), 1 of µL 25 mM MgCl2 (Life Technologies), 1 µL of 2 mM deoxynucleoside 5'-triphosphates, 1 µL 4 mg/mL bovine serum albumin (BSA; Fisher Scientific), 1 µL of 20 µM forward primer (F15), 1 µL of 20 µM reverse primer (R499), 3 µL water, 1 unit AmpliTaq Gold® polymerase (Life Technologies), and 1 µL template DNA. Cycling parameters were 10 min at 94 °C, 38 cycles of 30 s at 94 °C, 30 s at 60 °C, and 30 s at 72 °C, and a final extension of 5 min at 72 °C. Five microliters of PCR product was electrophoresed on a 1% agarose gel.

Statistical Analysis

Statistical tests were performed using XLSTAT 2014.2.01 (Addinsoft, Paris, France) with a significance level of 0.05. A Shapiro-Wilk test was conducted to determine normality for all nuclear DNA data (including quantification and STR results), a Kruskal-Wallis test was used to make multiple comparisons, and pairwise comparisons were made using Mann-Whitney. A Bonferroni correction was applied to account for multiple pairwise comparisons within a set of data. Box plots were constructed to illustrate the distribution of the data. Fisher's exact test was conducted to determine whether quantitation level, swabbing strategy, and cartridge caliber had a statistically significant effect on mtDNA profile classification.

RESULTS

Loading, Collecting, Cyanoacrylate Fuming, and DNA Isolation of Cartridge Casings

Volunteers had a broad range of experience with loading cartridges, and as a result the amount of time spent handling each cartridge varied from several seconds to a couple of minutes. The microscope cover used to capture casings during Collection 1 was relatively inefficient, as multiple casings fell out and made contact with the firing range floor. The pop-up hamper utilized in Collection 2 was simpler to use, as the mesh made it easier to find and grasp casings, and resulted in few or none of them being dropped.

The casings fumed at MSP had a white residue coating the outer surface, exemplified in Figure 1. The first set of casings fumed at MSU did not have a change in appearance, though a similar white residue was present on the second and third sets.

Figure 1. Example of a non-fumed (left) and fumed (right) casing covered in a white residue.

White flecks were present in the interface between the organic and aqueous layers during the phenol and occasionally the chloroform extractions, which were not transferred to the Amicon® column. The extraction of DNA from both fumed and non-fumed casings resulted in

black residue at the bottom of the tube following centrifugation of the spin baskets and during the phenol extraction. Solutions were clear after the chloroform extraction and Amicon® purification.

Reagent Blanks: Quantitation and MtDNA Amplification Results

DNA quantities of reagent blanks were low, with a median concentration of 0.009 pg/µL. One reagent blank quantified much higher than all of the others (2.11 pg/µL), however mtDNA from it failed to amplify. DNA from only one reagent blank, which had the second highest concentration, amplified mtDNA, and the resulting band (visualized via gel electrophoresis) was faint.

Collection 1: Effect of Fuming on DNA Recovery and Analysis from Spent Casings

Comparison of DNA Yields from Fumed and Non-Fumed Casings

The median DNA yields of fumed and non-fumed casings and the distributions of the data are shown in Figures 2 and 3, respectively. The non-fumed casings resulted in a DNA yield of 25.86 pg, while 11.53 was recovered from the MSU-fumed, and 4.95 pg from the MSP-fumed casings. Descriptive statistics are in Table 4. DNA yields were not normally distributed (Shapiro-Wilk, $p < 0.0001$), and there was a significant difference among the non-fumed, MSU-fumed, and MSP-fumed casings (Kruskal-Wallis, $p < 0.0001$). Pairwise comparisons (Table 5) showed that all differences in DNA yield were significant. Appendix B contains the DNA concentration and yield from each casing.

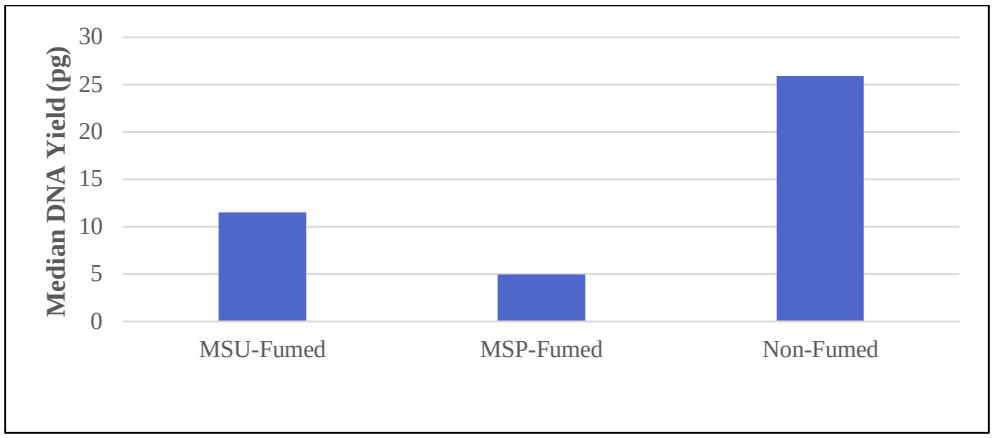

Figure 2. Median DNA (pg) yields among the fumed and non-fumed casings. Data for the non-fumed casings taken from Mottar (2014).

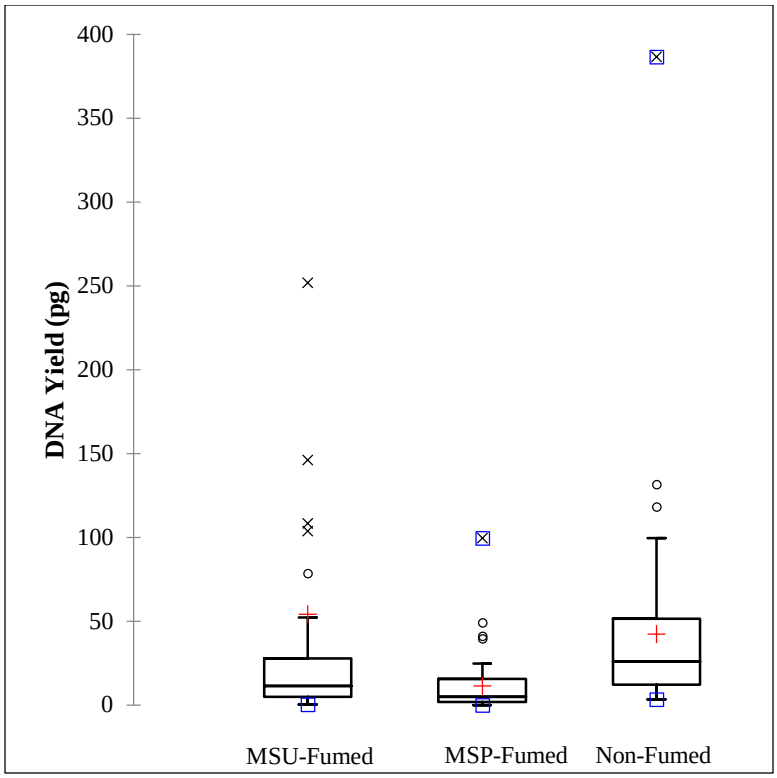

Figure 3. Box plots displaying the distribution of the DNA yields (pg) of fumed and non-fumed casings. The box encompasses the interquartile range (the distance between the lower and upper quartiles), with the line through the box symbolizing the median. The mean is represented by a red +, extreme outliers are represented by x, mild outliers are represented by ◦, and maximum/minimum values are represented by blue squares. The whiskers represent the maximum/minimum values that are not outliers. The MSU-fumed casings contained an extreme outlier at 1420.2 pg that is not shown. Data for the non-fumed casings taken from Mottar (2014).

Table 4. Descriptive statistics of quantitation results of fumed and non-fumed casings. Data for the non-fumed casings taken from Mottar (2014).

		MSU-Fumed	MSP-Fumed	Non-Fumed
DNA Concentration (pg/uL)	**Median**	0.57	0.18	0.99
	Average	2.15	0.41	1.65
	Standard Deviation	7.46	0.58	2.37
DNA Yield (pg)	**Median**	11.53	4.95	25.86
	Average	53.94	11.47	42.33
	Standard Deviation	199.97	16.88	57.95
	n	51	51	51

Table 5. Pairwise comparisons of DNA yields (pg) for fumed and non-fumed casings (Mann-Whitney, Bonferroni corrected $\alpha = 0.0167$). Data for the non-fumed casings taken from Mottar (2014).

Pair		P-Value
MSU-Fumed	MSP-Fumed	0.0065
MSU-Fumed	Non-Fumed	0.0024
MSP-Fumed	Non-Fumed	< 0.0001

The median DNA concentration was 0.33 pg/μL for the first set of casings fumed at MSU, 0.82 pg/μL for the second set, and 0.58 pg/μL for the third. The DNA concentrations were not normally distributed, and there was no significant difference among the groups (Kruskal-Wallis, $p = 0.561$).

Comparison of Commercial STR Kits: MiniFiler™ and Fusion

Table 6 displays the median number of handler alleles, non-handler alleles, and percent profile produced using MiniFiler™ and Fusion from the subset of casings that were amplified using both kits. MiniFiler™ produced a median of 2 alleles consistent with the handler from casings fumed both at MSU and at MSP and 11 for non-fumed casings, while Fusion resulted in medians of 10 (MSU-fumed), 15 (MSP-fumed), and 23 (non-fumed). MiniFiler™ produced a median of a 13% profile for both MSU and MSP fumed casings, while Fusion produced 25% (MSU-fumed) and 36% (MSP-fumed) profiles. The non-fumed casings resulted in median profiles of 67% (MiniFiler) and 60.5% (Fusion). The MSU-fumed casings, MSP-fumed casings, and non-fumed casings produced median percent Fusion profiles of 31%, 42%, and 70%, respectively, when only the loci smaller than 300 bp were examined. Fusion also generated a higher number of non-handler alleles, resulting in medians of 10 (MSU-fumed), 4 (MSP-fumed) and 3 (non-fumed), compared to medians of 2 (MSU-fumed), 0 (MSP-fumed), and 1 (non-

fumed) amplified using MiniFiler™. Pairwise comparisons (Table 7) showed that all but three differences between MiniFiler™ and Fusion were significant (percent profiles from the MSU-fumed casings, percent profiles from the non-fumed casings, and number of non-handler alleles from the non-fumed casings). Appendix C contains the STR profile for each DNA extract (from casings and buccal swabs) amplified with MiniFiler™ and Fusion, and Appendix D summarizes the number of handler alleles, non-handler alleles, and percent profiles produced using each kit.

Table 6. Median number of handler (H) alleles, non-handler (NH) alleles, and percent profiles produced from fumed and non-fumed casings using MiniFiler™ and Fusion. Data for the non-fumed casings taken from Mottar (2014).

	MSU-Fumed		MSP-Fumed		Non-Fumed	
	MiniFiler™	**Fusion**	**MiniFiler™**	**Fusion**	**MiniFiler™**	**Fusion**
Median #H Alleles	2	10	2	15	11	23
Median #NH Alleles	2	10	0	4	1	3
Median % Profile	13	25	13	36	67	61
n	22	22	19	19	11	11

Table 7. Mann-Whitney pairwise comparisons between the number of handler (H) alleles, non-handler (NH) alleles, and percent profiles generated using MiniFiler™ and Fusion (Bonferroni corrected $\alpha = 0.0167$). Data for the non-fumed casings taken from Mottar (2014).

Pair	#H Alleles (p-value)	#NH Alleles (p-value)	% Profile (p-value)
MiniFiler™ vs. Fusion (MSU-Fumed)	< 0.0001	< 0.0001	0.0606
MiniFiler™ vs. Fusion (MSP-Fumed)	< 0.0001	< 0.0001	0.0003
MiniFiler™ vs. Fusion (Non-Fumed)	0.0005	0.1912	0.6862

Comparison of Fusion STR Profiles from Fumed and Non-Fumed Casings

Figure 4 displays the median number of alleles consistent and not consistent with the handler (amplified with Fusion) for the full set of casings (DNA from three casings was not

amplified due to low extract volumes), and descriptive statistics are in Table 8. The non-fumed casings generated the greatest number of alleles consistent with the handler, with a median of 12, while the fumed casings generated medians of 5 (MSU) and 5.5 (MSP). Pairwise comparisons (Table 9) showed that the number of handler alleles did not differ significantly between the MSU-fumed and the MSP-fumed casings, while significantly more were produced from the non-fumed casings. MSU and MSP-fumed casings both resulted in a median percent profile of 13.2%, while non-fumed casings produced 30.8% of a full profile. The percent profile from non-fumed casings was significantly higher than the MSU and MSP-fumed casings. Unlike the number of handler alleles, the MSU-fumed casings resulted in the largest number of non-handler alleles with a median of 7, which was significantly greater than those produced from the MSP-fumed and non-fumed casings. The distributions of the number of handler and non-handler alleles are shown in Figures 5 and 6, respectively. The STR profile for each casing is in Appendix E, and a summary of the data is in Appendix F.

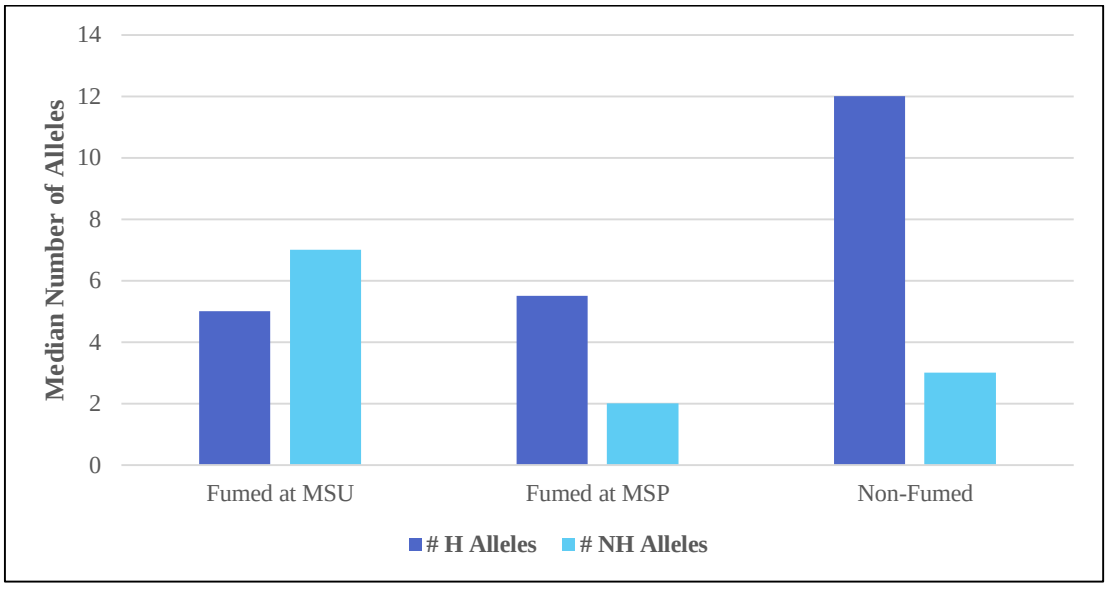

Figure 4. Median number of handler (H) and non-handler (NH) alleles amplified from fumed and non-fumed casings using Fusion. Data for the non-fumed casings taken from Mottar (2014).

Table 8. Descriptive statistics of the number of handler alleles (H), non-handler alleles (NH), and percent profile from fumed and non-fumed casings. Data for the non-fumed casings taken from Mottar (2014).

		MSU-Fumed	MSP-Fumed	Non-Fumed
#H Alleles	**Median**	5.0	5.5	12.0
	Average	7.9	7.6	13.0
	Standard Deviation	8.5	7.4	9.7
#NH Alleles	**Median**	7.0	2.0	3.0
	Average	8.6	3.6	4.5
	Standard Deviation	8.1	4.6	4.9
% Profile	**Median**	13.2	13.2	30.8
	Average	18.8	18.4	31.0
	Standard Deviation	19.4	17.9	22.9
	n	49	50	51

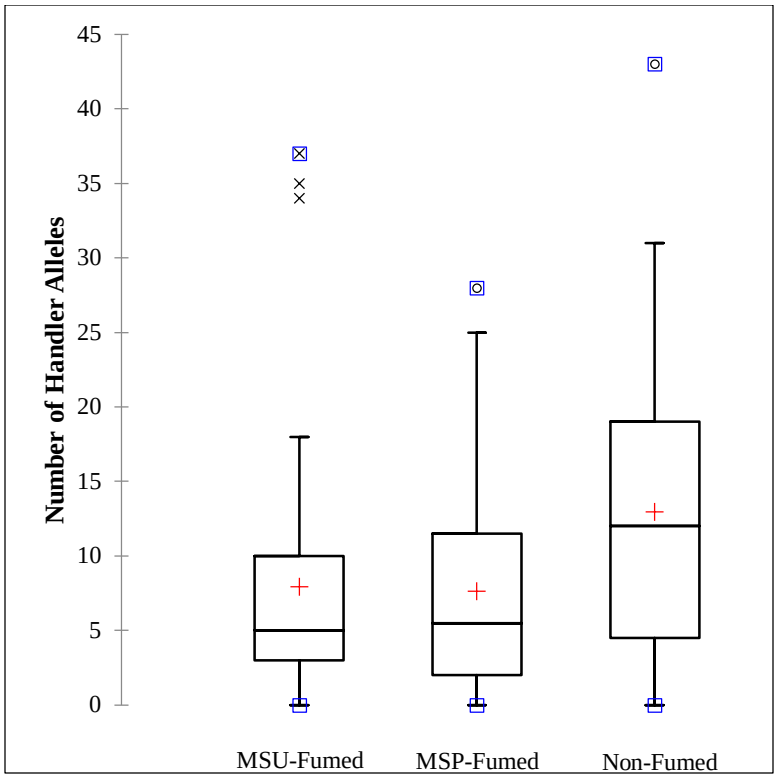

Figure 5. Box plots displaying the distribution of the number of handler alleles from fumed and non-fumed casings. The box encompasses the interquartile range (the distance between the lower and upper quartiles), with the line through the box symbolizing the median. The mean is represented by a red +, extreme outliers are represented by x, mild outliers are represented by ◦, and maximum/minimum values are represented by blue squares. The whiskers represent the maximum/minimum values that are not outliers. Data for the non-fumed casings taken from Mottar (2014).

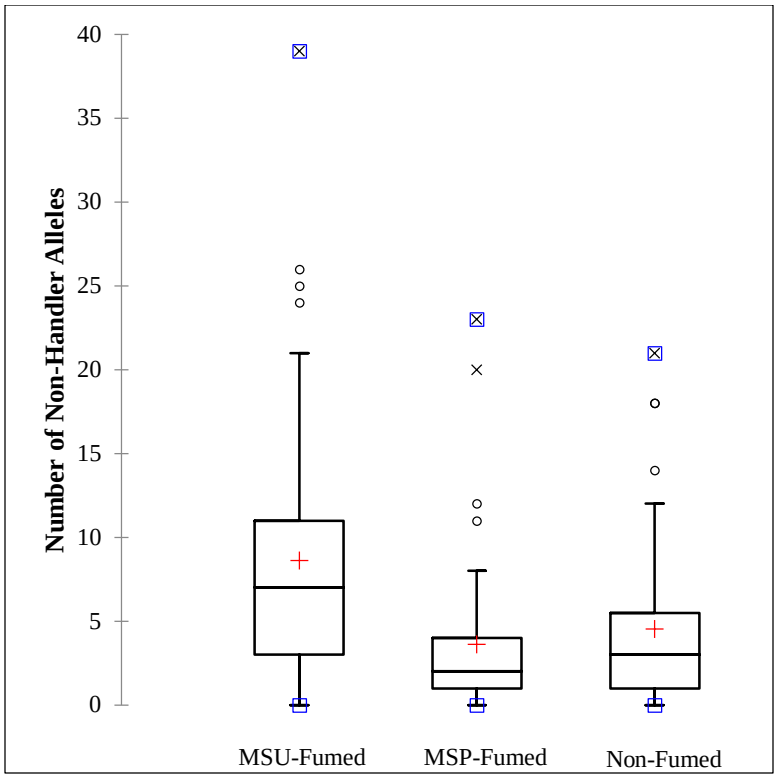

Figure 6. Box plots displaying the distribution of the number of non-handler alleles from fumed and non-fumed casings. The box encompasses the interquartile range (the distance between the lower and upper quartiles), with the line through the box symbolizing the median. The mean is represented by a red +, extreme outliers are represented by x, mild outliers are represented by ◦, and maximum/minimum values are represented by blue squares. The whiskers represent the maximum/minimum values that are not outliers. Data for the non-fumed casings taken from Mottar (2014).

Table 9. Pairwise comparisons of the number of handler alleles (H) and non-handler alleles (NH) and the percent profiles using the Mann-Whitney test (Bonferroni corrected $\alpha = 0.0167$). Data for the non-fumed casings taken from Mottar (2014).

Pair		**#H Alleles (p-value)**	**#NH Alleles (p-value)**	**% Profile (p-value)**
MSU-Fumed	MSP-Fumed	0.8690	< 0.0001	0.8418
MSU-Fumed	Non-Fumed	0.0025	0.0027	0.0038
MSP-Fumed	Non-Fumed	0.0034	0.2383	0.0056

Influence of Loading/Firing Order on DNA Yields and STRs

The median DNA yield and number of handler alleles of casings from each set of cartridges fired is shown in Table 10. Cartridges 10 – 12 were not consistently loaded into the same magazine, and were either the last fired cartridges from magazine 1 or the first fired cartridges from magazine 2. Consequently, casings 10 – 12 were not included in pairwise comparisons, the p-values for which are in Table 11. No differences were significant.

Table 10. Median DNA yields (pg) and number of handler (H) alleles from spent casings. Casing number 1 – 3 refers to casings from the 1st, 2nd, and 3rd cartridges fired, 4 – 6 refers the 4th, 5th, and 6th, etc. Cartridges 10 – 12 were sometimes loaded into magazine 1 and fired. Data for the non-fumed casings taken from Mottar (2014).

		Magazine 1			**Magazine 2**			
Casing #		**1 – 3**	**4 – 6**	**7 – 9**	**10 – 12**	**13 – 15**	**16 – 18**	**19 – 21**
MSU-Fumed	**Yield (pg)**	16.54	2.54	15.89	11.53	3.65	77.99	3.96
	#H Alleles	6.0	3.0	5.0	8.0	2.5	24.5	3.0
MSP-Fumed	**Yield (pg)**	3.18	15.63	5.13	3.60	15.38	1.77	3.96
	#H Alleles	3.0	3.0	6.0	4.0	17.0	2.0	8.5
Non-Fumed	**Yield (pg)**	12.90	38.47	17.13	37.26	32.10	7.59	72.62
	#H Alleles	3.5	17.0	11.0	18.0	15.0	2.5	26.0

Table 11. Mann-Whitney pairwise comparisons between DNA yields and number of handler (H) alleles of casings from the first and last cartridges fired (Bonferroni corrected $\alpha = 0.008$). Data for the non-fumed casings taken from Mottar (2014).

		Casings 1 – 3 vs. 7 – 9	**Casings 13 – 15 vs. 19 – 21**
MSU-Fumed	**DNA Yield (p-value)**	0.953	0.158
	#H Alleles (p-value)	0.991	0.613
MSP-Fumed	**DNA Yield (p-value)**	1.000	0.316
	#H Alleles (p-value)	0.414	0.866
Non-Fumed	**DNA Yield (p-value)**	0.776	0.050
	#H Alleles (p-value)	0.118	0.120

Correlation Between DNA Concentration and STR Profiles

The correlation coefficient between DNA concentration and the number of handler alleles generated using Fusion for fumed and non-fumed casings is shown in Table 12. The MSP and non-fumed casings had similar, moderate correlations (0.7432 and 0.7051, respectively), while the MSU-fumed casings had a weaker correlation (0.5850).

Table 12. The correlation coefficient (r) between DNA concentration (pg/μL) and the number of handler alleles generated using Fusion for fumed and non-fumed casings. Data for the non-fumed casings taken from Mottar (2014).

	Correlation Coefficient (r)
MSU-Fumed	0.5850
MSP-Fumed	0.7432
Non-Fumed	0.7051

Collection 2: Effect of Swabbing Strategy and Cartridge Caliber on DNA Recovery and Analysis

Comparison of DNA Yields Based on Swabbing Strategy and Cartridge Caliber

Figure 7 displays the median DNA yields based on swabbing strategy and cartridge caliber, and the distribution of the data is in Figure 8. More DNA was recovered from 0.45 caliber casings than from 0.22 caliber casings, and cumulative swabbing resulted in higher yields than individual swabbing. Cumulatively swabbed 0.45 casings resulted in the largest median DNA yield (46.41 pg), followed by individually swabbed 0.45 casings (18.13 pg), cumulatively swabbed 0.22 casings (17.40 pg), and individually swabbed 0.22 casings (13.31 pg). The DNA concentration of approximately 37% of individually swabbed 0.22, 15% of individually swabbed 0.45, 13% of cumulatively swabbed 0.22, and 2% of cumulatively swabbed 0.45 caliber casings fell at or below the lowest quantitation standard. Descriptive statistics and pairwise comparisons are shown in Tables 13 and 14, respectively. All differences were significant with the exception

of individually swabbed 0.45 and cumulatively swabbed 0.22 casings. The quantitation results for each casing are in Appendix G.

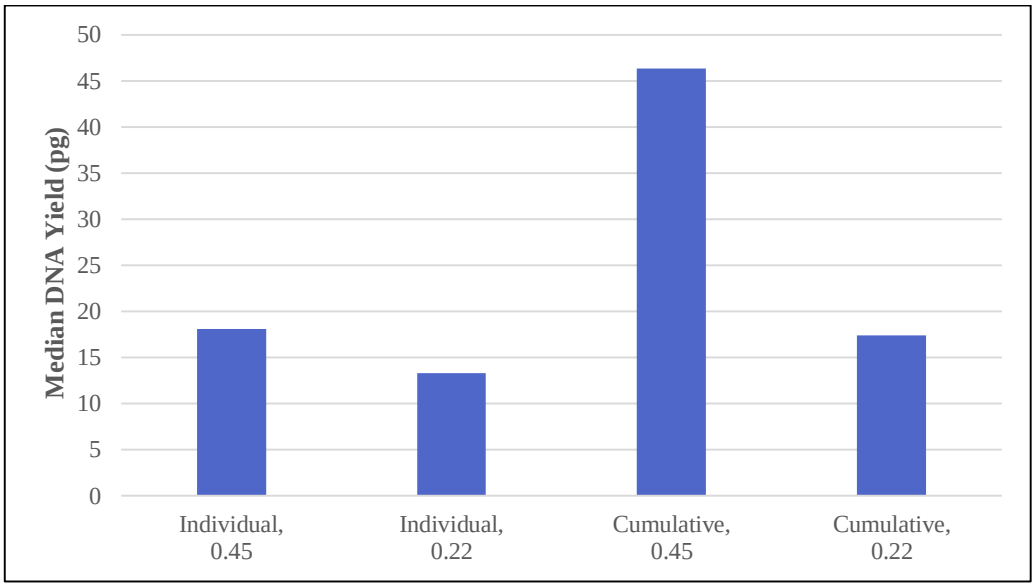

Figure 7. Median DNA yield (pg) based on swabbing strategy and cartridge caliber. Individual/cumulative refers to the swabbing strategy and 0.45/0.22 refers to the caliber.

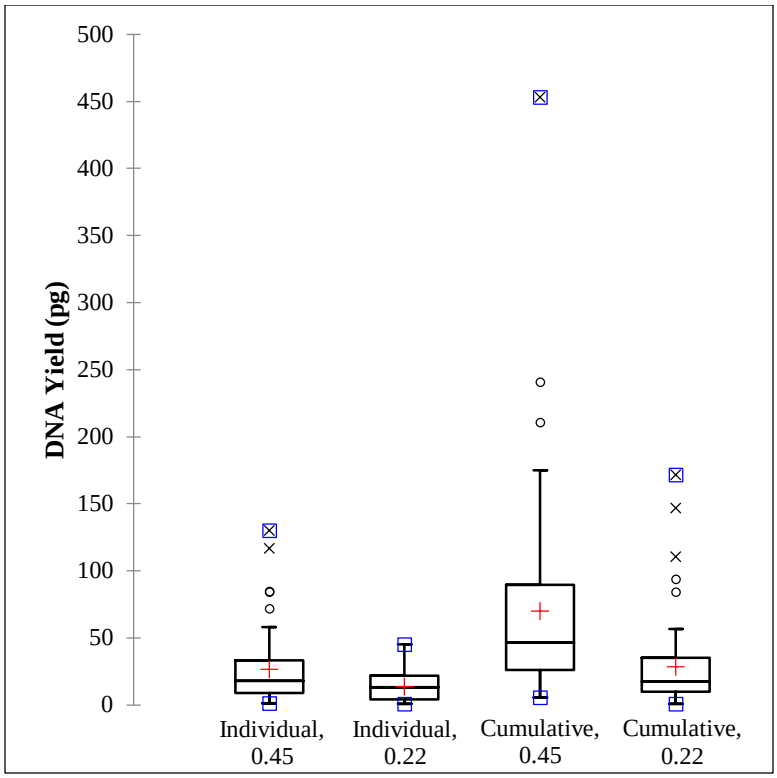

Figure 8. Box plots displaying the distribution of the DNA yield (pg) based on swabbing strategy and cartridge caliber. The box encompasses the interquartile range (the distance between the lower and upper quartiles), with the line through the box symbolizing the median. The mean is represented by a red +, extreme outliers are represented by x, mild outliers are represented by ◦, and maximum/minimum values are represented by blue squares. The whiskers represent the maximum/minimum values that are not outliers. Individual/cumulative refers to the swabbing strategy and 0.45/0.22 refers to the caliber.

Table 13. Descriptive statistics of quantitation results based on swabbing strategy and cartridge caliber. Individual/cumulative refers to the swabbing strategy and 0.45/0.22 refers to the caliber.

	Individual, 0.45	**Individual, 0.22**	**Cumulative, 0.45**	**Cumulative, 0.22**
Median DNA Yield (pg)	18.13	13.31	46.41	17.4
Average DNA Yield (pg)	26.52	13.94	70.22	28.33
Standard Deviation (yield)	26.21	11.25	80.00	33.02
Median Concentration (pg/µL)	0.57	0.42	1.60	0.57
Average Concentration (pg/µL)	0.89	0.45	2.25	0.92
Standard Deviation (concentration)	0.85	0.36	2.16	1.08
n	60	59	60	60

Table 14. Mann-Whitney pairwise comparisons for DNA yield (pg) (Bonferroni corrected $\alpha = 0.0083$). Individual/cumulative refers to the swabbing strategy, and 0.45/0.22 refers to the caliber.

Pair		P-Value
Individual, 0.45	Individual, 0.22	0.0023
Individual, 0.45	Cumulative, 0.45	< 0.0001
Individual, 0.45	Cumulative, 0.22	0.9728
Individual, 0.22	Cumulative, 0.22	0.0039
Individual, 0.22	Cumulative, 0.45	< 0.0001
Cumulative, 0.45	Cumulative, 0.22	< 0.0001

Influence of Handling 0.45 or 0.22 Caliber Cartridges First on DNA Yields from Spent Casings

The median DNA yields from casings handled first and second are compared in Figure 9. More DNA was recovered from the 0.22 caliber casings (both individually and cumulatively swabbed) when they were handled first than when they were handled second. Similarly, more DNA was recovered from cumulatively swabbed 0.45 casings when they were handled first than when they were handled second. In contrast, more DNA was recovered from the individually swabbed 0.45 casings that were handled second rather than first. Pairwise comparisons between DNA yields of casings handled first and second for each caliber and swabbing strategy (Table 15) revealed that the only significant difference was in the cumulatively swabbed 0.22 casings.

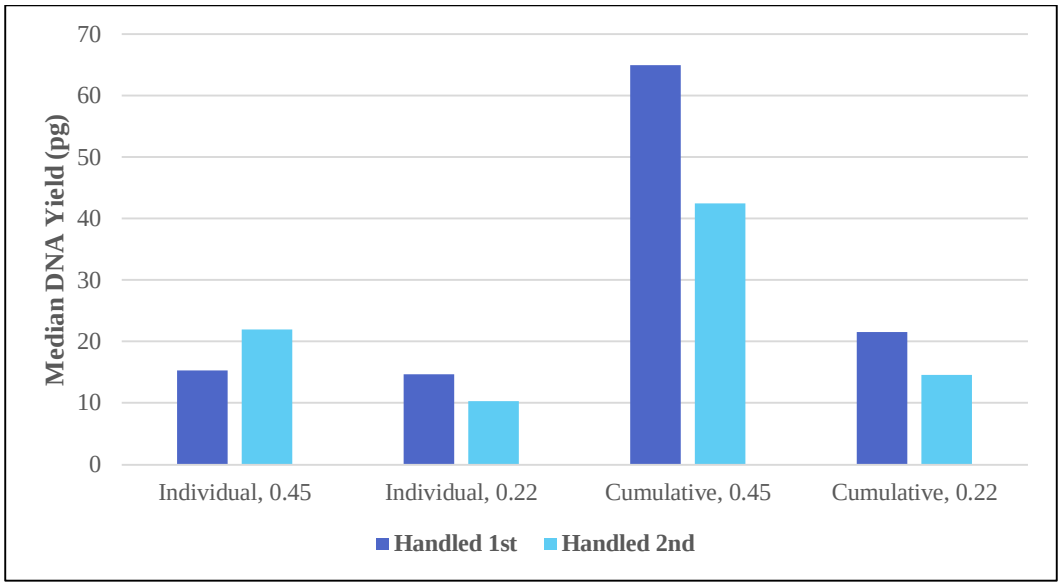

Figure 9. Median DNA yields (pg) of 0.45/0.22 caliber casings based on swabbing strategy. Individual/cumulative refers to the swabbing strategy, and 0.45/0.22 refers to the caliber.

Table 15. Mann-Whitney pairwise comparisons between the DNA yields (pg) from casings handled first and second based on swabbing strategy and cartridge caliber (Bonferroni corrected $\alpha = 0.013$). Individual/cumulative refers to the swabbing strategy, and 0.45/0.22 refers to the caliber.

	P-Value
Individual, 0.45	0.947
Individual, 0.22	0.462
Cumulative, 0.45	0.109
Cumulative, 0.22	0.006

Comparison of Fusion STR Profiles

The median number of handler and non-handler alleles based on swabbing strategy and cartridge caliber and descriptive statistics are displayed in Figure 10 and Table 16, respectively. The cumulatively swabbed 0.45 casings resulted in the largest median number of handler alleles (17.5), followed by cumulatively swabbed 0.22 casings (8.5), individually swabbed 0.45 casings (6.0), and individually swabbed 0.22 casings (4.0). The distribution of the handler alleles is in Figure 11. Pairwise comparisons (Table 17) showed that the only non-significant difference was

between individually swabbed 0.45 and cumulatively swabbed 0.22 casings. The median number of non-handler alleles was 4.5 for cumulatively swabbed 0.45 caliber casings, 2.5 for individually swabbed 0.45 caliber casings, 2.0 for cumulatively swabbed 0.22 caliber casings, and 1.0 for individually swabbed 0.22 caliber casings. The only pairs that were not significantly different the individually swabbed 0.45 and 0.22 caliber casings, individually swabbed 0.45 and cumulatively swabbed 0.22 caliber casings, and cumulatively swabbed 0.45 and 0.22 caliber casings. The distribution of the non-handler alleles is in Figure 12. Median profiles of 41.5% (cumulative, 0.45), 20.0% (cumulative, 0.22), 15.0% (individual, 0.45), and 10.0% (individual, 0.22) were produced. The percent profile differed significantly between all groups except for individually swabbed 0.45 and cumulatively swabbed 0.22 casings. All STR profiles are in Appendix H, and are summarized in Appendix I.

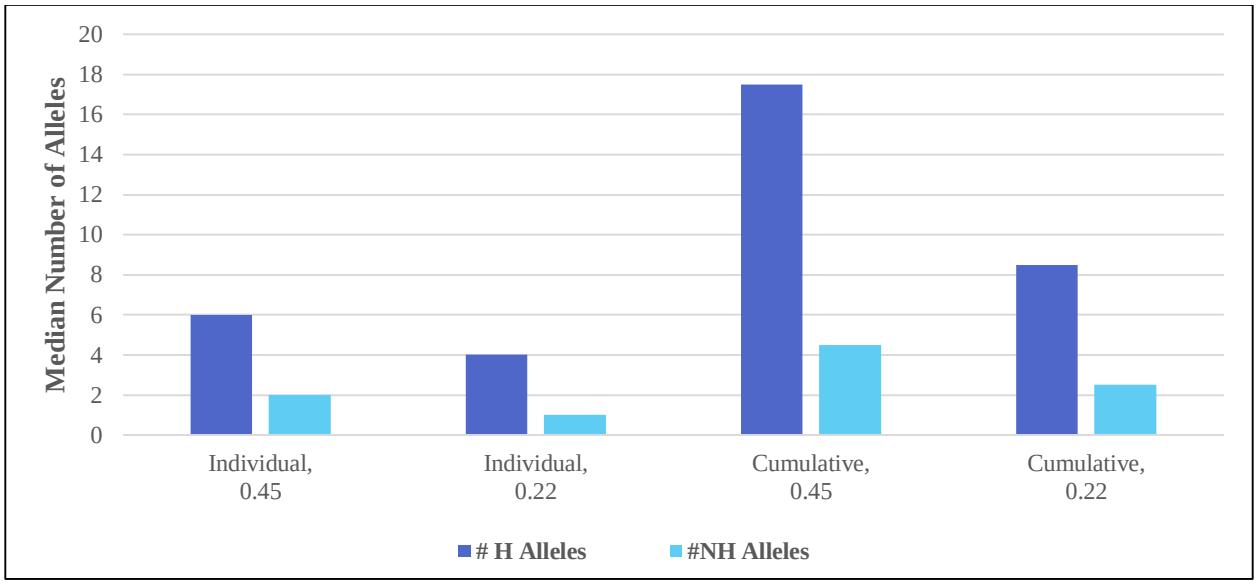

Figure 10. Median number of handler (H) and non-handler (NH) alleles based on swabbing strategy and cartridge caliber. Individual/cumulative refers to the swabbing strategy and 0.45/0.22 refers to the caliber.

Table 16. Descriptive statistics of the number of handler alleles (H), non-handler alleles (NH), and percent STR profile based on swabbing strategy and cartridge caliber. Individual/cumulative refers to the swabbing strategy and 0.45/0.22 refers to the caliber.

		Individual, 0.45	**Individual, 0.22**	**Cumulative, 0.45**	**Cumulative, 0.22**
#H Alleles	**Median**	6.0	4.0	17.5	8.5
	Average	6.8	5.2	18.8	11.1
	Standard Deviation	6.9	4.7	10.5	9.1
#NH Alleles	**Median**	2.0	1.0	4.5	2.5
	Average	2.6	1.7	6.2	3.8
	Standard Deviation	3.1	1.9	5.5	3.8
% Profile	**Median**	15.6	10.0	41.5	20.0
	Average	20.6	12.7	45.8	26.9
	Standard Deviation	16.6	11.1	25.8	21.7
	n	60	59	60	60

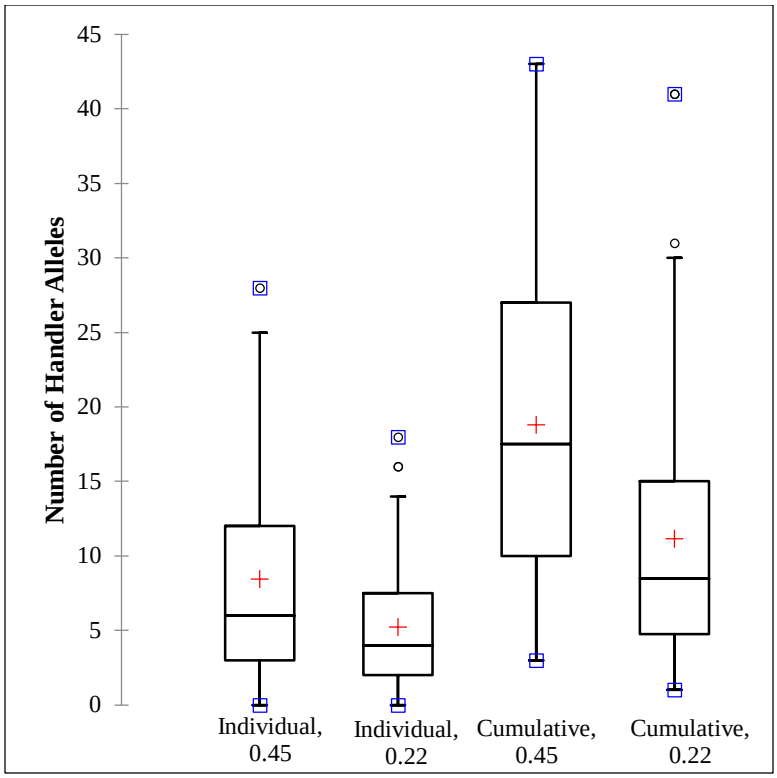

Figure 11. Box plots displaying the distribution of the number of handler alleles based on swabbing strategy and cartridge caliber. The box encompasses the interquartile range (the distance between the lower and upper quartiles), with the line through the box symbolizing the median. The mean is represented by a red +, mild outliers are represented by ◦, and maximum/minimum values are represented by blue squares. The whiskers represent the maximum/minimum values that are not outliers. Individual/cumulative refers to the swabbing strategy and 0.45/0.22 refers to the caliber.

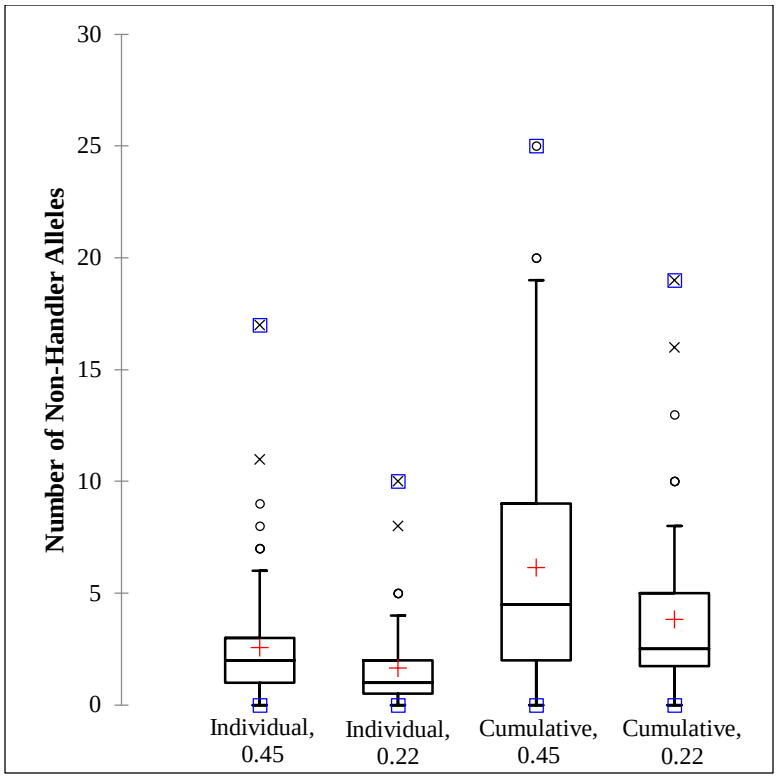

Figure 12. Box plots displaying the distribution of the number of non-handler alleles based on swabbing strategy and cartridge caliber. The box encompasses the interquartile range (the distance between the lower and upper quartiles), with the line through the box symbolizing the median. The mean is represented by a red +, extreme outliers are represented by x, mild outliers are represented by ◦, and maximum/minimum values are represented by blue squares. The whiskers represent the maximum/minimum values that are not outliers. Individual/cumulative refers to the swabbing strategy and 0.45/0.22 refers to the caliber.

Table 17. Pairwise comparisons of the number of handler alleles (H), non-handler alleles (NH), and percent profile (Mann-Whitney test, Bonferroni corrected $\alpha = 0.008$). Individual/cumulative refers to the swabbing strategy and 0.45/0.22 refers to the caliber.

Pair		#H Alleles (p-value)	#NH Alleles (p-value)	% Profile (p-value)
Individual, 0.45	Individual, 0.22	0.0062	0.0964	0.0070
Individual, 0.45	Cumulative, 0.45	< 0.0001	< 0.0001	< 0.0001
Individual, 0.45	Cumulative, 0.22	0.0860	0.0125	0.0930
Individual, 0.22	Cumulative, 0.45	< 0.0001	< 0.0001	< 0.0001
Individual, 0.22	Cumulative, 0.22	< 0.0001	< 0.0001	< 0.0001
Cumulative, 0.45	Cumulative, 0.22	< 0.0001	0.0145	< 0.0001

Influence of Loading/Firing Order on DNA Yields and STRs

Table 18 displays the median DNA yield and number of handler alleles from casings from each magazine based on swabbing strategy and caliber. Pairwise comparisons of DNA yields and number of handler alleles between the first and last casings (Table 19) showed that no differences were significant.

Table 18. Median DNA yields (pg) and number of handler (H) alleles from spent casings. Casing number 1 – 3 refers to the casings from the 1st, 2nd, and 3rd cartridges fired, 4 – 6 refers to the 4th, 5th, and 6th, etc. Individual/cumulative refers to the swabbing strategy and 0.45/0.22 refers to the caliber.

		Magazine 1		**Magazine 2**	
Casing #		**1 – 3**	**4 – 6**	**7 – 9**	**10 – 12**
Individual, 0.45	**Yield (pg)**	12.26	17.83	29.04	25.28
	#H Alleles	3.0	6.0	9.0	10.0
Individual, 0.22	**Yield (pg)**	4.75	9.32	14.24	24.77
	#H Alleles	3.0	5.0	6.0	3.5
Cumulative, 0.45	**Yield (pg)**	43.55	64.78	31.82	44.71
	#H Alleles	20.0	22.0	14.0	15.0
Cumulative, 0.22	**Yield (pg)**	18.14	16.63	18.71	14.88
	#H Alleles	8.0	9.0	8.0	10.0

Table 19. Mann-Whitney pairwise comparisons of DNA yields (pg) and number of handler (H) alleles between casings from the first and last fired cartridges from each magazine based on swabbing strategy and cartridge caliber (Bonferroni corrected $\alpha = 0.006$). Individual/cumulative refers to the swabbing strategy and 0.45/0.22 refers to the caliber.

		Casings 1 – 3 vs. 4 – 6	**Casings 7 – 9 vs. 10 – 12**
Individual, 0.45	**DNA Yield (p-value)**	0.46	0.68
	#H Alleles (p-value)	0.16	0.98
Individual, 0.22	**DNA Yield (p-value)**	0.28	0.01
	#H Alleles (p-value)	0.03	0.72
Cumulative, 0.45	**DNA Yield (p-value)**	0.56	0.74
	#H Alleles (p-value)	0.75	0.90
Cumulative, 0.22	**DNA Yield (p-value)**	0.74	0.07
	#H Alleles (p-value)	0.87	0.48

Correlation Between DNA Concentration and STR Profiling

The correlation coefficient between DNA concentration and the number of handler alleles generated using Fusion for each swabbing strategy and caliber is shown in Table 20. Correlations were highly variable, ranging from 0.3030 (individual, 0.22) to 0.8061 (cumulative, 0.22).

Table 20. The correlation coefficient (r) between DNA concentration (pg/μL) and the number of handler alleles generated using Fusion for each caliber and swabbing strategy. Individual/cumulative refers to the swabbing strategy, and 0.45/0.22 refers to the caliber.

	Correlation Coefficient (r)
Individual, 0.45	0.5647
Individual, 0.22	0.3030
Cumulative, 0.45	0.5825
Cumulative, 0.22	0.8061

Comparison of MtDNA Profiles

MtDNA profiles were successfully generated from all 96 DNA extracts tested. Figure 13 displays the classification results from all profiles, of which 50 were consistent (52%), 25 were mixed-consistent (26%), 18 were inconsistent (19%), and 3 were mixed-inconsistent (3%). In total, 78% of the generated profiles included the handler and 29% contained a mixture. All mtDNA profiles are in Appendix J.

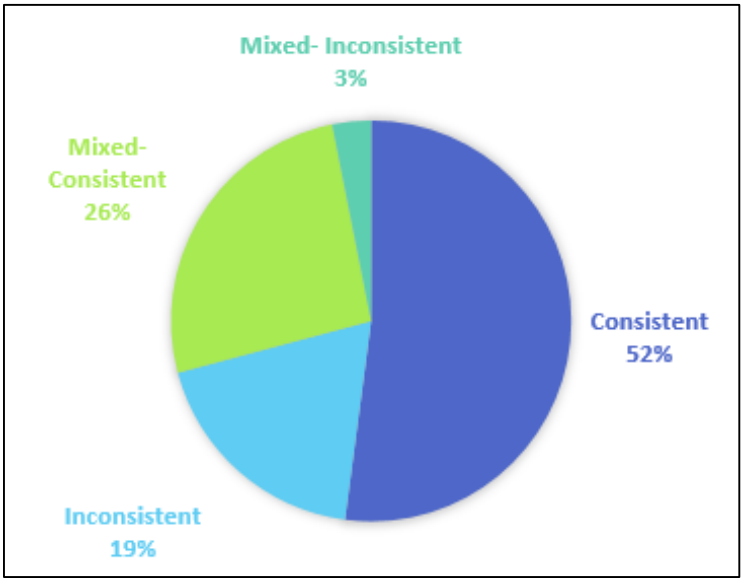

Figure 13. Classification of mtDNA profiles for all samples (n=96).

A major contributor could be identified in some mixtures, exemplified in Figure 14, when one peak was higher than the other in both strands. A major contributor could not be identified in other mixtures, when the two peaks were of approximately equal height (e.g., Figure 15).

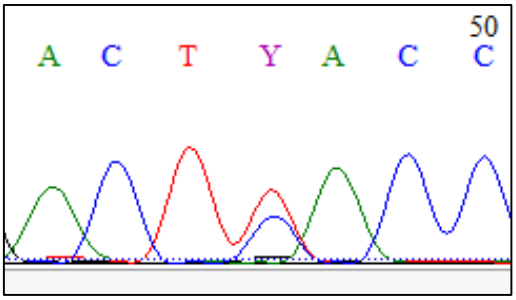

Figure 14. MtDNA containing a mixture in which a major contributor could be determined (major = ACTTACC, minor = ACTCACC). Similar peak heights were observed in both strands.

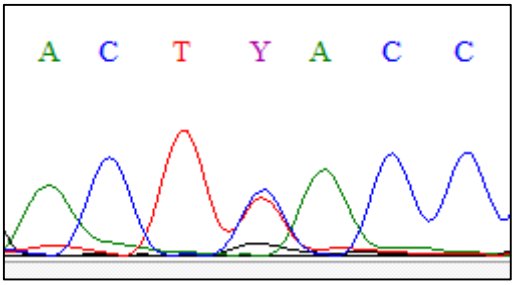

Figure 15. MtDNA containing a mixture in which a major contributor could not be determined. Similar peak heights were observed in both strands.

Figure 16 displays the mtDNA profile classifications for each DNA quantitation level (high, medium, and low). The highest DNA yields resulted in 21 consistent, 4 mixed-consistent, 6 inconsistent, and 1 mixed-inconsistent profile. The medium DNA yields resulted in 18 consistent, 9 mixed-consistent, 4 inconsistent, and 1 mixed-inconsistent profile. The lowest DNA yields resulted in 11 consistent, 12 mixed-consistent, 8 inconsistent, and 1 mixed-inconsistent profile. Fisher's exact test indicated that mtDNA profile classification was independent of quantitation level ($p = 0.433$).

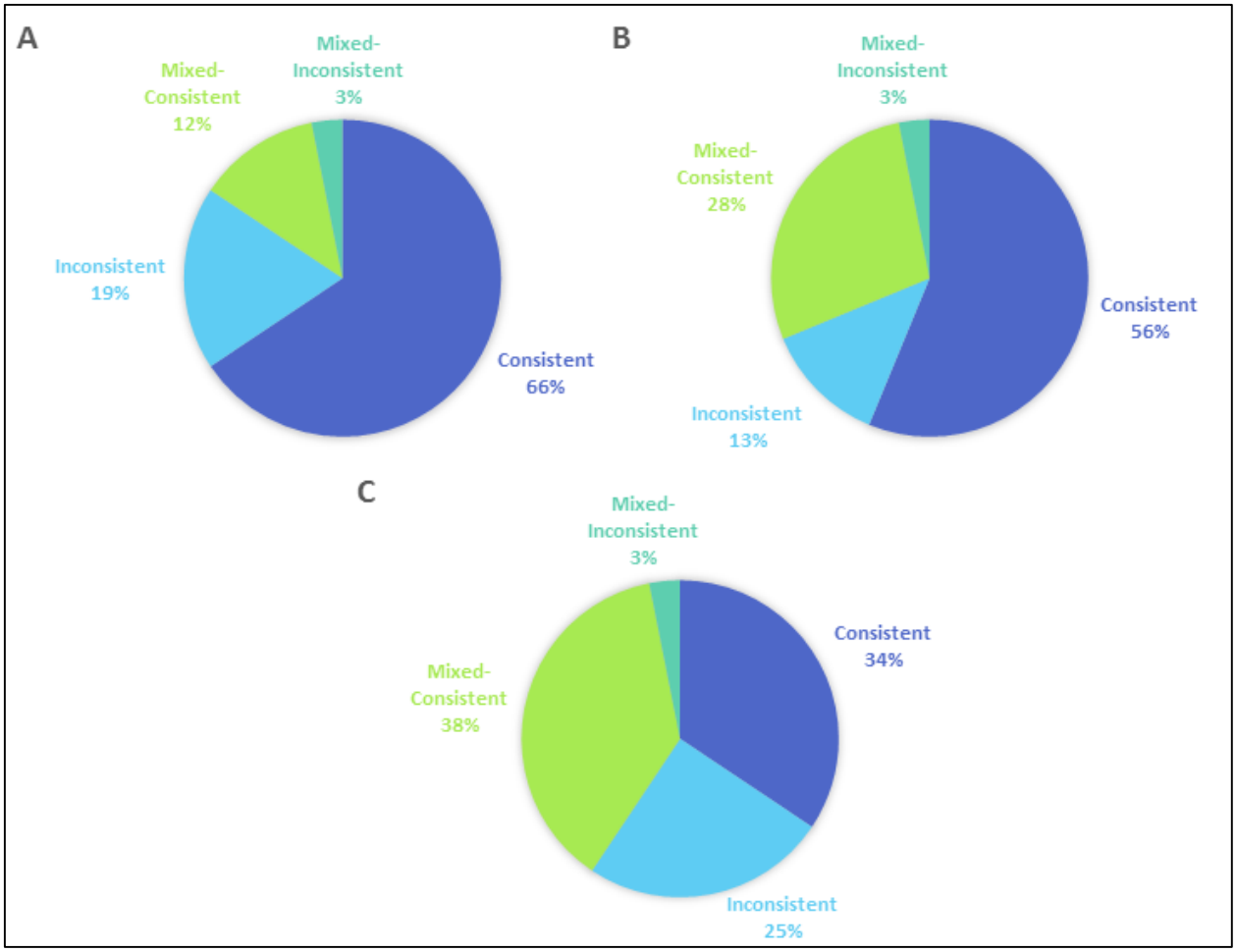

Figure 16. Classification of mtDNA profiles for the high (A), medium (B), and low (C) DNA quantity samples (n = 32 for each chart).

Cumulatively and individually swabbed casings produced similar numbers of inconsistent profiles, however individually swabbed casings had fewer mixtures (Figure 17). The cumulatively swabbed casings resulted in 29 consistent, 19 mixed-consistent, 10 inconsistent, and no mixed-inconsistent profiles. The individually swabbed casings resulted in 31 consistent, 6 mixed-consistent, 8 inconsistent, and 3 mixed-inconsistent profiles. Fisher's exact test produced a p-value of 0.035, indicating the two variables are dependent/linked. Individually swabbed samples produced significantly more consistent profiles and significantly fewer mixed-consistent

profiles, while the number of inconsistent and mixed-inconsistent profiles did not differ significantly.

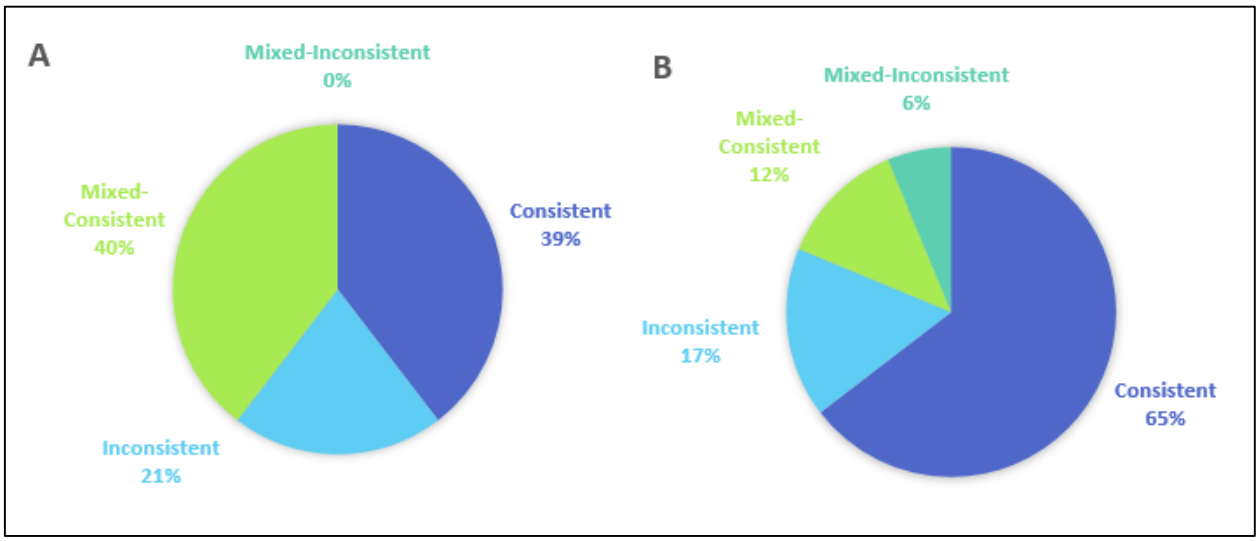

Figure 17. Percentage of each mtDNA profile category for cumulatively swabbed casings (A) and individually swabbed casings (B) (n = 48 for each chart).

Figure 18 displays the mtDNA profile classifications for 0.45 and 0.22 caliber casings. Forty-five caliber casings resulted in 26 consistent, 14 mixed-consistent, 6 inconsistent, and 2 mixed-inconsistent profiles. Twenty-two caliber casings resulted in 24 consistent, 11 mixed-consistent, 12 inconsistent, and 1 mixed-inconsistent profile. Fisher's exact test produced a p-value of 0.321, indicating that mtDNA profile classification was independent of cartridge caliber.

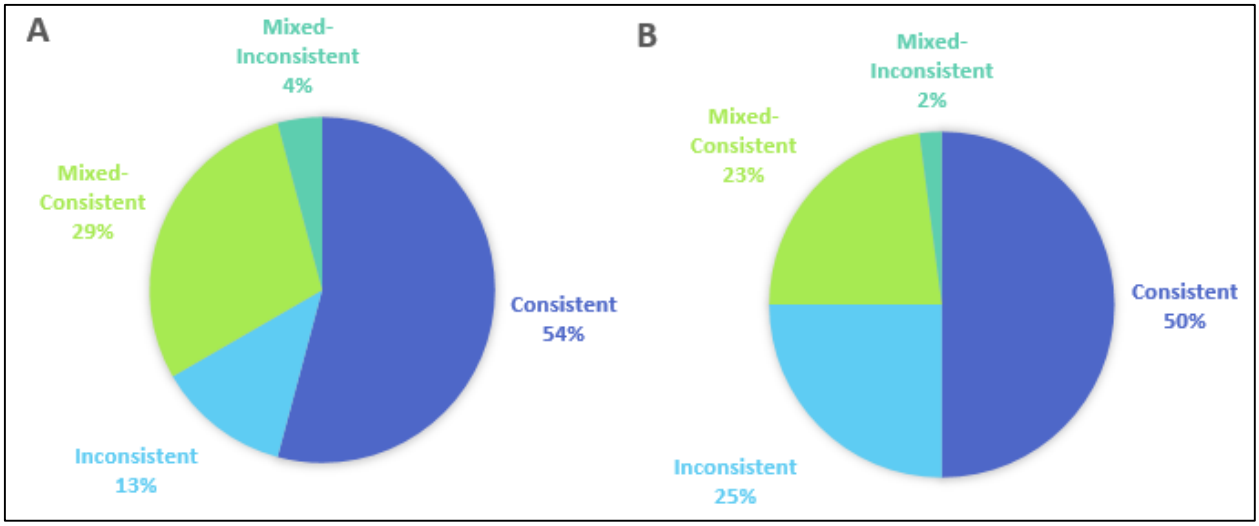

Figure 18. Percentage of each mtDNA profile category for 0.45 caliber casings (A) and 0.22 caliber casings (B) (n = 48 for each chart).

Comparison of STR and mtDNA Results

Overall, the STR and mtDNA results corresponded well with one another. Figure 19 shows the median number of handler and non-handler STR alleles for samples of each mtDNA profile classification. DNA extracts that produced an inconsistent mtDNA profile also had a relatively high number of non-loader alleles when compared to extracts that resulted in a mtDNA profile consistent with the handler. Consistent mtDNA profiles had a median of 11 handler and 2 non-handler alleles, mixed-consistent profiles had a median of 9 handler and 3 non-handler alleles, and inconsistent profiles had a median of 7.5 handler and 3 non-handler alleles. The classification of each mtDNA profile and the corresponding number of handler and non-handler alleles for each sample are in Appendix K.

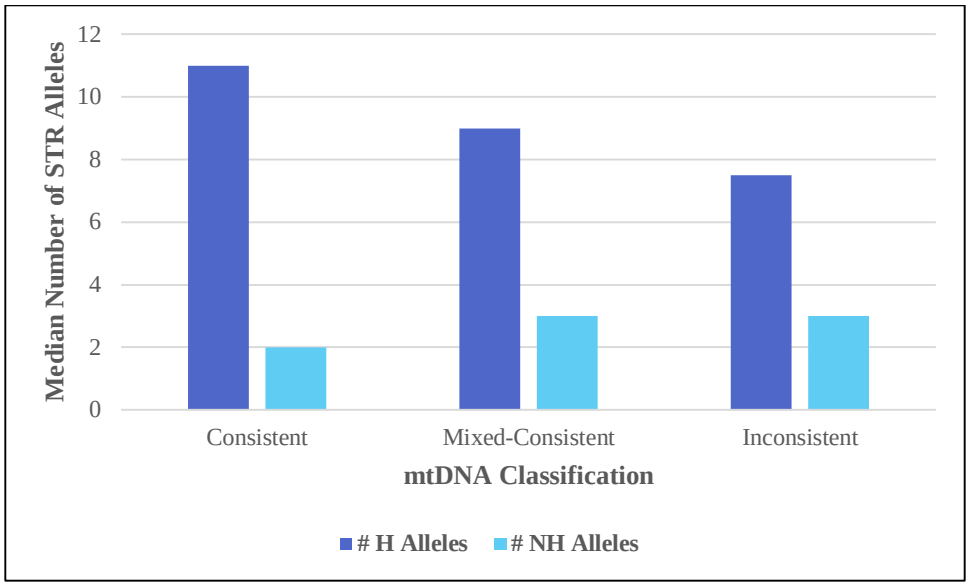

Figure 19. Medium number of handler (H) and non-handler (NH) STR alleles for each classification of mtDNA profile. Only three samples were classified as mixed-inconsistent, which are not included in this graph.

DISCUSSION

DNA profiling from spent cartridge casings has been suggested as a method for identifying the individual who loaded a weapon, but thus far it has not been highly successful (e.g. Horsman-Hall et al., 2009; Branch, 2010). Optimization of DNA extraction can greatly improve its recovery (Mottar, 2014), however there are a variety of other factors that may also affect DNA isolation and profiling. The goals of this research were to evaluate several of these to determine what effect, if any, they have on DNA yields and analysis from spent casings, in an attempt to better establish the quantity and quality of genetic information that can be obtained from them.

Touch DNA samples are inherently variable, which presents challenges in a research setting. Some authors have stated that individuals differ greatly in the quantity of DNA they transfer through contact (e.g. Lowe et al., 2002), while others have shown that variation exists within an individual (Thomasma and Foran, 2013), and that the number of cells deposited through touch is dependent on many factors, including the surface being handled and the condition of one's skin (e.g. Phipps and Petricevic, 2007). Meakin and Jamieson (2013) proposed that any activity liable to remove cells from a person's hands may influence the transfer of touch DNA; for example, less DNA is generally transmitted through contact immediately after hand washing (Lowe et al., 2002; Phipps and Petricevic, 2007). These same variables had the potential to affect the touch samples in this research, but there are other factors to consider as well. For instance, the volunteers in this study were largely from the firearms and DNA units of the MSP Forensic Laboratory, and therefore many had likely been wearing disposable gloves prior to (but not while) handling ammunition. It is possible that the action of removing gloves strips off loose cells, causing less DNA to be transferred through touch.

Alternatively, wearing gloves for an extended period could prevent cell loss, increasing the quantity of DNA deposited onto a surface. Consequently, the amount of DNA transmitted to the casings probably differed between individuals who had been wearing gloves and those who had not. Another factor that might have influenced DNA yields was cartridge handling time, which varied widely because volunteers were simply instructed to load the cartridges into a magazine, rather than hold them for a specific amount of time as other researchers have done (e.g. Horsman-Hall et al., 2009; Branch, 2010). Some volunteers had a great deal of experience working with firearms and quickly and effortlessly loaded the cartridges, while others had never handled ammunition and expended more time and energy. For example, one volunteer from Collection 1 took up to two minutes to load each cartridge and was not capable of loading 12 into a single magazine, and instead loaded three or six cartridges at a time. Volunteers typically found it easier to load the 0.22 caliber cartridges than the 0.40 and 0.45 caliber cartridges, and required less time and force to load them, although experience seemed to have a bigger impact on handling time. Balogh et al. (2003a) recovered DNA from paper that was touched for varying lengths of time (from 1 to 60 s), and reported that handling time did not affect STR profiling (using a Profiler Plus® kit), however in that study volunteers placed single fingerprints on paper using the same amount of pressure. The individuals in the current research who spent extra time handling the cartridges usually moved them around in their hand, likely resulting in more cells being deposited.

Another variable that had the potential to influence the amount of DNA recovered from casings was the order in which the cartridges were placed into the magazine and subsequently fired. However, loading/firing order did not have a significant effect on DNA yield in the current research, for which there are several potential explanations. One is that the order the cartridges

were loaded in simply did not influence DNA yield. However, Van Oorschot et al. (2003) stated that the amount of DNA transferred to items "can drop significantly after the initial touch", although their experiment involved only four volunteers placing their hand on 30 sheets of plastic. DNA was then extracted and amplified using Profiler Plus®, and STR peak heights were used as a metric of the amount of DNA deposited, which is not a generally accepted method for determining DNA yield. The quantity of DNA transmitted to a surface therefore may not systematically decrease as multiple surfaces are touched. Another factor related to loading order is the force needed to place the cartridges into the magazine; although the last cartridge required more force to load, this might not have resulted in increased cell deposition. Additionally, it is possible that firing order did not significantly affect DNA yields because the temperature of the casings did not get high enough to have a degradative effect. The temperature of the barrel of a fired gun can reportedly reach 1,200 °C (Lawton, 2001), but the casing likely does not get that hot as it is quickly ejected from the firearm. Or, it may be that both loading and firing order do have an effect on DNA yield, but one counteracts the other. For example, the first loaded cartridge might contain the most DNA prior to firing, but the temperature inside the gun degrades it to the point that the amount recovered is similar to the last loaded/first fired cartridge.

An alternate explanation for why loading/firing order did not affect DNA yield is that aspects of the experimental design of this research masked its true effect. Cartridges were fired in sets of three, so it was not known which casing was the first or last fired in each set. Instead, the sets of three were compared; if the amount of DNA deposited dropped substantially after the first cartridge loaded, this could have affected the results. For example, if the first cartridge had a large quantity of DNA and the second and third had much less, the average of the three would be much smaller than that of the first alone. The manner in which the cartridges were fired might

also have mitigated the effect of heat, as the temperature inside the chamber of the gun when the last cartridges were fired was less than it would have been had they all been fired in quick succession. Finally, because casings from each volunteer were assigned different treatment methods (i.e. fuming methods or swabbing strategies), DNA yields from the first and last fired cartridges loaded by the same individual could not be directly compared. Whether any of these variables affected the results is unclear, but since loading/firing order did not have a measurable effect on DNA recovery in the current research, knowing the order of casings collected from a crime scene does not appear to be critical to their analysis. This is fortunate, as it is unlikely that the order in which cartridges were fired will be determined when casings are found at a crime scene.

There are also several characteristics of a casing itself that might influence DNA recovery. Cartridge casings can be made of brass, nickel, aluminum, and steel, and spent casings submitted to a crime laboratory as evidence are likely to vary in metal composition. All casings in the current research were brass, an alloy of copper and zinc. Copper has antimicrobial properties and causes membrane damage when it makes contact with cells, which can be followed by DNA degradation (Grass et al., 2011). It is possible that the composition of the cartridge casing caused damage to touch DNA prior to and/or post firing, lowering the amount that was recovered. Bille et al. (n.d.) reported that when controlled quantities of cells were placed on unfired cartridges, significantly more DNA resulted from those made of nickel, aluminum, and steel than from brass. Similarly, Wan et al. (2015) had volunteers handle clean brass and nickel cartridges, and the latter produced significantly higher DNA yields. Given the findings of these studies, DNA analysts may consider directing their efforts to casings made of metals other

than brass when a crime laboratory receives them as evidence. It also might be better to process brass casings for DNA as soon as possible, or store them at -20 °C until processing can occur.

Another characteristic of cartridges that had an effect on the recovery of DNA in this research was caliber. There are two potential reasons for why the 0.45 caliber casings yielded significantly more DNA than did the 0.22 caliber casings. First, the former have a greater surface area on which DNA can be deposited, and second, the increased force required to load the bigger cartridges may have resulted in additional cells being transferred to the casing surface. Or, both may have had an effect. Spear et al. (2005) reported similar findings when investigating the recovery of fingerprints from spent casings. The authors placed prints on cartridges ranging in size from 0.22 to 0.45 caliber, and half were fired. Only one identifiable print was obtained from the spent casings and five from the unfired cartridges, all six of which were 9 mm (approximately 0.35 in) or 0.45 caliber. Based on the results of the current research, as well as previous studies, forensic examiners should consider cartridge caliber when deciding whether to attempt DNA recovery from a casing, and when determining what methods to use during processing (discussed below).

Since more DNA was recovered from 0.45 than from 0.22 caliber casings, it was expected that 0.40 caliber casings would generate an intermediate amount. Instead, the 0.40 caliber casings resulted in the highest DNA yields in this study (a median of 23 pg, compared to 18 pg from the 0.45 caliber casings). However, it is difficult to compare the 0.40 and 0.45 caliber casings directly, because they were from separate collections. The groups of volunteers who participated in each collection differed (although some individuals overlapped), and Collection 1 took place in December while Collection 2 was in June, so the environmental conditions were dissimilar, which could have influenced DNA deposition and recovery. Bright and Petricevic

(2004) reported that when the hands of different individuals were directly swabbed, volunteers who repeatedly resulted in higher DNA yields had comparatively drier skin (although how this was determined was not described). People tend to have drier skin during winter (Rudikoff, 1998), potentially increasing the amount of DNA transferred to the casings in Collection 1. Furthermore, the data for the non-fumed 0.40 caliber casings were taken from Mottar (2014) so the casings were processed by another individual, though the analyst followed the same procedures. Given these differences, the higher yields of the 0.40 caliber casings should likely not be used to refute the idea that more DNA can be recovered from larger casings.

On the other hand, it is possible that more DNA actually can be recovered from 0.40 than from 0.45 caliber casings. The latter are much bigger than 0.22 caliber casings (having surface areas of approximately 9 cm^2 and 3 cm^2, respectively), while 0.40 caliber casings are very close in size (approximately 8 cm^2), thus similar amounts of DNA may be deposited on the larger two. However, bigger cartridges hold more gunpowder, and therefore likely reach a higher temperature when they are fired, which could have increased DNA degradation. Additionally, it is feasible that more DNA was recovered from the earlier loaded 0.40 caliber casings because volunteers who participated in both collections improved at the loading process, and spent less time and effort placing the 0.45 caliber cartridges in the magazine.

Gunshot residue is expelled when cartridges are fired, so while it is not a characteristic of the casing itself it is likely always present on spent casings, and has the potential to influence DNA processing. The components of gunshot residue include burnt and unburnt powder, metals from the cartridge or firearm, and elements from the primer (including lead, antimony, and barium), smoke, and lubricant (reviewed by Dalby et al., 2010), which produce a black layer of soot coating the casing and could interfere with the recovery and/or analysis of DNA. Horsman-

Hall et al. (2009) stated that gunshot residue might inhibit PCR, and when the authors added control DNA to DNA recovered from 28 shotgun shells, inhibition was observed in three of them (11%), although there was no evidence of PCR inhibition in the current research or that of Mottar (2014). Most or all of the gunshot residue appeared to have been removed from solution in this study, as many of the components are not water soluble and pelleted when DNA was extracted. Troy et al. (2015) added a gunshot residue suspension to purified DNA and extracted it using either a robotic silica-based technique (a Qiagen®'s EZ1® Advanced XL system) or a manual organic procedure. Peak height suppression occurred in several of the samples extracted using the silica-based technique, while the organically extracted samples resulted in peaks that were "consistently high and well-balanced", showing that gunshot residue can inhibit PCR if it is not removed.

Once a casing has been collected from a shooting scene, it is likely to be transported to a crime laboratory for analysis such as visualization of fingerprints, microscopic examination, and DNA profiling. How the casing is handled upon discovery and collection has the potential to affect the amount of DNA recovered from it. Fingerprints are typically enhanced using chemical or physical means that might remove touch DNA, trap it in place, introduce contaminant DNA, or introduce substances that interfere with DNA extraction or analysis. If the casing is manipulated by a firearms examiner, any cells that were deposited onto the casing when the cartridge was loaded could be inadvertently lost, and may even be replaced by DNA from the examiner if precautions are not taken. Additionally, the manner in which the casing is processed for DNA can also affect yields, as there are many extraction and analysis techniques employed by forensic scientists to analyze DNA. Several of these factors were examined in the current research.

Cyanoacrylate fuming is a common fingerprint enhancement technique that can be performed using a portable or stationary fuming chamber. The purpose of testing two methods in this study was to determine not only if cyanoacrylate fuming had an effect on the recovery and analysis of DNA from spent casings, but also whether fuming casings on site was more beneficial than transporting them prior to fuming. For example, fuming casings immediately after collection may glue cells to the surface and prevent loss during transportation. On the other hand, cyanoacrylate itself might interfere with DNA retrieval and/or analysis. Transportation did not affect DNA recovery from fumed casings in the current research, as the MSU-fumed casings (which were transported before fuming) resulted in significantly higher DNA yields. However, the MSP-fumed casings were coated in a heavier layer of cyanoacrylate than those fumed at MSU, so it is possible that the amount of cyanoacrylate affected the DNA yields. Even more DNA was recovered from the non-fumed casings, thus it is likely that the cyanoacrylate residue hindered DNA extraction, potentially because the cells were trapped in the cyanoacrylate, which remained in the interface between the organic and aqueous layers.

Another disadvantage of cyanoacrylate fuming was that casings fumed at MSU produced a large number of non-handler alleles. This may have resulted from the extra handling that was required to insert and remove them from the portable fuming chamber (though the investigator's DNA could not be identified, if present, due to the small number of alleles that were generated). It was difficult to position casings inside of the chamber, and they therefore spent a longer period of time exposed to the atmosphere of the laboratory, which might have contributed to the increased number of non-handler alleles. In contrast, the casings fumed in the commercial chamber at the MSP laboratory were kept isolated from one another on paper bags and none fell over and made contact with the surface of the fuming chamber, while the non-fumed casings

were not removed from their paper bags until just before swabbing. Consequently, if casings are to be fumed prior to DNA processing, precautions such as cleaning the chamber and fuming different evidence samples separately should be taken to avoid contamination.

Several researchers have examined the influence of cyanoacrylate fuming on DNA recovery and also found it to be deleterious. Von Wurmb et al. (2001) placed blood and saliva on glass slides, half of which were fumed with cyanoacrylate, cells/DNA was removed (details not given) and a Chelex extraction performed. STRs were amplified using Profiler Plus®, and while profiles were generated from all samples, cyanoacrylate "had a negative effect on the signal intensity". The authors also reported that when cyanoacrylate was directly added to controlled amounts of purified DNA, PCR was inhibited, although this probably occurred because Chelex does not separate it from substances like cyanoacrylate in the solution. Pitilertpanya and Palmback (2007) placed fingerprints on soda cans, fumed them with cyanoacrylate, extracted DNA using a QiaAmp extraction kit, and amplified STRs using an AmpFℓSTR® COfiler™ kit. No quantitative data or statistics were presented, but the authors stated that more cyanoacrylate resulted in "worse" profiles and non-fumed prints produced "better DNA profiles", which is consistent with the findings of the current study. In contrast, other researchers have found that cyanoacrylate fuming did not have an effect on DNA analysis. Gicale (2011) examined the recovery of DNA from deflagrated pipe bombs fumed with cyanoacrylate by having volunteers assemble bombs, fuming half on site for 15 min following deflagration, and extracting DNA; there was no statistical difference in DNA yields between the two. Bille et al. (2009) also examined cyanoacrylate fuming of pipe bombs, but used a cell suspension to deliver a constant amount of DNA, and deflagrated bombs were exposed to cyanoacrylate for 10 min. Only six bombs were tested and no statistics were reported, although DNA yields from fumed and un-

fumed bombs were similar. These conflicting results are likely due to variations in experimental design, sample type (blood, saliva, touch samples, etc.), fuming method, and DNA extraction technique. Cyanoacrylate fuming may therefore not have the same effect on all types of samples, so both the sample and processing methods must be taken into account by the DNA analyst, and extraction procedures that do not separate molecules such as cyanoacrylate from the DNA (e.g. Chelex) should be avoided.

Based on the results of the current study, cyanoacrylate fuming spent casings is not recommended for several reasons. The purpose of this technique is to enhance latent fingerprints, which is rarely successful when working with casings (e.g. Bentsen et al., 1995). Furthermore, even when fingerprints are visible on spent casings they are often partial or have poor ridge detail, and are not easily identifiable (Bentsen et al., 1995). Fingerprints were not observed on any casings in this study while STR results were produced from most, indicating that DNA is far more likely to provide investigative information from spent casings. However, cyanoacrylate fuming casings prior to DNA processing was detrimental to the identification of the loader, as it resulted in lower DNA yields and more non-handler alleles.

The isolation and extraction method used to recover DNA can also affect the yields obtained from spent casings. One goal of the current study was to further the work of Mottar (2014), who optimized extraction methods and compared soaking and swabbing as means of isolating DNA from spent casings, by examining whether swabbing casings cumulatively or individually was advantageous. Cumulative swabbing resulted in higher DNA yields than did individual swabbing, however they were not three times larger, so cumulative swabbing recovered more DNA per swab but not per casing. The most probable reason for this is that DNA retrieved from one casing was deposited onto subsequently swabbed casings. Hebda et al. (2014)

examined the effects of cumulative swabbing when studying the collection and analysis of DNA from fingernail evidence. Blood from a male volunteer was placed on two of four female fingernails, which were cumulatively swabbed with a single swab wetted with digestion buffer, alternating between bloody and clean. The clean nails were re-swabbed using the double swab technique and DNA was extracted, quantified, and Y-STRs were amplified to assay blood transfer; enough blood was transmitted to the clean nails to produce full Y-STR profiles. It is likely that DNA was also transferred in the current research, as cells picked up from an earlier swabbed casing were deposited onto those that were subsequently swabbed, resulting in the reduction of important genetic evidence.

An interesting finding regarding swabbing strategy in this research was that while cumulative swabbing did not triple DNA yields for either caliber, the increase over swabbing a single casing was much larger for the 0.45 caliber casings. This probably stemmed from the difference in surface area between the two calibers. The surface area of a 0.45 caliber casing is approximately three times bigger than that of a 0.22 caliber casing, so when multiple 0.45 caliber casings are swabbed there is a greater increase in the total surface area (the surface area increases by 18 cm^2 and 6 cm^2 for 0.45 and 0.22 caliber casings, respectively). This larger increase in surface area would logically result in a bigger difference in DNA yield. Another factor that may have caused this discrepancy is the accuracy of the DNA quantitation. If a sample falls outside the range of a standard curve, the calculation of its DNA concentration is likely not as accurate as samples that fall within the curve. The lowest quantitation standard in this research was near the limit of detection of the assay, so adding another standard dilution was not an option. More individually swabbed 0.22 caliber samples fell at or below the smallest quantitation standard than any other caliber/swabbing strategy, so the calculated DNA concentrations for these samples

were probably less accurate. The median DNA yield for the individually swabbed 0.22 casings could thus be artificially high, making it appear as though there was not a large difference between individually and cumulatively swabbed 0.22 caliber casings.

Once DNA has been extracted, two analysis methods are commonly used by forensic scientists: STR profiling and mtDNA sequencing. The Fusion STR amplification kit greatly outperformed MiniFiler™ in the current research (consistent with the findings of Mottar [2014]) because the design of the Fusion kit makes it better suited to overcome the challenges associated with analyzing DNA from spent casings, the first of which is the small amount that is present. Fusion has a lower limit of detection, and thus allows smaller quantities of DNA to be amplified. Promega recommends 0.25 – 0.50 ng of template DNA for Fusion reactions, although Oostdik et al. (2014) showed that full Fusion profiles could be produced from 100 pg of DNA, and the percentage of alleles recovered dropped only slightly to 97.2% when 50 pg of DNA was amplified. Mulero et al. (2008), in contrast, reported that 125 pg input DNA was required to generate a full MiniFiler™ profile, while 500 – 750 pg was determined to be optimal. The amount of DNA added to each STR reaction in the current study was almost always below 50 pg, so using a more sensitive kit was highly beneficial.

There are several other strategies that can be employed to improve the amount of genetic information produced when analyzing small quantities of DNA. One way to increase the DNA yield is whole genome amplification (WGA), in which all of the DNA present in an extract is amplified prior to analysis. There are several methods for performing WGA (reviewed by Zheng et al., 2011) and multiple commercial WGA kits have been developed, including the Qiagen REPLI-g Kit and the Illustra GenomiPhi V2 DNA Amplification Kit. Both these kits were tested by Schneider et al. (2004), who found that full profiles could be obtained from 500 pg of starting

DNA, while drop-out started to occur at 50 pg (drop-in was not observed). However, this research was accomplished using high quality DNA from human cell lines. Barber and Foran (2006) examined the feasibility of using WGA for the analysis of forensic samples, testing control DNA, artificially degraded DNA, and DNA extracted from fresh blood, aged blood, hair, and aged bones. WGA was performed using improved primer extension pre-amplification and multiple displacement amplification, and different primer sets were used to assay various lengths of nuclear and mtDNA both prior to and following WGA. STR analysis was conducted using an Identifiler® kit. The results showed that while WGA was successful when working with high quality DNA, it did not perform well with degraded samples, and in some instances allelic drop-out/amplification failure became worse following WGA. This likely occurred because both improved primer extension pre-amplification and multiple displacement amplification utilize random primers, which decrease the size of the DNA as amplification proceeds. If the DNA fragments are small initially, this reduction may prevent their subsequent amplification. Consequently, the authors stated that WGA was of limited use when working with degraded forensic samples.

Another method for improving analysis success of low quantity DNA is to concentrate it prior to amplification (e.g. Hudlow et al., 2011), which allows for more DNA to be added to an STR reaction, and may result in additional alleles being detected. However, any PCR inhibitors present in solution may also be concentrated, causing amplification failure. Furthermore, increasing the concentration of the DNA decreases solution volume, potentially preventing replicate testing. Following amplification, the signal intensity of STR products from low copy number DNA can be strengthened using post-PCR clean-up. The purpose of this procedure is to remove remaining primers, dNTPs, and salts from solution, all of which are small molecules that

could be preferentially injected into a capillary, reducing the amount of DNA that is loaded. Smith and Ballantyne (2007) extracted DNA from blood, then serially diluted and amplified it using Identifiler®. Four post-PCR purification methods were tested and full STR profiles were produced from 20 pg of DNA using a MinElute column, although it also caused increased stutter and drop-in. More DNA can likewise be loaded into a capillary by increasing the voltage and time of the injection. Westen et al. (2009) tested various capillary electrophoresis conditions, including injection voltages and times ranging from 3 to 15 kV and 10 to 300 s, respectively. The standard setting was 3 kV/10 s, and an increase to 9 kV/15 s improved sensitivity while maintaining "peak shapes that still allowed correct binning and discrimination from background structures like spikes and blobs". A comparison was also made between boosted injection and elevated PCR cycle number, and the authors reported that though drop-out, peak imbalance, and drop-in occurred using both methods, elevated PCR cycle number resulted in increased stutter whereas boosted injection did not.

The other problem faced when analyzing DNA from the spent casings was that the DNA was highly degraded. The size of the targets affects the successful amplification of alleles, and Mottar (2014) observed that, when using Fusion to analyze DNA from spent casings, alleles were more frequently detected at the smaller loci. The MiniFiler™ kit amplifies nine loci that are all less than 300 bp in size, while Fusion targets 24 loci, of which 14 are below 300 bp. MiniFiler™ was designed to amplify the eight largest loci in the Identifiler® kit, so that more genetic information could be obtained from degraded samples than through Identifiler® alone. However, even when examining only the Fusion loci smaller than 300 bp, Fusion still produced higher percent profiles than MiniFiler™. Consequently, Fusion generated far more genetic

information, and it (and other similar mega-STR kits) may be better suited for use in forensic laboratories when working with DNA from spent casings.

There are several methods for decreasing amplicon size to improve the success of STR analysis of highly degraded DNA. Primers can be redesigned to anneal closer to the actual STR (Wiegand and Kleiber, 2001), however there are limits to how far they may be moved, as primers cannot be placed inside the STR itself. It is also not feasible for every STR to be small; if loci overlap in size, it is impossible to distinguish them from one another. One way to overcome this is to alter the mobility of the DNA fragments in a capillary by attaching non-nucleotide linkers to the primers (Grossman et al., 1994); this allows loci to be differentiated even though the actual amplicon length is the same. Alternatively, additional fluorescent dyes can be used to discriminate between similar sized fragments. Most modern commercial STR kits utilizes a five dye system (e.g. MiniFiler™, Fusion, Identifiler®), although GlobalFiler™ has six dyes, which permits the amplification of more small loci; 17 of the 24 loci targeted by GlobalFiler™ are less than 300 bp in size, and 23 are smaller than 400 bp.

If a sample has a relatively high quantity of DNA, it would logically generate a more complete profile than one with less. However, the correlation coefficients between DNA yield and number of handler alleles were moderate in the current research (ranging from 0.3 to 0.8), meaning that while yield does affect STR analysis, it cannot always be used to accurately predict whether or not profiling will be successful. Samples that resulted in greater amounts of DNA (e.g. non-fumed casings, cumulatively swabbed 0.45 casings, etc.) typically produced more handler alleles, with one exception. The large number of non-handler alleles generated from the MSU-fumed casings lowered the correlation between yield and handler alleles, as DNA from sources other than the handler contributed to the total DNA. The relationship between DNA

quantity and STR alleles is important to consider when working with evidence, because samples with very little DNA can still produce genetic information. Not all quantitation assays are as sensitive as the *Alu* assay utilized in the current study, so crime laboratories may commonly encounter samples that appear to have no DNA. This research shows that such low yielding samples could actually contain enough DNA to generate a partial profile.

The relationship between DNA yield and profiling results did not exist when mtDNA was sequenced, as profiles were generated for all samples tested, even those with very little DNA and/or produced few or no STR alleles. However, the DNA quantitation assay used in this research does not measure mtDNA, thus its level in each sample was unknown. MtDNA sequencing is more sensitive than nuclear DNA analysis, as there are hundreds of copies of mtDNA per cell (Robin and Wong, 1988) compared to the two copies of nuclear DNA, potentially making its amplification more successful when analyzing touch samples (e.g. Balogh et al., 2003b). Additionally, mtDNA is better protected from degradation than nuclear DNA (Foran, 2006), which is beneficial when working with degraded samples such as DNA from spent casings. Consequently, all casings had enough high quality mtDNA for HV1 and HV2 to successfully amplify. It should be noted, though, that samples with a high DNA yield generated more accurate sequences (i.e. consistent with the handler) than low yielding samples, but the difference was not significant.

One of the major advantages of mtDNA analysis over STRs in the current research was that the mtDNA sequences were simpler to analyze. HV1 and HV2 were each analyzed as a single fragment, thus they either amplified or did not, and there was no drop-out, like there was in the STR profiles. Furthermore, mtDNA sequences were easier to compare to the handler than STR profiles. STR analysis involves the examination of many fragments of DNA as opposed to a

continuous sequence, and individual alleles were classified as consistent with or not consistent with the handler rather than the whole profile. Whether a mixture was present could not easily be ascertained when partial profiles were produced, which was the case for nearly all samples. For instance, if an STR profile consisted of six alleles, and four of them were consistent with the handler, it was impossible to determine if the profile was a mixture of the handler and another individual or resulted from a single individual who had four alleles in common with the handler. In contrast, a mixture was recognized in mtDNA when more than one peak was present at a given position, and determining if it could include the handler was straightforward. For example, if a mixture contained the polymorphisms 16126Y and 16147Y (with Y representing a mixture of C and T) and the handler's profile was 16126C and 16147T, the mixture was classified as including the handler. The only mixtures that were particularly difficult to interpret were those that involved different length polymorphisms in the first C-stretch of HV2 (302 – 310); in such samples, the sequences become out of phase downstream of the C-stretch causing multiple bases to appear at every nucleotide position, which is irresolvable. Other researchers have also had problems sequencing this region, and Stewart et al. (2001) reported various lengths of that C-stretch in separate hairs from the same individual, so they proposed that length polymorphisms not be used for exclusionary purposes. Several authors have stated that when a mtDNA mixture is present in a forensic sample, no attempt should be made to interpret it at all (e.g. Andréasson et al., 2006; Butler 2011), thus complex mixtures involving C-stretch polymorphisms would simply be classified as inconclusive.

Only ~30% of the spent casings in the current research produced mtDNA mixtures, however it is possible that this number was underestimated as not all mixtures can be detected through Sanger sequencing. Mixtures of a 1:5 ratio may be readily visualized in a sequence, but

more extreme ratios ranging from 1:20 to 1:300 are typically indistinguishable from background noise (Holland et al., 2011). Additionally, mixtures can consist of DNA from two individuals who have the same mtDNA sequence, which would appear as a single source sample. These would probably have no bearing on forensic evidence, though, because if a minor contributor is not detected, it will not complicate interpretation.

Most sequences produced in this research were clean and easy to read, although low quality sequences, comprised of irregularly shaped or spaced peaks, were sometimes generated. This problem was generally solved by re-sequencing, often using less amplified DNA, potentially because when too much was added to the reaction, non-specific binding of the sequencing primer occurred. Primer specificity is very important; if there are multiple template regions of DNA, it is impossible to obtain a single, clean sequence. The sequencing protocol used in the current study has an annealing time of only 5 s to help prevent primers from binding non-specifically to the DNA. Too much template likely creates a greater opportunity for non-specific primer binding, resulting in low quality sequences.

Overall, mtDNA sequencing was more effective for accurately "identifying" the loader of a cartridge, and therefore might be the better option for examining DNA from evidentiary casings. However, there are drawbacks to analyzing mtDNA in a crime laboratory that do not exist for STR analysis. MtDNA profiles are not unique, and the most common Caucasian and African American haplotypes have frequencies of approximately 4% and 2.7%, respectively (Holland and Parsons, 1999). Consequently, mtDNA cannot be utilized to positively identify the loader of a firearm, although it can be used to include, and more importantly to completely exclude, a suspect. Sequencing is also much more time consuming than STR analysis. Further, few crime laboratories perform mtDNA analysis, and may instead have to send samples to

agencies such as the regional FBI laboratories for testing, which already have a backlog of cases (U.S. Department of Justice, 2012), or private laboratories.

Another shortcoming of mtDNA sequencing is that an evidentiary profile is only informative when it can be compared to a suspect, as there is currently no searchable database for forensic mtDNA profiles (although the FBI has stated it plans to add mtDNA to CODIS [Federal Bureau of Investigation, 2010]). In contrast, the existing database can be searched for possible matches to an STR profile, giving STRs more investigative value when there is no suspect. CODIS includes an index of arrestees and convicted offenders, so if an individual whose DNA is already in the database commits another crime, searching their profile will identify them. Several studies have shown that most individuals involved in firearm violence are repeat offenders, therefore their DNA would be in the system. For example, 60% of all youth homicides in Boston involve chronic offenders, and in Indianapolis, homicide suspects from 1997 – 1998 averaged 11.5 prior arrests (reviewed by McGarrell et al., 2006). Additionally, the investigation of the Brightwood Gang in Indianapolis in 1999 resulted in the arrest of 16 individuals, who combined had more than 20 prior convictions for violent felonies and over 70 other convictions (McGarrell et al., 2006). DNA from spent cartridge casings would provide valuable evidence in such instances, as the results of a database search would often include the individual who loaded the weapon. STRs thus have the potential to provide greater investigative leads when no information about a suspect is available, even though mtDNA analysis is more sensitive and better for helping to identify the individual who loaded a firearm when a suspect's DNA is available for comparison.

The greatest impediment to accurately identifying the loader in this research was the presence of DNA not consistent with the handler, as 85% of STR profiles and 48% of mtDNA

sequences contained at least one non-handler allele/polymorphism. There were multiple potential sources of these non-handler DNAs. Cartridges were not cleaned prior to loading, and DNA might have already been present on them. Some volunteers placed the cartridges on the table top prior to loading them into a magazine, which was not a clean surface and could have held DNA from other individuals. The same magazines were loaded by each volunteer and all cartridges of the same caliber were fired by a single weapon, so DNA may have been transferred between the casings and the magazine/firearm. The shooter wore gloves when firing the cartridges, but the casings sometimes made contact with the individual's lab coat. DNA from the shooter could not be identified due to the small number of alleles produced and the anonymous method used to collect buccal swabs, and the non-handler alleles did not appear to be from one consistent individual. The casings within each collection were captured using a single apparatus (a microscope cover for Collection 1 or a pop-up hamper for Collection 2), and as a result DNA might have been transferred between the collection apparatus and the casings. Additionally, many casings fell onto the floor during collection. Although some of these sources of inconsistent alleles/polymorphisms may be a product of the research setting (e.g. it is unlikely that 20 different individuals would load the same gun over the course of a few hours), others are likely to be present in a forensic scenario. For instance, it is doubtful that a criminal will clean ammunition prior to loading it, and casings are going to fall to the ground when ejected from a weapon.

Non-handler DNAs were present in all types of casings, however they were most numerous in the MSU-fumed casings (as discussed above) and the cumulatively swabbed casings. This was a severe disadvantage to cumulative swabbing; although it resulted in higher DNA yields, it also held a stronger risk of inaccurate information. Individual swabbing was thus

superior as a method of accurately identifying the individual who loaded a firearm, because it produced less non-handler alleles/polymorphisms. This was especially true when analyzing mtDNA, since both strategies yielded the same amount of information (i.e. both generated complete sequences), but individual swabbing gave rise to fewer mixed and inconsistent profiles. However, when STRs were amplified, the decrease in the number of non-handler alleles produced from the individual swabbed casings was accompanied by a decrease in the number of handler alleles and the loss of valuable evidence. If an STR profile contains only a few alleles, there might not be enough information to hold any real evidentiary value, even when no non-handler alleles are present. Searching a database for profiles consistent with a small amount of alleles will likely return too many hits to be useful if there is no other information to narrow down the list of potential suspects. Conversely, if casings are cumulatively swabbed and generate a profile containing 20 alleles, some number of which are inconsistent with the handler, the information could still provide investigative leads. For example, a database may be searched using low stringency conditions (e.g. search for profiles that are 75% consistent) to compensate for the possibility of the profile containing non-handler alleles; this can still result in many consistent profiles, but the number might be small enough to determine if any of the individuals were potentially involved in the crime. Due to this trade-off between many handler and few non-handler alleles, cumulative swabbing could be more advantageous if STRs are to be analyzed from spent casings, particularly when working with smaller caliber (e.g. 0.22) casings, which did not frequently yield more than a few alleles when individually swabbed. Another trade-off of individual swabbing is the amount of time it takes. If a crime scene involves a large number of casings it may not be feasible to swab them all individually, even if it will generate less mixtures, as swabbing casings individually is time consuming.

Non-handler DNA was much more abundant in the STR profiles than in the mtDNA sequences, and many casings produced several non-handler alleles but no non-handler polymorphisms. It is thus likely that some of the non-handler STR alleles were the product of artifacts such as drop-in and stutter, rather than contaminant DNA. A recommended method to overcome the challenges presented by artifacts is replicate analysis (not used in this study), in which DNA is amplified multiple times from the same extract and only alleles that are observed at least twice are reported (Taberlet et al., 1996; Budowle et al., 2009). This might remove drop-in from a profile, but it may also eliminate correct alleles. Stochastic sampling can result in different DNA being transferred to separate STR reactions, and therefore dissimilar alleles being amplified. Consequently, low copy number samples are often not reproducible (reviewed by Budowle et al., 2009), so distinguishing between correct alleles and artifacts is difficult. One STR artifact that could be reproducible is stutter. A general observation in the current research was that many non-handler STR alleles were in a stutter position, however classification of these alleles as stutter was not attempted due to peak height imbalance, which is extremely prevalent in low copy number samples (e.g. Gill et al., 2000), although the GeneMapper® software does identify and filter out some stutter peaks. Additionally, it is typical for non-artifact alleles to be in a stutter position of one another, since the frequencies of STR alleles for each locus are generally normally distributed (i.e. form a bell curve), not random (e.g. Díaz et al., 2008). Thus, the most common allele is at or near the center of the distribution, with the next most frequent alleles occurring in its stutter positions. For instance, 12 might be the most common allele at a particular locus, with 11 and 13 following in frequency, so many individuals in a population will have some combination of those alleles. Consequently, just because an allele in a low copy number sample is located in a stutter position of another allele does not mean that it actually is

stutter. It may therefore be advisable to perform replicate analysis when working with spent casings in a forensic setting in an attempt to eliminate STR artifacts from evidentiary profiles and identify all of the alleles that are present in a sample.

Alleles could easily be classified as consistent or not consistent with the handler in this study, which is extremely valuable in a research setting, as variables (e.g. swabbing strategy and STR kit) can be manipulated and the amount of accurate genetic information that is produced can be measured. If cumulative and individual swabbing were compared in the current research without knowing the handler's profile, the fact that the former resulted in a larger number of non-handler alleles/polymorphisms would not have been recognized. Another group of researchers, Dieltjes et al. (2011), developed an extraction procedure for cartridges, bullets, and casings and reported that profiles were generated from 6.9% of the 4,085 items tests, but whether the alleles were consistent with the individual who loaded or shot the firearm could not be established because the items were from criminal investigations for which the handler was not known, thus the true effectiveness of the method is unclear. Profiles from evidence are typically compared to that of a suspect, although they may not be the individual who loaded the weapon. If an examiner assumed the evidence and the suspect are consistent, they would be biased and could attribute any inconsistent alleles to drop-in, even if they are not. Conversely, if all alleles are presumed to be correct, the presence of even one non-handler allele might result in the exclusion of the individual who loaded the firearm. Guidelines regarding the conclusions that are made from DNA from spent casings must therefore be developed/adopted and strictly followed. As a hypothetical example, a laboratory policy could state that 20% of the alleles in a profile can be inconsistent with a suspect who is said to be included as a possible source of the DNA, and that 50% must be inconsistent to completely exclude the suspect, while anything in between is

inconclusive. Additionally, the laboratory may require that a profile includes a minimum number of alleles (e.g. 10) in order for any conclusions to be made. If these policies were applied to the this research, approximately 62% of the Collection 2 profiles would have been classified as inconclusive/not enough information, 30% as including the loader, and 8% as excluding the loader. However, due to the prevalence of non-handler alleles in the MSU-fumed casings, only 13% of Collection 2 profiles would have included the loader, while 18% would have excluded them and 69% were inconclusive/not enough information.

The presence of alleles/polymorphisms that are inconsistent with a suspect could have serious consequences in court. The prosecution's goal is to convince a jury that the suspect committed the crime, so being aware of potential sources of contamination would allow them to classify a profile as being consistent with the suspect even when alleles inconsistent with the defendant are present. For instance, if a partial STR profile is produced that includes 20 alleles consistent with the suspect and two that are not, it is possible that the inconsistent alleles are from the surface the casing fell on, the manufacturing process, or any of the other contamination sources mentioned previously, so the prosecution would likely argue that the DNA still came from the suspect. On the other hand, the defense could contend that even one inconsistent allele excludes the suspect as the source of the DNA. Additionally, they may argue that the entire profile was the result of contamination, and the presence of the suspect's DNA on a spent casing does not mean that they fired the gun, or even that they loaded the cartridge. Instead, the defense might reason that the casing came in contact with the suspect's cells when it fell onto the floor, or that the suspect had handled the gun prior to the commission of the crime. Consequently, DNA analysts need to be aware of the possibility of contamination/artifacts in a profile from a spent casing and be cautious when making claims about the consistency of the profile.

There are many other variables that remain to be investigated regarding the recovery and analysis of DNA from spent cartridge casings. First, experiments could be conducted to further explore the effect of loading and firing order, which were not examined individually in the current study. Whether loading order alone actually affects the amount of DNA deposited on casings may be determined by having volunteers load cartridges into a magazine, then manually cycling the weapon (without firing it) to eject them. Cartridges from each volunteer would be collected individually and processed for DNA in order to directly compare them. Firing order might then be examined by repeating the experiment, this time firing the weapon, and comparing the spent casings to the cartridges to see if the results differed. Second, though fuming certainly had a negative impact on DNA recovery and analysis from spent casings, whether transportation had an effect was not as clear due to the differences in the fuming methods. Fuming casings on site immediately following firing and after a period of time during which the casings were transported using the same or a similar fuming chamber would reduce or eliminate variables such as the amount of cyanoacrylate deposited on the casings, making comparisons more straightforward. Next, additional techniques for analyzing low copy number DNA could be tested to improve the success of DNA profiling. For instance, post-PCR clean-up might increase the quantity of genetic information produced utilizing commercial STR kits. It would also be beneficial to better understand the sources of non-handler DNA. If a portion of the non-handler alleles are due to STR artifacts and not contaminant DNA, it is possible that techniques such as replicate analysis will eliminate them. Additionally, using the same magazines, gun, and collection apparatus for all volunteers may have contributed to non-handler alleles/polymorphisms in the current research, and exaggerated the likelihood of DNA contamination on casings collected from a crime scene, which would be valuable knowledge in

court. Examination of all these variables could greatly improve the accuracy of using DNA profiling from spent cartridge casings to identify the loader of a firearm.

CONCLUSIONS

Overall, a variety of factors were examined in this research to determine what, if any, effect they have on the recovery and analysis of DNA from spent casings. Several important facts were determined over the course of this study. Among them are:

- Significantly more DNA was recovered from 0.45 than from 0.22 caliber casings.
- Loading/firing order did not have a significant effect on the recovery and analysis of DNA from spent casings.
- Cyanoacrylate fuming spent casings prior to DNA processing was detrimental to DNA recovery/analysis.
- Cumulative swabbing recovered more DNA than individual swabbing, but also resulted in more non-handler alleles/polymorphisms.
- The Fusion STR kit generated more genetic information than MiniFiler™.
- MtDNA was more sensitive than STR analysis, and resulted in fewer mixed/incorrect profiles.

Knowledge of these facts can aid law enforcement in the accurate identification of the individual who loaded a firearm based on DNA from spent casings, providing valuable evidence that may be used during the investigation of a firearm offense.

APPENDICES

APPENDIX A. ASSIGNMENT OF FUMING METHODS AND SWABBING STRATEGIES FOR COLLECTIONS 1 AND 2

Table A1. Assignment of fuming methods for Collection 1**.**

Bag #	Buccal Letter	Casing # Fired	Fuming Method
2-1	U	1 thru 3	Fumed at MSP
2-2	U	4 thru 6	Fumed at MSU
2-3	U	7 thru 9	Not Fumed
2-4	U	10 thru 12	Used in a different study
2-5	U	13 thru 15	Used in a different study
2-6	U	16 thru 18	Used in a different study
2-7	U	19 thru 21	Used in a different study
3-1	MM	1 thru 3	Used in a different study
3-2	MM	4 thru 6	Fumed at MSP
3-3	MM	7 thru 9	Fumed at MSU
3-4	MM	10 thru 12	Not Fumed
3-5	MM	13 thru 15	Used in a different study
3-6	MM	16 thru 18	Used in a different study
3-7	MM	19 thru 21	Used in a different study
8-1	S	1 thru 3	Used in a different study
8-2	S	4 thru 6	Used in a different study
8-3	S	7 thru 9	Fumed at MSP
8-4	S	10 thru 12	Fumed at MSU
8-5	S	13 thru 15	Not Fumed
8-6	S	16 thru 18	Used in a different study
8-7	S	19 thru 21	Used in a different study
10-1	VV	1 thru 3	Used in a different study
10-2	VV	4 thru 6	Used in a different study
10-3	VV	7 thru 9	Used in a different study
10-4	VV	10 thru 12	Fumed at MSP
10-5	VV	13 thru 15	Fumed at MSU
10-6	VV	16 thru 18	Not Fumed
10-7	VV	19 thru 21	Used in a different study
13-1	V	1 thru 3	Used in a different study
13-2	V	4 thru 6	Used in a different study

Table A1 (cont'd)

13-3	V	7 thru 9	Used in a different study
13-4	V	10 thru 12	Used in a different study
13-5	V	13 thru 15	Fumed at MSP
13-6	V	16 thru 18	Fumed at MSU
13-7	V	19 thru 21	Not Fumed
15-1	HH	1 thru 3	Not Fumed
15-2	HH	4 thru 6	Used in a different study
15-3	HH	7 thru 9	Used in a different study
15-4	HH	10 thru 12	Used in a different study
15-5	HH	13 thru 15	Used in a different study
15-6	HH	16 thru 18	Fumed at MSP
15-7	HH	19 thru 21	Fumed at MSU
23-1	L	1 thru 3	Fumed at MSU
23-2	L	4 thru 6	Not Fumed
23-3	L	7 thru 9	Used in a different study
23-4	L	10 thru 12	Used in a different study
23-5	L	13 thru 15	Used in a different study
23-6	L	16 thru 18	Used in a different study
23-7	L	19 thru 21	Fumed at MSP
25-1	T	1 thru 3	Fumed at MSP
25-2	T	4 thru 6	Fumed at MSU
25-3	T	7 thru 9	Not Fumed
25-4	T	10 thru 12	Used in a different study
25-5	T	13 thru 15	Used in a different study
25-6	T	16 thru 18	Used in a different study
25-7	T	19 thru 21	Used in a different study
26-1	XX	1 thru 3	Used in a different study
26-2	XX	4 thru 6	Fumed at MSP
26-3	XX	7 thru 9	Fumed at MSU
26-4	XX	10 thru 12	Not Fumed
26-5	XX	13 thru 15	Used in a different study
26-6	XX	16 thru 18	Used in a different study
26-7	XX	19 thru 21	Used in a different study
27-1	N	1 thru 3	Used in a different study
27-2	N	4 thru 6	Used in a different study

Table A1 (cont'd)

27-3	N	7 thru 9	Fumed at MSP
27-4	N	10 thru 12	Fumed at MSU
27-5	N	13 thru 15	Not Fumed
27-6	N	16 thru 18	Used in a different study
27-7	N	19 thru 21	Used in a different study
24-1	OO	1 thru 3	Used in a different study
24-2	OO	4 thru 6	Used in a different study
24-3	OO	7 thru 9	Used in a different study
24-4	OO	10 thru 12	Fumed at MSP
24-5	OO	13 thru 15	Fumed at MSU
24-6	OO	16 thru 18	Not Fumed
24-7	OO	19 thru 21	Used in a different study
33-1	B	1 thru 3	Used in a different study
33-2	B	4 thru 6	Used in a different study
33-3	B	7 thru 9	Used in a different study
33-4	B	10 thru 12	Used in a different study
33-5	B	13 thru 15	Fumed at MSP
33-6	B	16 thru 18	Fumed at MSU
33-7	B	19 thru 21	Not Fumed
36-1	D	1 thru 3	Not Fumed
36-2	D	4 thru 6	Used in a different study
36-3	D	7 thru 9	Used in a different study
36-4	D	10 thru 12	Used in a different study
36-5	D	13 thru 15	Used in a different study
36-6	D	16 thru 18	Fumed at MSP
36-7	D	19 thru 21	Fumed at MSU
38-1	WW	1 thru 3	Fumed at MSU
38-2	WW	4 thru 6	Not Fumed
38-3	WW	7 thru 9	Used in a different study
38-4	WW	10 thru 12	Used in a different study
38-5	WW	13 thru 15	Used in a different study
38-6	WW	16 thru 18	Used in a different study
38-7	WW	19 thru 21	Fumed at MSP
40-1	SS	1 thru 3	Fumed at MSP
40-2	SS	4 thru 6	Fumed at MSU

Table A1 (cont'd)

40-3	SS	7 thru 9	Not Fumed
40-4	SS	10 thru 12	Used in a different study
40-5	SS	13 thru 15	Used in a different study
40-6	SS	16 thru 18	Used in a different study
40-7	SS	19 thru 21	Used in a different study
41-1	Y	1 thru 3	Used in a different study
41-2	Y	4 thru 6	Fumed at MSP
41-3	Y	7 thru 9	Fumed at MSU
41-4	Y	10 thru 12	Not Fumed
41-5	Y	13 thru 15	Used in a different study
41-6	Y	16 thru 18	Used in a different study
41-7	Y	19 thru 21	Used in a different study
50-1	II	1 thru 3	Used in a different study
50-2	II	4 thru 6	Used in a different study
50-3	II	7 thru 9	Fumed at MSP
50-4	II	10 thru 12	Fumed at MSU
50-5	II	13 thru 15	Not Fumed
50-6	II	16 thru 18	Used in a different study
50-7	II	19 thru 21	Used in a different study

Table A2. Assignment of swabbing strategies for Collection 2.

Bag #	Caliber	Buccal Letter	Casing # Fired	Swabbing Strategy
70–1	0.45 and 0.22	OOO	1 – 3	Cumulative
70–2	0.45 and 0.22	OOO	4 – 6	Cumulative
70–3	0.45 and 0.22	OOO	7 – 9	Individual
70–4	0.45 and 0.22	OOO	10 – 12	Cumulative
53–1	0.45 and 0.22	B	1 – 3	Cumulative
53–2	0.45 and 0.22	B	4 – 6	Cumulative
53–3	0.45 and 0.22	B	7 – 9	Cumulative
53–4	0.45 and 0.22	B	10 – 12	Individual
67–1	0.45 and 0.22	VVV	1 – 3	Individual
67–2	0.45 and 0.22	VVV	4 – 6	Cumulative
67–3	0.45 and 0.22	VVV	7 – 9	Cumulative

Table A2 (cont'd)

67–4	0.45 and 0.22	VVV	10 – 12	Cumulative
51–1	0.45 and 0.22	NN	1 – 3	Cumulative
51–2	0.45 and 0.22	NN	4 – 6	Individual
51–3	0.45 and 0.22	NN	7 – 9	Cumulative
51–4	0.45 and 0.22	NN	10 – 12	Cumulative
58–1	0.45 and 0.22	J	1 – 3	Cumulative
58–2	0.45 and 0.22	J	4 – 6	Cumulative
58–3	0.45 and 0.22	J	7 – 9	Individual
58–4	0.45 and 0.22	J	10 – 12	Cumulative
56–1	0.45 and 0.22	AA	1 – 3	Cumulative
56–2	0.45 and 0.22	AA	4 – 6	Cumulative
56–3	0.45 and 0.22	AA	7 – 9	Cumulative
56–4	0.45 and 0.22	AA	10 – 12	Individual
66–1	0.45 and 0.22	DDD	1 – 3	Individual
66–2	0.45 and 0.22	DDD	4 – 6	Cumulative
66–3	0.45 and 0.22	DDD	7 – 9	Cumulative
66–4	0.45 and 0.22	DDD	10 – 12	Cumulative
69–1	0.45 and 0.22	R	1 – 3	Cumulative
69–2	0.45 and 0.22	R	4 – 6	Individual
69–3	0.45 and 0.22	R	7 – 9	Cumulative
69–4	0.45 and 0.22	R	10 – 12	Cumulative
52–1	0.45 and 0.22	ZZZ	1 – 3	Cumulative
52–2	0.45 and 0.22	ZZZ	4 – 6	Cumulative
52–3	0.45 and 0.22	ZZZ	7 – 9	Individual
52–4	0.45 and 0.22	ZZZ	10 – 12	Cumulative
61–1	0.45 and 0.22	I	1 – 3	Cumulative
61–2	0.45 and 0.22	I	4 – 6	Cumulative
61–3	0.45 and 0.22	I	7 – 9	Cumulative
61–4	0.45 and 0.22	I	10 – 12	Individual
68–1	0.45 and 0.22	XXX	1 – 3	Individual
68–2	0.45 and 0.22	XXX	4 – 6	Cumulative
68–3	0.45 and 0.22	XXX	7 – 9	Cumulative
68–4	0.45 and 0.22	XXX	10 – 12	Cumulative
59–1	0.45 and 0.22	KKK	1 – 3	Cumulative
59–2	0.45 and 0.22	KKK	4 – 6	Individual

Table A2 (cont'd)

59–3	0.45 and 0.22	KKK	7 – 9	Cumulative
59–4	0.45 and 0.22	KKK	10 – 12	Cumulative
62–1	0.45 and 0.22	YYY	1 – 3	Cumulative
62–2	0.45 and 0.22	YYY	4 – 6	Cumulative
62–3	0.45 and 0.22	YYY	7 – 9	Individual
62–4	0.45 and 0.22	YYY	10 – 12	Cumulative
60–1	0.45 and 0.22	JJJ	1 – 3	Cumulative
60–2	0.45 and 0.22	JJJ	4 – 6	Cumulative
60–3	0.45 and 0.22	JJJ	7 – 9	Cumulative
60–4	0.45 and 0.22	JJJ	10 – 12	Individual
57–1	0.45 and 0.22	A	1 – 3	Individual
57–2	0.45 and 0.22	A	4 – 6	Cumulative
57–3	0.45 and 0.22	A	7 – 9	Cumulative
57–4	0.45 and 0.22	A	10 – 12	Cumulative
63–1	0.45 and 0.22	EE	1 – 3	Cumulative
63–2	0.45 and 0.22	EE	4 – 6	Individual
63–3	0.45 and 0.22	EE	7 – 9	Cumulative
63–4	0.45 and 0.22	EE	10 – 12	Cumulative
54–1	0.45 and 0.22	BBB	1 – 3	Cumulative
54–2	0.45 and 0.22	BBB	4 – 6	Cumulative
54–3	0.45 and 0.22	BBB	7 – 9	Individual
54–4	0.45 and 0.22	BBB	10 – 12	Cumulative
39–1	0.45 and 0.22	SSS	1 – 3	Cumulative
39–2	0.45 and 0.22	SSS	4 – 6	Cumulative
39–3	0.45 and 0.22	SSS	7 – 9	Cumulative
39–4	0.45 and 0.22	SSS	10 – 12	Individual
55–1	0.45 and 0.22	C	1 – 3	Individual
55–2	0.45 and 0.22	C	4 – 6	Cumulative
55–3	0.45 and 0.22	C	7 – 9	Cumulative
55–4	0.45 and 0.22	C	10 – 12	Cumulative
65–1	0.45 and 0.22	JJ	1 – 3	Cumulative
65–2	0.45 and 0.22	JJ	4 – 6	Individual
65–3	0.45 and 0.22	JJ	7 – 9	Cumulative
65–4	0.45 and 0.22	JJ	10 – 12	Cumulative

APPENDIX B. DNA QUANTITIES RECOVERED FROM SPENT CARTRIDGE CASINGS FROM COLLECTION 1

Table B1. Quantitation results of casings fumed at MSU from Collection 1.

Sample	Extract Volume (µL)	DNA Concentration (pg/µL)	DNA Yield (pg)
2-2a	27.00	1.01E+00	27.27
2-2b	24.20	1.05E+00	25.41
2-2c	22.50	1.12E+01	252.00
3-3a	23.50	6.76E-01	15.89
3-3b	25.50	6.10E-01	15.56
3-3c	27.00	5.79E-01	15.63
8-4a	26.00	1.58E+00	41.08
8-4b	19.40	5.61E-01	10.88
8-4c	21.00	7.21E-01	15.14
10-5a	26.50	3.10E-02	0.82
10-5b	23.80	1.42E-01	3.38
10-5c	21.60	7.33E-02	1.58
13-6a	24.80	4.18E+00	103.66
13-6b	22.50	4.81E+00	108.23
13-6c	24.00	2.18E+00	52.32
15-7a	26.60	1.83E-01	4.87
15-7b	26.40	3.14E-01	8.29
15-7c	21.00	1.25E+00	26.25
23-1a	22.00	1.05E+00	23.10
23-1b	25.50	3.17E-01	8.08
23-1c	27.00	1.04E+00	28.08
24-5a	24.00	6.20E-01	14.88
24-5b	26.40	1.90E-01	5.02
24-5c	27.00	1.45E-01	3.92
25-2a	27.20	2.71E-02	0.74
25-2b	22.50	1.06E-01	2.39
25-2c	26.00	2.14E-02	0.56
26-3a	27.20	1.31E+00	35.63
26-3b	27.50	8.69E-01	23.90
26-3c	23.00	6.36E+00	146.28
27-4a	21.70	2.88E-01	6.25
27-4b	20.50	3.83E+00	78.52
27-4c	23.00	2.05E+00	47.15
33-6a	26.20	1.61E+00	42.18

Table B1 (cont'd)

33-6b	27.00	5.26E+01	1420.20
33-6c	25.50	4.94E-01	12.60
36-7a	30.00	4.85E-02	1.46
36-7b	27.50	1.11E-01	3.05
36-7c	26.70	1.48E-02	0.40
38-1a	22.80	4.37E-01	9.96
38-1b	24.20	4.12E-01	9.97
38-1c	22.80	1.49E+00	33.97
40-2a	27.80	7.25E-02	2.02
40-2b	29.70	8.56E-02	2.54
40-2c	25.00	3.57E-01	8.93
41-3a	25.50	2.05E-01	5.23
41-3b	24.00	2.34E-01	5.62
41-3c	25.80	6.21E-01	16.02
50-4a	7.00	8.61E-01	6.03
50-4b	24.00	2.68E-01	6.43
50-4c	20.40	5.65E-01	11.53

Table B2. Quantitation results of casings fumed at MSP from Collection 1.

Sample	Extract Volume (µL)	DNA Concentration (pg/µL)	DNA Yield (pg)
2-1a	29.30	1.32E-01	3.87
2-1b	30.00	7.02E-03	0.21
2-1c	27.00	8.43E-01	22.76
3-2a	20.00	2.61E-07	0.00
3-2b	26.30	4.29E-04	0.01
3-3c	28.60	5.18E-03	0.15
8-3a	28.40	6.72E-01	19.08
8-3b	27.80	7.99E-01	22.21
8-3c	25.00	4.14E-01	10.35
10-4a	26.70	2.85E-03	0.08
10-4b	28.80	2.61E-01	7.52
10-4c	26.00	2.46E-01	6.40
13-5a	29.20	5.63E-01	16.44
13-5b	30.30	1.31E+00	39.69
13-5c	26.00	1.89E+00	49.14
15-6a	26.00	3.60E-02	0.94
15-6b	26.70	5.55E-02	1.48
15-6c	24.40	4.52E-04	0.01
23-7a	32.00	1.28E+00	40.96

Table B2 (cont'd)

23-7b	29.80	3.34E+00	99.53
23-7c	26.30	1.18E-01	3.10
24-4a	25.70	8.07E-03	0.21
24-4b	27.50	7.81E-01	21.48
24-4c	27.40	2.92E-02	0.80
25-1a	27.00	2.48E-01	6.70
25-1b	28.40	1.00E-01	2.84
25-1c	22.20	7.15E-02	1.59
26-2a	26.50	7.83E-01	20.75
26-2b	22.60	4.56E-01	10.31
26-2c	22.10	1.07E+00	23.65
27-3a	28.20	1.18E-01	3.33
27-3b	23.20	1.09E-01	2.53
27-3c	27.30	1.45E-01	3.96
33-5a	27.60	6.95E-02	1.92
33-5b	23.00	2.15E-01	4.95
33-5c	27.80	5.15E-01	14.32
36-6a	30.00	6.87E-02	2.06
36-6b	27.50	4.01E-01	11.03
36-6c	26.70	3.72E-01	9.93
38-7a	25.80	1.72E-01	4.44
38-7b	27.00	1.87E-01	5.05
38-7c	25.00	6.57E-02	1.64
40-1a	27.80	1.77E-01	4.92
40-1b	29.70	7.88E-02	2.34
40-1c	25.00	1.27E-01	3.18
41-2a	27.80	4.95E-01	13.76
41-2b	29.00	5.45E-01	15.81
41-2c	26.50	5.83E-01	15.45
50-3a	28.50	8.62E-01	24.57
50-3b	29.00	1.77E-01	5.13
50-3c	28.80	8.98E-02	2.59

Table B3. Quantitation results of non-fumed casings from Collection 1.

Sample	Extract Volume (µL)	DNA Concentration (pg/µL)	DNA Yield (pg)
2-3a	28.80	2.21E+00	63.65
2-3b	33.00	5.19E-01	17.13
2-3c	26.00	8.25E-01	21.45
3-4a	29.00	2.42E+00	70.18
3-4b	26.00	5.35E-01	13.91
3-4c	24.00	3.40E-01	8.16
8-5a	27.00	9.57E-01	25.84
8-5b	25.00	1.66E+00	41.50
8-5c	29.00	1.97E+00	57.13
10-6a	26.20	2.59E-01	6.79
10-6b	27.80	3.14E-01	8.73
10-6c	28.40	2.28E-01	6.48
13-7a	25.20	3.69E+00	92.99
13-7b	24.00	1.61E+01	386.40
13-7c	27.50	2.93E+00	80.58
15-1a	25.20	1.74E-01	4.38
15-1b	30.80	3.67E-01	11.30
15-1c	27.60	3.81E-01	10.52
23-2a	25.60	5.14E+00	131.58
23-2b	24.00	4.15E+00	99.60
23-2c	25.20	1.38E+00	34.78
24-6a	26.80	1.24E-01	3.32
24-6b	27.00	4.73E-01	12.77
24-6c	27.40	3.06E-01	8.38
25-3a	26.80	2.95E-01	7.91
25-3b	24.80	3.54E-01	8.78
25-3c	24.00	4.79E-01	11.50
26-4a	24.50	1.70E+00	41.65
26-4b	26.80	1.78E+00	47.70
26-4c	27.00	1.38E+00	37.26
27-5a	21.20	1.38E+00	29.26
27-5b	18.80	1.39E+00	26.13
27-5c	24.50	1.31E+00	32.10
33-7a	24.50	2.16E+00	52.92
33-7b	24.40	2.65E+00	64.66
33-7c	25.60	1.19E+00	30.46
36-1a	22.20	9.91E-01	22.00
36-1b	25.00	5.80E-01	14.50
36-1c	28.00	8.94E-01	25.03

Table B3 (cont'd)

38-2a	26.20	9.87E-01	25.86
38-2b	27.20	1.55E+00	42.16
38-2c	26.00	7.87E-01	20.46
40-3a	27.00	6.83E-01	18.44
40-3b	28.80	1.83E+00	52.70
40-3c	27.20	3.53E-01	9.60
41-4a	24.00	5.42E-01	13.01
41-4b	29.30	4.04E+00	118.37
41-4c	25.50	7.36E-01	18.77
50-5a	25.70	1.94E+00	49.86
50-5b	25.50	3.68E+00	93.84
50-5c	27.40	9.58E-01	26.25

APPENDIX C. COMPARISON OF FUSION AND MINIFILER™ STR PROFILES

Red font: non-loader allele

**: allele was above the threshold using OSIRIS, but below the threshold using GeneMapper®.*

†: off-ladder allele

Blank cell: no alleles were amplified

Gray cell: locus not amplified

N/A: not applicable

Table C1. Fusion and MiniFiler™ profiles generated from spent cartridge casings loaded by individual U.

Locus	2-1c (Fusion)	2-1c (MiniFiler™)	2-2a (Fusion)	2-2a (MiniFiler™)	U
Amel			X		X,X
D3			15		15,15
D1			15.3,16.3		11,17.3
D2					10,15
D10					12,14
D13	13		9		9,13
Penta E			12		12,15
D16	11,13		6		11,13
D18	14,15	14			14,15
D2		17	17		17,25
CSF					10,12
Penta D			11		10,11
THO1	6		9,9.3		6,7
vWA	14,20		18		14,20
D21					28,30
D7			11		11,11
D5	11				11,11
TPOX					8,11
DYS391					N/A
D8	12		†,†,13,15		12,12
D12	17,23				17,23
D19	15				13,13
FGA					24,25
D22			16		16,16
Fuming Method	MSP-Fumed	MSP-Fumed	MSU-Fumed	MSU-Fumed	Buccal

Table C1 (cont'd)

Locus	2-2c (Fusion)	2-2c (MiniFiler™)	2-3a (MiniFiler™)	2-3a (Fusion)	U
Amel	X	X	X	X	X,X
D3	14,15,18		15		15,15
D1	14,17.3		11,17.3		11,17.3
D2	10,11,11.3		10,15		10,15
D10	12,14		12		12,14
D13	10,11	9,10,11		9	9,13
Penta E	12				12,15
D16	10,12		11,13	11	11,13
D18	12,17	12,17	13,14,15	14,15	14,15
D2	17,18,25	17,18	25	17,25	17,25
CSF	10	9,10	12	12	10,12
Penta D	16		10		10,11
THO1	6,7,9.3		6,7		6,7
vWA	16		14		14,20
D21	30,33.2	28,30,33.2	28,30,31	28,30	28,30
D7	10,12			11	11,11
D5	12		11		11,11
TPOX	8,11		8,11		8,11
DYS391					N/A
D8	12,14,15		12		12,12
D12	20		23		17,23
D19	13,15		13		13,13
FGA	20	20,†	17.2,24,25	24,†,†,†	24,25
D22	11,16		16		16,16
Fuming Method	MSU-Fumed	MSU-Fumed	Non-Fumed	Non-Fumed	Buccal

Table C2. Fusion and MiniFiler™ profiles generated from spent cartridge casings loaded by individual MM.

Locus	3-3b (Fusion)	3-3b (MiniFiler™)	3-4a (Fusion)	3-4a (MiniFiler™)	MM
Amel	X		Y		X,X
D3			18		14,16
D1	12				12,16
D2	14				10,11
D10					14,15
D13					8,12
Penta E	12				7,21
D16	11,12		12		12,12
D18	13.2,16	12			14,14.2
D2	17,18,22	17,23			17,23
CSF		12		†	12,13
Penta D	11				13,13
THO1	9.3				9,9.3
vWA					17,17
D21					29,31.2
D7					9,11
D5					9,10
TPOX	12				8,8
DYS391					N/A
D8	10,15,15.1,†,†				13,15
D12			22		18,22
D19	13				14,15.2
FGA	23.2	22,25,26.2	17.2	47.2,†,†,†	22,26
D22	16				11,12
Fuming Method	MSU-Fumed	MSU-Fumed	Non-Fumed	Non-Fumed	Buccal

Table C3. Fusion and MiniFiler™ profiles generated from spent cartridge casings loaded by individual S.

Locus	8-3a (Fusion)	8-3a (MiniFiler™)	8-3b (Fusion)	8-3b (MiniFiler™)	S
Amel	Y				X,X
D3	18				18,18
D1					12,15
D2	16				11,11.3
D10					13,15
D13	13	13	14		12,13
Penta E					12,13
D16	11		11		11,11
D18	12,17			12,16	12,16
D2	17,25	17,25		17	17,25
CSF					10,11
Penta D	12				10,13
THO1	6,9.3				6,9
vWA	15,17				17,18
D21	28,30	28	28		28,28
D7	10,12				10,10
D5					10,12
TPOX	11				8,11
DYS391					N/A
D8	12,15		13		13,16
D12	18,18.3		18		18,18.3
D19	14,15				13.2,15
FGA	23,24		22*		22,23
D22					15,15
Fuming Method	MSP-Fumed	MSP-Fumed	MSP-Fumed	MSP-Fumed	Buccal

Table C3 (cont'd)

Locus	8-3c (Fusion)	8-3c (MiniFiler™)	8-4a (Fusion)	8-4a (MiniFiler™)	S
Amel	X		XY		X,X
D3			16		18,18
D1	12				12,15
D2	11,11.3				11,11.3
D10	15		13		13,15
D13	12		10,13	13	12,13
Penta E					12,13
D16	11		9,11,13	†	11,11
D18					12,16
D2	17		18		17,25
CSF				6	10,11
Penta D					10,13
THO1	6		6,7		6,9
vWA	17		17		17,18
D21	28			28	28,28
D7	10				10,10
D5					10,12
TPOX			8		8,11
DYS391					N/A
D8	13,16		10,13,15,16		13,16
D12			17,18		18,18.3
D19	13.2				13.2,15
FGA					22,23
D22					15,15
Fuming Method	MSP-Fumed	MSP-Fumed	MSU-Fumed	MSU-Fumed	Buccal

Table C4. Fusion and MiniFiler™ profiles generated from spent cartridge casings loaded by individual VV.

Locus	10-4b (Fusion)	10-4b (MiniFiler™)	VV
Amel	X		X,Y
D3	15,18		14,17
D1	12,14,15,16.3		15,17.3
D2			11,14
D10	14		12,13
D13	9,11	12	11,11
Penta E			7,8
D16	12,13		12,12
D18	13,16	14	12,16
D2		17	17,18
CSF	10,12		11,11
Penta D	12		9,12
THO1	6,9.3		9.3,9.3
vWA	15,18		17,17
D21	29,30.2,32.2		28,32.2
D7			10,11
D5			11,13
TPOX	8		11,11
DYS391			11
D8	8,10		8,12
D12	23		15,25
D19	14		14,15.2
FGA	24		22,23
D22			11,15
Fuming Method	MSP-Fumed	MSP-Fumed	Buccal

Table C5. Fusion and MiniFiler™ profiles generated from spent cartridge casings loaded by individual V.

Locus	13-5a (Fusion)	13-5a (MiniFiler™)	13-5b (Fusion)	13-5b (MiniFiler™)	V
Amel	XY		XY		X,Y
D3	14		14		14,14
D1	16,16.3		15,16,16.3, 17.3		16.3,17.3
D2	11		11,11.3		11,11.3
D10	15		13,15		15,16
D13	12	†	10,12		10,12
Penta E			14		5,14
D16	12		11,12,13		11,12
D18	16	16	14		16,17
D2	†	20			20,22
CSF	11				10,11
Penta D			12		11,12
THO1	9,9.3		9,9.3		9,9.3
vWA	16		16,18		16,18
D21					28,32.2
D7			12		11,12
D5	12		12		12,12
TPOX	8		8		8,8
DYS391	11		11		11
D8	12		9,12		9,12
D12	23		21,23		21,23
D19	12		12,16		12,14
FGA	21.2,22.2	21.2			21.2,22
D22	11		11		11,16
Fuming Method	MSP-Fumed	MSP-Fumed	MSP-Fumed	MSP-Fumed	Buccal

Table C5 (cont'd)

Locus	13-5c (Fusion)	13-5c (MiniFiler™)	13-6a (Fusion)	13-6a (MiniFiler™)	V
Amel	Y		XY		X,Y
D3			14		14,14
D1			16.3,17.3		16.3,17.3
D2	11.3		11		11,11.3
D10	16		15,16		15,16
D13	10,12	10,12	10,12	10,12	10,12
Penta E					5,14
D16			11,12		11,12
D18		16	16,17	16,17	16,17
D2	20	20,22	20,22	20,22	20,22
CSF	10	10,11	11	10	10,11
Penta D	11				11,12
THO1	9,9.3		9,9.3		9,9.3
vWA	16		16,18		16,18
D21		32.2	32.2	28,33.2	28,32.2
D7			11,12		11,12
D5			12		12,12
TPOX			8		8,8
DYS391	11				11
D8	9,12		9,12		9,12
D12	23		21,23		21,23
D19	12		12,14		12,14
FGA	21.2,22	21.2	21.2,22,22.2	16.2,21.2,22	21.2,22
D22	11				11,16
Fuming Method	MSP-Fumed	MSP-Fumed	MSU-Fumed	MSU-Fumed	Buccal

Table C5 (cont'd)

Locus	13-6b (Fusion)	13-6b (MiniFiler™)	13-6c (Fusion)	13-6c (MiniFiler™)	V
Amel	XY		XY		X,Y
D3	14		14,15		14,14
D1	15.3,16.3, 17.3		14,17.3		16.3,17.3
D2	11,11.3				11,11.3
D10	15		13,16		15,16
D13	10,12	10,12	12	10	10,12
Penta E	5,14				5,14
D16	11,12	11,12	11,13		11,12
D18	16,17	16,17,18	12		16,17
D2	20,22	20,22,23	23.3	17,20,22	20,22
CSF	11	10,11	12	10,12	10,11
Penta D	11,12				11,12
THO1	7,9, 9.3		9		9,9.3
vWA	17,18		17		16,18
D21	28,32.2	28,33.2			28,32.2
D7	11,12		9,11		11,12
D5	12				12,12
TPOX			7		8,8
DYS391					11
D8	9,10,11,12		12,14,15		9,12
D12	17,21,23		21, 23		21,23
D19	12,14		14		12,14
FGA	22,23	21,22,23	17	20,22	21.2,22
D22	11				11,16
Fuming Method	MSU-Fumed	MSU-Fumed	MSU-Fumed	MSU-Fumed	Buccal

Table C5 (cont'd)

Locus	13-7a (Fusion)	13-7a (MiniFiler™)	13-7b (Fusion)	13-7b (MiniFiler™)	V
Amel	X,Y		X,Y	X,Y	X,Y
D3	14,18		14		14,14
D1	16.3,17.3		16.3,17.3		16.3,17.3
D2	11,11.3		11,11.3		11,11.3
D10			15,16		15,16
D13	10	12	10,12	10,12	10,12
Penta E	5,14		5,14		5,14
D16	11,12	11,12	11,12	11,12	11,12
D18	17	16	16,17	16,17	16,17
D2	20,22	17,20,22, 25	20,22	20,22	20,22
CSF	11	†	10,11	10,11	10,11
Penta D	12		11,12		11,12
THO1	9,9.3		9,9.3		9,9.3
vWA	16,18		16,18		16,18
D21	28,32.2	32.2	28,32.2	28,32.2	28,32.2
D7	12		11,12	11	11,12
D5	12		12		12,12
TPOX			8		8,8
DYS391	11		11		11
D8	9,12		9,12		9,12
D12	20,21,23		21,23		21,23
D19			12		12,14
FGA	22	21.2,31.2,†,†	21.2,22	21.2,22,†	21.2,22
D22			11,16		11,16
Fuming Method	Non-Fumed	Non-Fumed	Non-Fumed	Non-Fumed	Buccal

Table C5 (cont'd)

Locus	13-7c (Fusion)	13-7c (MiniFiler™)	V
Amel	X,Y		X,Y
D3	14		14,14
D1			16.3,17.3
D2	11,11.3		11,11.3
D10	15,16		15,16
D13		12	10,12
Penta E	5,14		5,14
D16	12	11,12	11,12
D18	16,17	16	16,17
D2	20	20,22	20,22
CSF	10	10,11	10,11
Penta D	12		11,12
THO1	9,9.3		9,9.3
vWA	14,17,18		16,18
D21	28	28	28,32.2
D7	11,12	11	11,12
D5			12,12
TPOX			8,8
DYS391	11		11
D8	9,12		9,12
D12	23		21,23
D19	12		12,14
FGA	22,23,32.2	21.2,†,†	21.2,22
D22	11,16		11,16
Fuming Method	Non-Fumed	Non-Fumed	Buccal

Table C6. Fusion and MiniFiler™ profiles generated from spent cartridge casings loaded by individual HH.

Locus	15-7c (Fusion)	15-7c (MiniFiler™)	HH
Amel			X,X
D3	14		14,18
D1	15.3		16,17.3
D2			11,14
D10			13,15
D13	†		10,11
Penta E			10,14
D16			9,12
D18	12, †		16,16
D2		17,18,19	17,19
CSF	†		11,13
Penta D	†		10,10
THO1	6,7		9,9
vWA			14,16
D21			30,31
D7	11		11,12
D5			9,12
TPOX			9,11
DYS391			N/A
D8	10*,15*		10,13
D12	18*		20,21
D19	14		13,14
FGA		20	22,25
D22			16,16
Fuming Method	MSU-Fumed	MSU-Fumed	Buccal

Table C7. Fusion and MiniFiler™ profiles generated from spent cartridge casings loaded by individual L.

Locus	23-1a (Fusion)	23-1a (MiniFiler™)	23-7a (Fusion)	23-7a (MiniFiler™	L
Amel	X		X	X	X,X
D3	17				16,16
D1	15.3				16,17.3
D2	11		11		11,11
D10	13		13,15		13,15
D13		12,13	13	13	13,13
Penta E			7		7,7
D16	11		11	11	11,11
D18	12,16	12	15,16	19	15,16
D2		16,17,18,19, 24	17	17	17,17
CSF	12	11,12			12,13
Penta D	10,14				9,11
THO1	9,9.3		8,9.3		8,9.3
vWA	17,19		14,17,18		14,18
D21	29	31.2	24,30		27,30
D7	8		10		8,10
D5			11		11,12
TPOX					8,8
DYS391	10				N/A
D8			13,14		13,14
D12	21		18*		18,20
D19			15		14,15
FGA		23,25	21,23	23	21,23
D22	16				15,16
Fuming Method	MSU-Fumed	MSU-Fumed	MSP-Fumed	MSP-Fumed	Buccal

Table C7 (cont'd)

Locus	23-7b (Fusion)	23-7b (MiniFiler™)	23-7c (Fusion)	23-7c (MiniFiler™)	L
Amel	X		X		X,X
D3	16		16		16,16
D1	16				16,17.3
D2	11				11,11
D10	13				13,15
D13	13	13			13,13
Penta E	7				7,7
D16	11		11		11,11
D18	16				15,16
D2	17,23	17			17,17
CSF		12			12,13
Penta D					9,11
THO1	8,9.3				8,9.3
vWA	14,18		14		14,18
D21	30	30	27		27,30
D7	8,10		12		8,10
D5	12		11,12		11,12
TPOX					8,8
DYS391					N/A
D8	11,13,14		13,14		13,14
D12	18,20		20		18,20
D19	15				14,15
FGA		23			21,23
D22					15,16
Fuming Method	MSP-Fumed	MSP-Fumed	MSP-Fumed	MSP-Fumed	Buccal

Table C7 (cont'd)

Locus	23-2a (Fusion)	23-2a (MiniFiler™)	23-2b (Fusion)	23-2b (MiniFiler™)	L
Amel	X	X	X	X	X,X
D3	16		16,17		16,16
D1	16,17.3		16,17.3		16,17.3
D2	11		11		11,11
D10			15		13,15
D13	13	13		13	13,13
Penta E	7		7		7,7
D16	11	11	11	11	11,11
D18	15,16	15,16	15	15,16	15,16
D2	17	17	17	17,19	17,17
CSF	13	12,13,†,†		12,13,†,†	12,13
Penta D			8.2		9,11
THO1	8,9.3		8,9.3		8,9.3
vWA	14		14,18		14,18
D21	30	27,30	30		27,30
D7	8	8,10			8,10
D5					11,12
TPOX					8,8
DYS391					N/A
D8	13,14		13,14		13,14
D12	20		18,20		18,20
D19			14,15		14,15
FGA	21,23	23.2,30,†,†,†	21,†,†	21,23	21,23
D22			†,16		15,16
Fuming Method	Non-Fumed	Non-Fumed	Non-Fumed	Non-Fumed	Buccal

Table C8. Fusion and MiniFiler™ profiles generated from spent cartridge casings loaded by individual OO.

Locus	24-4b (Fusion)	24-4b (MiniFiler™)	24-5a (Fusion)	24-5a (MiniFiler™)	OO
Amel			X		X,X
D3	14,16		15		15,18
D1	15.3				14,18.3
D2	11.3,14				11,14
D10					14,15
D13	10			11,12	9,12
Penta E	12				10,13
D16	13		11	13	12,12
D18	12		12	†	11,14
D2	22	22,25			17,25
CSF	10,12			10	10,11
Penta D	11				9,12
THO1	9.3		6		6,9
vWA	17		17		17,18
D21	28,33.2				28,31.2
D7	10				10,10
D5	12,13		†		11,11
TPOX	12		8		8,8
DYS391					N/A
D8	10		14*,15		12,17
D12	18,21				18.3,20
D19	14,15		14		14,16
FGA	21,23		21,23	20,†	18,24
D22	16				11,15
Fuming Method	MSP-Fumed	MSP-Fumed	MSU-Fumed	MSU-Fumed	Buccals

Table C9. Fusion and MiniFiler™ profiles generated from spent cartridge casings loaded by individual XX.

Locus	26-2a (Fusion)	26-2a (MiniFiler™)	26-2b (Fusion)	26-2b (MiniFiler™)	XX
Amel			X		X,X
D3	15		14,15,16,18		14,15
D1	12		14,17.3		14,17.3
D2					12,14
D10					14,16
D13	10		13	13	12,13
Penta E			12		12,12
D16	13		13		11,13
D18			12,17	18	17,18
D2	16				17,17
CSF		10,12	10	10,12	10,12
Penta D					12,12
THO1	9,9.3		9.3		9,9.3
vWA			17		17,19
D21	29				29,32
D7	12		12		9,12
D5			10		10,13
TPOX	8				8,12
DYS391					N/A
D8	10,13		10		10,13
D12	18,21		20		18,22
D19	14				13,14
FGA	23				21,23
D22					16,17
Fuming Method	MSP-Fumed	MSP-Fumed	MSP-Fumed	MSP-Fumed	Buccal

Table C9 (cont'd)

Locus	26-2c (Fusion)	26-2c (MiniFiler™)	26-3a (Fusion)	26-3a (MiniFiler™)	XX
Amel	X		X		X,X
D3	14,15		†,16		14,15
D1	15.3,17.3		15,15.3		14,17.3
D2	12,14		11		12,14
D10					14,16
D13			12		12,13
Penta E			7		12,12
D16	OL5.1,11,13		11		11,13
D18	17		12,15,16	13,24,†	17,18
D2	17	17	20,24		17,17
CSF	10,12				10,12
Penta D			3.2,10,11		12,12
THO1	7,9.3		8,9.3		9,9.3
vWA	17,19		17		17,19
D21	30			30	29,32
D7	9,12		9		9,12
D5	9,13		10		10,13
TPOX	8		8		8,12
DYS391					N/A
D8	10,13,15		13,14		10,13
D12	18,22				18,22
D19			15		13,14
FGA	23,24		21		21,23
D22	16		15		16,17
Fuming Method	MSP-Fumed	MSP-Fumed	MSU-Fumed	MSU-Fumed	Buccal

Table C9 (cont'd)

Locus	26-3c (Fusion)	26-3c (MiniFiler™)	XX
Amel	XY	X	X,X
D3	15,16,17		14,15
D1	11		14,17.3
D2	10,11		12,14
D10	13,15		14,16
D13	10	10,11,12,13	12,13
Penta E	5,14		12,12
D16	9,11,11.3	9,11	11,13
D18	17,21	15,17,21	17,18
D2	20,24	17,20,24	17,17
CSF	10,12,13	10	10,12
Penta D	9,11		12,12
THO1	9		9,9.3
vWA	14,16,18		17,19
D21	31,32.2	31,33.2	29,32
D7	8,9,11		9,12
D5	7		10,13
TPOX	11,12		8,12
DYS391	10		N/A
D8	10,12,13,14, 16		10,13
D12	18,21		18,22
D19	14.2,15		13,14
FGA	20,22,24	21,22,23	21,23
D22	15,16		16,17
Fuming Method	MSU-Fumed	MSU-Fumed	Buccal

Table C10. Fusion and MiniFiler™ profiles generated from spent cartridge casings loaded by individual N.

Locus	27-4b (Fusion)	27-4b (MiniFiler™)	27-4c (Fusion)	27-4c (MiniFiler™)	N
Amel	X		X		X,X
D3	15,17,18				16,17
D1	12,17.3				15.3,17.3
D2	11,11.3				11,11
D10	13,15				13,13
D13	11,13				12,14
Penta E	13				13,15
D16	11		10		12,13
D18	12,16				13,14
D2	17,25	17,25			20,23
CSF	10	11			11,12
Penta D	10				10,12
THO1	6,9,9.3				6,9.3
vWA	17,18				17,18
D21	28	28			30,32.2
D7	10				11,12
D5	10,12				12,12
TPOX	8				8,8
DYS391					N/A
D8	12,13		13		13,13
D12	18,18.3				19,20
D19	13.2,15				13,14
FGA	22,29.1				21,25
D22	15				11,15
Fuming Method	MSU-Fumed	MSU-Fumed	MSU-Fumed	MSU-Fumed	Buccal

Table C11. Fusion and MiniFiler™ profiles generated from spent cartridge casings loaded by individual B.

Locus	33-5c (Fusion)	33-5c (MiniFiler™)	33-6a (Fusion)	33-6a (MiniFiler™)	B
Amel			X		X,Y
D3	14,16		18		16,18
D1	14,16.3				16.3,17.3
D2			11.3,14		14,15
D10	16		15		13,15
D13			10	10	10,12
Penta E					7,18
D16	9,11,12		11,12,13		9,13
D18			12,13,19.2		13,15
D2	17		22	20,22	20,25
CSF	10,12	12	12		10,12
Penta D					12,13
THO1	8		6,8,9.3		8,9.3
vWA	16,17,18		17,18		17,18
D21	28,29,30		31		29,31
D7	10				9,12
D5	13		12,13		11,13
TPOX	12		8,12		8,8
DYS391					11
D8	8,13		8,13		8,13
D12	23		21		22,23
D19	14,15				13,15
FGA					21,23
D22	16				15,16
Fuming Method	MSP-Fumed	MSP-Fumed	MSU-Fumed	MSU-Fumed	Buccal

Table C11 (cont'd)

Locus	33-6b (Fusion)	33-6b (MiniFiler™)	33-6c (Fusion)	33-6c (MiniFiler™)	B
Amel	X				X,Y
D3	14,16,18		18		16,18
D1	14,15.3,16.3,17.3		15.3,16.3		16.3,17.3
D2	11.3,14,15				14,15
D10	15				13,15
D13	10,12	10,12			10,12
Penta E	7,12				7,18
D16	11,13		9,13		9,13
D18	12	12	16,18		13,15
D2	18,22,25	18,20,22,25		22,25	20,25
CSF	10,12	12	12		10,12
Penta D	9,11,12,13				12,13
THO1	6,9.3		9.3		8,9.3
vWA	17		17		17,18
D21	28,29,31,33.2	28,29,32,33.2	32.2		29,31
D7	9,10,12		12		9,12
D5	11,12,13				11,13
TPOX	8,12		8		8,8
DYS391	11				11
D8	8,10,15		8,13		8,13
D12	18,21,23		22		22,23
D19	13,14,15				13,15
FGA	21,23	21,23		19.2	21,23
D22	15,16		17		15,16
Fuming Method	MSU-Fumed	MSU-Fumed	MSU-Fumed	MSU-Fumed	Buccal

Table C11 (cont'd)

Locus	33-7a (Fusion)	33-7a (MiniFiler™)	33-7b (Fusion)	33-7b (MiniFiler™)	B
Amel	X,Y		X,Y		X,Y
D3	16,17,18		16		16,18
D1	16.3,17.3				16.3,17.3
D2	14,15				14,15
D10					13,15
D13	10	12		10,12	10,12
Penta E					7,18
D16	9,13		9,12,13	9,13	9,13
D18	15	15	13,15	13,15	13,15
D2	25			20	20,25
CSF	12	10,†	12	10,12,†,†	10,12
Penta D					12,13
THO1	8,9,9.3		8,9.3		8,9.3
vWA	18		17,18		17,18
D21	29	29		29	29,31
D7	9				9,12
D5	13				11,13
TPOX					8,8
DYS391					11
D8	8,13		8,13		8,13
D12			22,23		22,23
D19	13,14				13,15
FGA	31,†	20.2,23,†,†		21,23,48.2	21,23
D22					15,16
Fuming Method	Non-Fumed	Non-Fumed	Non-Fumed	Non-Fumed	Buccal

Table C12. Fusion and MiniFiler™ profiles generated from spent cartridge casings loaded by individual WW.

Locus	38-1b (Fusion)	38-1b (MiniFiler™)	38-1c (Fusion)	38-1c (MiniFiler™)	WW
Amel	X		X		X,X
D3	16				16,18
D1	15				11,12
D2					11,14
D10	15,16				15,16
D13		12	10,12		8,9
Penta E					11,12
D16	†,11,12,13				12,12
D18	12				12,15
D2	18	18		18	17,21
CSF	11,12		†		11,12
Penta D					10,12
THO1	7,9.1,9.3		6		9.3,9.3
vWA	16,17				15,17
D21					28,30
D7					10,11
D5					13,13
TPOX					8,12
DYS391					N/A
D8	10				10,12
D12	18,23		17*,21		18,19.3
D19	15		14		13,14
FGA	23		21		20,24
D22			16		16,16
Fuming Method	MSU-Fumed	MSU-Fumed	MSU-Fumed	MSU-Fumed	Buccal

Table C13. Fusion and MiniFiler™ profiles generated from spent cartridge casings loaded by individual SS.

Locus	40-1a (Fusion)	40-1a (MiniFiler™)	SS
Amel			X,Y
D3			14,18
D1	12,17.3		14,17.3
D2	10		11,11
D10			14,14
D13			10,12
Penta E			7,19
D16			11,12
D18			10,12
D2	20		17,20
CSF			11,12
Penta D			9,12
THO1			8,8
vWA	15,17		17,17
D21	29,32.2		29,32.2
D7			12,12
D5			13,13
TPOX	8		9,9
DYS391	11		11
D8	13		12,13
D12	15		15,24
D19			14,15
FGA			22,24
D22	18		16,16
Fuming Method	MSP-Fumed	MSP-Fumed	Buccal

Table C14. Fusion and MiniFiler™ profiles generated from spent cartridge casings loaded by individual Y.

Locus	41-2c (Fusion)	41-2c (MiniFiler™)	41-3c (Fusion)	41-3c (MiniFiler™)	Y
Amel	X		X		X,Y
D3	14,16,17				16,17
D1	14		14,15		12,14
D2	14				14,15
D10	14				14,15
D13	11				13,14
Penta E	14,18				5,14
D16	11		10	11	11,12
D18	17		12,20.2		17,17
D2	17	17			17,24
CSF			12,14		OL,12,14
Penta D					8,13
THO1			9.3		9,9.3
vWA	14		14,18		14,16
D21	30.2				29,30.2
D7	8		8		8,10
D5					12,12
TPOX	8				8,8
DYS391					11
D8			†,10,13		10,14
D12	21		20,21		17,21
D19	13		14		13,16.2
FGA	21,27			22	22,27
D22					11,16
Fuming Method	MSP-Fumed	MSP-Fumed	MSU-Fumed	MSU-Fumed	Buccal

Table C14 (cont'd)

Locus	41-4b (Fusion)	41-4b (MiniFiler™)	Y
Amel	X,Y		X,Y
D3	14		16,17
D1	16.3,17.3		12,14
D2	11.3		14,15
D10	15,16		14,15
D13	12		13,14
Penta E	5,14		5,14
D16	11,12	11,12	11,12
D18	16,17	16,17	17,17
D2	20	20,22	17,24
CSF	10	7,10,11	OL,12,14
Penta D	12		8,13
THO1	9,9.3		9,9.3
vWA	16,18		14,16
D21	28,32.2	28,32.2	29,30.2
D7	11,12	11,12	8,10
D5	12		12,12
TPOX	8		8,8
DYS391	11		11
D8	9,12		10,14
D12	21,23		17,21
D19	12,14		13,16.2
FGA	21.2,22	25.2,†,†	22,27
D22	11,16		11,16
Fuming Method	Non-Fumed	Non-Fumed	Buccal

Table C15. Fusion and MiniFiler™ profiles generated from spent cartridge casings loaded by individual II.

Locus	50-3a (Fusion)	50-3a (MiniFiler™)	50-4c (Fusion)	50-4c (MiniFiler™)	II
Amel	X		X		X,Y
D3	15		16		17,17
D1					15,18.3
D2	14		11.3		10,11
D10			15		13,15
D13	12	12			11,12
Penta E					13,14
D16	11				12,12
D18	13	13		17	16,17
D2			23		19,21
CSF		10		11	12,12
Penta D					9,13
THO1	7,8,9.3				8,9.3
vWA	17		15		15,17
D21		31,31.2			29,31
D7					10,12
D5	12		12		11,12
TPOX	11				8,8
DYS391					11
D8	14				11,13
D12	20		18,20		18,20
D19	14		14		14,15.2
FGA		50.2			21,23
D22	17*		12		15,16
Fuming Method	MSP-Fumed	MSP-Fumed	MSU-Fumed	MSU-Fumed	Buccal

Table C15 (cont'd)

Locus	50-5b (Fusion)	50-5b (MiniFiler™)	II
Amel	Y	Y	X,Y
D3	17		17,17
D1	15,18.3		15,18.3
D2	10,11		10,11
D10	13		13,15
D13	11,12	12	11,12
Penta E	13,14		13,14
D16	11,12	12	12,12
D18	16	16,17	16,17
D2		19,21	19,21
CSF	12	12	12,12
Penta D			9,13
THO1	6,8,9,9.3		8,9.3
vWA	15,17		15,17
D21	31	29	29,31
D7	12	10	10,12
D5	12		11,12
TPOX	8		8,8
DYS391			11
D8	11,13		11,13
D12	18,20,†		18,20
D19	14		14,15.2
FGA	23,†	20,21,23,25.2,†,†,†,†,†	21,23
D22	15,16		15,16
Fuming Method	Non-Fumed	Non-Fumed	Buccal

APPENDIX D. COMPARISON OF HANDLER AND NON-HANDLER ALLELES AMPLIFIED WITH MINIFILER™ AND FUSION

Table D1. Comparison of the number of handler alleles (H) and percent profile produced by MiniFiler™ and Fusion from casings fumed at MSU.

	#H Alleles		#NH Alleles		% Profile	
Sample	**MiniFiler™**	**Fusion**	**MiniFiler™**	**Fusion**	**MiniFiler™**	**Fusion**
33-6b	8	35	6	21	44.0%	76.1%
2-2c	6	18	8	25	38.0%	46.2%
26-3c	9	13	10	39	56.0%	31.0%
13-6b	12	37	5	7	67.0%	84.1%
13-6a	10	34	2	1	56.0%	77.3%
27-4b	1	15	3	24	6.0%	37.5%
13-6c	5	15	3	11	28.0%	34.1%
27-4c	0	2	0	1	0.0%	5.0%
33-6a	2	14	1	13	11.0%	30.4%
8-4a	3	10	2	10	21.0%	25.0%
38-1c	0	3	1	7	0.0%	7.3%
26-3a	0	10	4	18	0.0%	23.8%
15-7c	2	4	2	10	13.0%	9.8%
23-1a	4	9	9	11	29.0%	23.7%
2-2a	0	8	0	10	0.0%	20.5%
41-3c	2	9	0	9	12.0%	20.5%
24-5a	2	6	5	7	13.0%	14.6%
3-3b	4	6	2	16	25.0%	14.6%
50-4c	1	7	1	4	6.0%	16.3%
33-6c	1	12	1	5	6.0%	26.1%
38-1b	0	12	2	11	0.0%	29.3%

Table D2. Comparison of the number of handler alleles (H) and percent profile produced by MiniFiler™ and Fusion from casings fumed at MSP.

	#H Alleles		#NH Alleles		% Profile	
Sample	**MiniFiler™**	**Fusion**	**MiniFiler™**	**Fusion**	**MiniFiler™**	**Fusion**
23-7b	5	23	0	2	36.0%	60.5%
13-5c	9	19	0	0	50.0%	43.2%
13-5b	0	28	0	6	0.0%	63.6%
23-7a	5	23	2	2	36.0%	60.5%
26-2c	1	25	0	7	6.0%	59.5%
50-3a	2	8	4	8	13.0%	18.6%
2-1c	2	12	0	1	13.0%	30.8%
8-3b	3	5	0	1	21.0%	12.5%
26-2a	2	12	0	4	13.0%	28.6%
24-4b	1	6	1	23	7.0%	14.6%
8-3a	4	15	0	12	29.0%	37.5%
41-2c	1	17	0	4	6.0%	40.5%
13-5a	3	21	1	3	17.0%	47.7%
33-5c	1	15	0	11	6.0%	32.6%
26-2b	4	15	0	4	25.0%	35.7%
8-3c	0	15	0	0	0.0%	37.5%
10-4b	1	10	2	20	7.0%	24.4%
40-1a	0	8	0	6	0.0%	20.5%
23-7c	0	10	0	1	0.0%	26.3%

Table D3. Comparison of the number of handler alleles (H) and percent profile produced by MiniFiler™ and Fusion from non-fumed casings.

	#H Alleles		#NH Alleles		% Profile	
Sample	**MiniFiler™**	**Fusion**	**MiniFiler™**	**Fusion**	**MiniFiler™**	**Fusion**
13-7b	17	43	0	0	94.4%	97.7%
23-2a	12	22	2	0	85.7%	57.9%
23-2b	10	23	3	2	71.4%	60.5%
41-4b	3	18	11	21	17.6%	40.9%
13-7a	8	31	3	2	44.4%	70.4%
50-5b	12	30	2	4	75.0%	69.8%
13-7c	11	29	0	4	61.1%	65.9%
33-7b	12	16	1	1	66.7%	34.8%
3-4a	0	2	1	3	0.0%	4.9%
2-3a	12	28	0	3	75.0%	71.8%
33-7a	5	23	1	4	27.8%	50.0%

APPENDIX E. FUSION STR PROFILES FROM COLLECTION 1.

Red font: non-loader allele

**: allele was above the threshold using OSIRIS, but below the threshold using GeneMapper®.*

†: off-ladder allele

Blank cell: no alleles were amplified

N/A: not applicable

Table E1. Fusion profiles generated from spent cartridge casings loaded by individual U.

Locus	**2-2a**	**2-2b**	**2-2c**	**2-1a**	**2-1b**	**2-1c**	**2-3a**	**2-3b**	**2-3c**	**U**
Amel	X	X	X		X		X	X,Y	X	X,X
D3	15		14,15,18				15	15	15	15,15
D1	15.3,16.3		14,17.3				11,17.3	11	17.3	11,17.3
D2			10,11, 11.3				10,15	15		10,15
D10			12,14				12	12	14	12,14
D13	9	11	10,11		12	13				9,13
Penta E	12		12					15		12,15
D16	6		10,12	13		11,13	11,13		11,13	11,13
D18			12,17		14	14,15	13,14,15		14,15	14,15
D2	17		17,18,25		19		25	17,25		17,25
CSF			10	10			12	10		10,12
Penta D	11		16				10			10,11
THO1	9,9.3	6	6,7,9.3	6,7	7	6	6,7	6,7	7	6,7
vWA	18		16	14		14,20	14	14	15	14,20
D21			30,33.2	30			28,30,31			28,30
D7	11		10,12	11						11,11
D5			12		11	11	11	11		11,11
TPOX			8,11				8,11	11		8,11
DYS391										N/A
D8	13,15,††	12	12,14,15		12	12	12	12	12,13,15	12,12
D12		18	20		17	17,23	23		17	17,23
D19			13,15			15	13	13		13,13
FGA			20				17.2,24, 25			24,25
D22	16		11,16				16			16,16
Fuming Method	MSU-Fumed	MSU-Fumed	MSU-Fumed	MSP-Fumed	MSP-Fumed	MSP-Fumed	Non-Fumed	Non-Fumed	Non-Fumed	Buccal

Table E2. Fusion profiles generated from spent cartridge casings loaded by individual MM.

Locus	3-3a	3-3b	3-3c	3-2a	3-2b	3-2c	3-4a	3-4b	3-4c	MM
Amel		X	X	Y			Y		X	X,X
D3							18			14,16
D1		12	11		16					12,16
D2		14							11	10,11
D10										14,15
D13			14						8	8,12
Penta E		12								7,21
D16		11,12	12,13		12		12	11		12,12
D18		13.2,16	16			14.2			15,17	14,14.2
D2		17,18,22								17,23
CSF			12							12,13
Penta D		11							13	13,13
THO1		9.3		6				9.3	6,9,9.3	9,9.3
vWA			17							17,17
D21										29,31.2
D7								8		9,11
D5									11	9,10
TPOX		12				8				8,8
DYS391										N/A
D8		10,15, 15.1,††			13			12	†,13,15	13,15
D12				22			22		18	18,22
D19		13								14,15.2
FGA		23.2					17.2			22,26
D22		16	16							11,12
Fuming Method	MSU-Fumed	MSU-Fumed	MSU-Fumed	MSP-Fumed	MSP-Fumed	MSP-Fumed	Non-Fumed	Non-Fumed	Non-Fumed	Buccal

Table E3. Fusion profiles generated from spent cartridge casings loaded by individual S.

Locus	8-4a	8-4c	8-3a	8-3b	8-3c	8-5a	8-5b	8-5c	S
Amel	XY	X	Y		X	X	X	X	X,X
D3	16	14,16	18			18	18	18	18,18
D1		14,15.3			12	11,15	12,15		12,15
D2		11.3	16		11,11.3			11	11,11.3
D10	13	13			15	15		15	13,15
D13	10,13	10,12	13	14	12	13	13		12,13
Penta E		12					13		12,13
D16	9,11,13	11,13	11	11	11	11	11	11	11,11
D18		12	12,17			12	12	12	12,16
D2	18	20,25	17,25		17			17	17,25
CSF		10,12				11	13		10,11
Penta D		11	12						10,13
THO1	6,7	6,9,9.3	6,9.3		6	7,9	6,9		6,9
vWA	17	17,18	15,17		17		18		17,18
D21		28,33.2	28,30	28	28	29,34			28,28
D7		9,10,11	10,12		10	12	10	10	10,10
D5		13					12	10	10,12
TPOX	8	12	11			8	11		8,11
DYS391									N/A
D8	10,13,15, 16	10,13,15	12,15	13	13,16	10,13,16	13,16	6,13,14,16	13,16
D12	17,18	17	18,18.3	18		18	18,18.3	18.3	18,18.3
D19		14,15,17	14,15		13.2		13.2,15	7,15,16, 19.2	13.2,15
FGA		21,23,†††	23,24	22*		21		23,†	22,23
D22		16,†							15,15
Fuming Method	MSU-Fumed	MSU-Fumed	MSP-Fumed	MSP-Fumed	MSP-Fumed	Non-Fumed	Non-Fumed	Non-Fumed	Buccal

Table E4. Fusion profiles generated from spent cartridge casings loaded by individual VV.

Locus	**10-5a**	**10-5b**	**10-5c**	**10-4a**	**10-4b**	**10-4c**	**10-6a**	**10-6b**	**10-6c**	**VV**
Amel			X	X	X			X		X,Y
D3			12		15,18			16		14,17
D1					12,14,15, 16.3					15,17.3
D2								14		11,14
D10					14					12,13
D13				12	9,11					11,11
Penta E			13							7,8
D16			11	12	12,13					12,12
D18			10,17		13,16					12,16
D2										17,18
CSF					10,12					11,11
Penta D				10	12					9,12
THO1		8	6	9,9.3	6,9.3					9.3,9.3
vWA				15,18	15,18					17,17
D21		28			29,30.2, 32.2					28,32.2
D7		9								10,11
D5		12						13		11,13
TPOX					8					11,11
DYS391										11
D8		14	10	11	8,10			8,13		8,12
D12		18,22		24	23					15,25
D19			14		14					14,15.2
FGA					24		32.2,†			22,23
D22										11,15
Fuming Method	MSU-Fumed	MSU-Fumed	MSU-Fumed	MSP-Fumed	MSP-Fumed	MSP-Fumed	Non-Fumed	Non-Fumed	Non-Fumed	Buccal

Table E5. Fusion profiles generated from spent cartridge casings loaded by individual V.

Locus	**13-6a**	**13-6b**	**13-6c**	**13-5a**	**13-5b**	**13-5c**	**13-7a**	**13-7b**	**13-7c**	**V**
Amel	XY	XY	XY	XY	XY	Y	X,Y	X,Y	X,Y	X,Y
D3	14	14	14,15	14	14		14,18	14	14	14,14
D1	16.3,17.3	15.3,16.3, 17.3	14,17.3	16,16.3	15,16, 16.3,17.3		16.3,17.3	16.3,17.3		16.3,17.3
D2	11	11,11.3		11	11,11.3	11.3	11,11.3	11,11.3	11,11.3	11,11.3
D10	15,16	15	13,16	15	13,15	16		15,16	15,16	15,16
D13	10,12	10,12	12	12	10,12	10,12	10	10,12		10,12
Penta E		5,14			14		5,14	5,14	5,14	5,14
D16	11,12	11,12	11,13	12	11,12,13		11,12	11,12	12	11,12
D18	16,17	16,17	12	16	14		17	16,17	16,17	16,17
D2	20,22	20,22	23.3	†		20	20,22	20,22	20	20,22
CSF	11	11	12	11		10	11	10,11	10	10,11
Penta D		11,12			12	11	12	11,12	12	11,12
THO1	9,9.3	7,9, 9.3	9	9,9.3	9,9.3	9,9.3	9,9.3	9,9.3	9,9.3	9,9.3
vWA	16,18	17,18	17	16	16,18	16	16,18	16,18	14,17,18	16,18
D21	32.2	28,32.2					28,32.2	28,32.2	28	28,32.2
D7	11,12	11,12	9,11		12		12	11,12	11,12	11,12
D5	12	12		12	12		12	12		12,12
TPOX	8		7	8	8			8		8,8
DYS391				11	11	11	11	11	11	11
D8	9,12	9,10,11, 12	12,14,15	12	9,12	9,12	9,12	9,12	9,12	9,12
D12	21,23	17,21,23	21, 23	23	21,23	23	20,21,23	21,23	23	21,23
D19	12,14	12,14	14	12	12,16	12		12	12	12,14
FGA	21.2,22, 22.2	22,23	17	21.2,22.2		21.2,22	22	21.2,22	22,23, 32.2	21.2,22
D22		11		11	11	11		11,16	11,16	11,16
Fuming Method	MSU-Fumed	MSU-Fumed	MSU-Fumed	MSP-Fumed	MSP-Fumed	MSP-Fumed	Non-Fumed	Non-Fumed	Non-Fumed	Buccal

Table E6. Fusion profiles generated from spent cartridge casings loaded by individual HH.

Locus	15-7a	15-7b	15-7c	15-6a	15-6b	15-6c	15-1a	15-1b	15-1c	HH
Amel		X					X	X		X,X
D3		16	14					15		14,18
D1		11,14	15.3							16,17.3
D2	11.3	11,11.3, 14		14						11,14
D10	15						13			13,15
D13		10,12	†							10,11
Penta E							12			10,14
D16		9,13			11		†			9,12
D18		12,17	12,†		13		12			16,16
D2										17,19
CSF			†							11,13
Penta D		11,13	†							10,10
THO1		6,7,9.3	6,7		8		9.3		9	9,9
vWA		17								14,16
D21										30,31
D7			11		10					11,12
D5							12			9,12
TPOX	8	12								9,11
DYS391										N/A
D8		10,13	10*,15*	12,13	13			10,11	14.1	10,13
D12		18,20	18*		22,23		20			20,21
D19	15	15	14							13,14
FGA		24		21	23					22,25
D22		16								16,16
Fuming Method	MSU-Fumed	MSU-Fumed	MSU-Fumed	MSP-Fumed	MSP-Fumed	MSP-Fumed	Non-Fumed	Non-Fumed	Non-Fumed	Buccal

Table E7. Fusion profiles generated from spent cartridge casings loaded by individual L.

Locus	**23-1a**	**23-1b**	**23-1c**	**23-7a**	**23-7b**	**23-7c**	**23-2a**	**23-2b**	**23-2c**	**L**
Amel	X	X		X	X	X	X	X	X	X,X
D3	17	14,16	16		16	16	16	16,17		16,16
D1	15.3	12,14	14		16		16,17.3	16,17.3		16,17.3
D2	11			11	11		11	11		11,11
D10	13	16		13,15	13			15	14	13,15
D13			8,10	13	13		13			13,13
Penta E				7	7		7	7		7,7
D16	11		12	11	11	11	11	11	11,12	11,11
D18	12,16	12		15,16	16		15,16	15	15	15,16
D2		23		17	17,23		17	17		17,17
CSF	12	10,12					13		12	12,13
Penta D	10,14		12,13					8.2	9	9,11
THO1	9,9.3	6,7	6,9.3	8,9.3	8,9.3		8,9.3	8,9.3	3,8,9.3	8,9.3
vWA	17,19	17	16	14,17,18	14,18	14	14	14,18	14	14,18
D21	29			24,30	30	27	30	30		27,30
D7	8			10	8,10	12	8			8,10
D5				11	12	11,12				11,12
TPOX										8,8
DYS391	10	10								N/A
D8		10,13,15	13	13,14	11,13,14	13,14	13,14	13,14	11,13,14	13,14
D12	21	21		18*	18,20	20	20	18,20	18	18,20
D19		14	15	15	15			14,15	15	14,15
FGA				21,23			21,23	21,†,†		21,23
D22	16							†,16		15,16
Fuming Method	MSU-Fumed	MSU-Fumed	MSU-Fumed	MSP-Fumed	MSP-Fumed	MSP-Fumed	Non-Fumed	Non-Fumed	Non-Fumed	Buccal

Table E8. Fusion profiles generated from spent cartridge casings loaded by individual OO.

Locus	24-5a	24-5b	24-5c	24-4a	24-4b	24-4c	24-6a	24-6b	24-6c	OO
Amel	X							X	Y	X,X
D3	15			16	14,16			15		15,18
D1			14		15.3					14,18.3
D2		15			11.3,14					11,14
D10										14,15
D13		12	12		10					9,12
Penta E			12		12			12		10,13
D16	11	12	8,13		13	9,11		11		12,12
D18	12				12			12	11,14	11,14
D2					22		20	20		17,25
CSF			10		10,12					10,11
Penta D					11			10		9,12
THO1	6		7		9.3	6	6,9	6,7,9,9.3	6,9.3	6,9
vWA	17		15		17	17		16		17,18
D21			28,33.2		28,33.2					28,31.2
D7		9,10			10		8	10		10,10
D5	†				12,13					11,11
TPOX	8		8		12			12		8,8
DYS391								8		N/A
D8	14*,15		9,10		10	10		13,16		12,17
D12					18,21			18	19,20	18.3,20
D19	14				14,15					14,16
FGA	21,23				21,23			22		18,24
D22		16			16					11,15
Fuming Method	MSU-Fumed	MSU-Fumed	MSU-Fumed	MSP-Fumed	MSP-Fumed	MSP-Fumed	Non-Fumed	Non-Fumed	Non-Fumed	Buccal

Table E9. Fusion profiles generated from spent cartridge casings loaded by individual T.

Locus	25-2a	25-2b	25-2c	25-1a	25-1b	25-1c	25-3a	25-3b	25-3c	T
Amel						X	X			X,X
D3							15		16,18	16,17
D1	14								11	16,17.3
D2									14	11,14
D10								14	15	14,17
D13		12			11		9			11,11
Penta E										11,12
D16		9,11					12			11,12
D18		12				13		13	15	13,17
D2										20,24
CSF			12							10,11
Penta D									12	8,10
THO1		8,9			6	6	6,7,9.3	6,8	9.3	6,7
vWA			18	18		15			15,17	19,20
D21	32.2							29		29,29
D7										8,10
D5		12							13	12,12
TPOX		8							12	8,11
DYS391										N/A
D8									10	13,14
D12								19,23	22	19,23
D19										13,16.2
FGA		21			24			24	24	24,24
D22							11			11,18
Fuming Method	MSU-Fumed	MSU-Fumed	MSU-Fumed	MSP-Fumed	MSP-Fumed	MSP-Fumed	Non-Fumed	Non-Fumed	Non-Fumed	Buccal

Table E10. Fusion profiles generated from spent cartridge casings loaded by individual XX.

Locus	26-3a	26-3b	26-3c	26-2a	26-2b	26-2c	26-4b	26-4b	26-4c	XX
Amel	X		XY		X	X	X	X	X	X,X
D3	16,†	†	15,16,17	15	14,15,16, 18	14,15	14,15	14,15	14,15	14,15
D1	15,15.3		11	12	14,17.3	15.3,17.3		14,17.3	14,17.3	14,17.3
D2	11	10	10,11			12,14	14	14	12	12,14
D10		15	13,15				14,16	13,14	14	14,16
D13	12		10	10	13			11,12		12,13
Penta E	7		5,14		12					12,12
D16	11	10,11,†	9,11,11.3	13	13	11,13, †	11,13	13	11,12,13	11,13
D18	12,15,16	16	17,21		12,17	17	17	17,17.2	15,17,18	17,18
D2	20,24		20,24	16		17		17	17	17,17
CSF			10,12,13		10	10,12		10		10,12
Penta D	3.2,10,11		9,11							12,12
THO1	8,9.3	8,9,9.3	9	9,9.3	9.3	7,9.3	9,9.3	7,9,9.3	9.3	9,9.3
vWA	17	14,15	14,16,18		17	17,19	17,19	17,19	17,19	17,19
D21			31,32.2	29		30			32	29,32
D7	9	8	8,9,11	12	12	9,12			9	9,12
D5	10		7		10	9,13	10		10	10,13
TPOX	8		11,12	8		8	12			8,12
DYS391			10							N/A
D8	13,14		10,12,13, 14,16	10,13	10	10,13,15	10,13	10,13	10,13,17	10,13
D12		17	18,21	18,21	20	18,22	18,22	19,22	18,22	18,22
D19	15	14	14.2,15	14			13	14	14	13,14
FGA	21		20,22,24	23		23,24	21,23	21.2,23	23	21,23
D22	15	11	15,16			16	6	16,18		16,17
Fuming Method	MSU-Fumed	MSU-Fumed	MSU-Fumed	MSP-Fumed	MSP-Fumed	MSP-Fumed	Non-Fumed	Non-Fumed	Non-Fumed	Buccal

Table E11. Fusion profiles generated from spent cartridge casings loaded by individual N.

Locus	27-4a	27-4b	27-4c	27-3a	27-3b	27-3c	27-5a	27-5b	27-5c	N
Amel		X	X	X	X		X	X,Y	X,Y	X,X
D3		15,17,18					16	17	14,17,18	16,17
D1		12,17.3					15.3	14	15.3,17.3	15.3,17.3
D2		11,11.3						11		11,11
D10		13,15							13	13,13
D13		11,13						14		12,14
Penta E		13			18					13,15
D16	14	11	10		13		11,12	12	11,12	12,13
D18	16,19.2	12,16		17	14		18	13,18	14,16	13,14
D2		17,25							20	20,23
CSF		10								11,12
Penta D		10		8					12	10,12
THO1	6,†	6,9,9.3			6,9.3	4	6,9.3	6,9.3	9.3	6,9.3
vWA	†	17,18			16		16,17,18	17	18	17,18
D21	30.1,34.1	28		28					32.2	30,32.2
D7		10								11,12
D5		10,12		10					11,13	12,12
TPOX		8								8,8
DYS391										N/A
D8	13	12,13	13		13		11,13	13,14	13,15	13,13
D12	20	18,18.3					25	17,19	20	19,20
D19		13.2,15			15.2				15.2	13,14
FGA		22,29.1					24	21,46.2		21,25
D22	19	15					17		11	11,15
Fuming Method	MSU-Fumed	MSU-Fumed	MSU-Fumed	MSP-Fumed	MSP-Fumed	MSP-Fumed	Non-Fumed	Non-Fumed	Non-Fumed	Buccal

Table E12. Fusion profiles generated from spent cartridge casings loaded by individual B.

Locus	**33-6a**	**33-6b**	**33-6c**	**33-5a**	**33-5b**	**33-5c**	**33-7a**	**33-7b**	**33-7c**	**B**
Amel	X	X		X	X		X,Y	X,Y	X,Y	X,Y
D3	18	14,16,18	18		16	14,16	16,17,18	16	18	16,18
D1		14,15.3, 16.3,17.3	15.3,16.3			14,16.3	16.3,17.3			16.3,17.3
D2	11.3,14	11.3,14,15		11			14,15		14	14,15
D10	15	15				16				13,15
D13	10	10,12					10		10	10,12
Penta E		7,12							7	7,18
D16	11,12,13	11,13	9,13	9		9,11,12	9,13	9,12,13		9,13
D18	12,13, 19.2	12	16,18	17			15	13,15	13	13,15
D2	22	18,22,25			17	17	25			20,25
CSF	12	10,12	12			10,12	12	12		10,12
Penta D		9,11,12,13								12,13
THO1	6,8,9.3	6,9.3	9.3		8,9,9.3,†	8	8,9,9.3	8,9.3	8	8,9.3
vWA	17,18	17	17			16,17,18	18	17,18	18	17,18
D21	31	28,29,31, 33.2	32.2	31		28,29,30	29			29,31
D7		9,10,12	12			10	9		12	9,12
D5	12,13	11,12,13				13	13			11,13
TPOX	8,12	8,12	8			12				8,8
DYS391		11								11
D8	8,13	8,10,15	8,13	8	13,†,†	8,13	8,13	8,13	8,13	8,13
D12	21	18,21,23	22			23		22,23		22,23
D19		13,14,15		15		14,15	13,14			13,15
FGA		21,23					31,†			21,23
D22		15,16	17	11		16				15,16
Fuming Method	MSU-Fumed	MSU-Fumed	MSU-Fumed	MSP-Fumed	MSP-Fumed	MSP-Fumed	Non-Fumed	Non-Fumed	Non-Fumed	Buccal

Table E13. Fusion profiles generated from spent cartridge casings loaded by individual D.

Locus	36-7a	36-7b	36-7c	36-6a	36-6b	36-6c	36-1a	36-1b	36-1c	D
Amel	X	X				X	X,Y	Y	Y	X,Y
D3		15	16	16			17		15	17,18
D1	17.3									15,15
D2										11,14
D10		13								13,14
D13							11			11,11
Penta E										7,13
D16		5,8,13		13		10,12,13			12,13	13,13
D18		12				14	14			12,14
D2			17			16			20	17,20
CSF										11,12
Penta D										9,11
THO1	9			9	8	8,9.3	8,9.3	9,9.3	9.3	8,9.3
vWA		17	16		15		15			15,17
D21										28,30
D7		9,10	12							9,12
D5									9	11,12
TPOX		9								9,11
DYS391										10
D8	15	8,15		13	8,16,18		8,13	13	9,13	8,13
D12			24				19		18	15,19
D19					18		15			14,15
FGA							22.1,†		†	21,26
D22							†			12,17
Fuming Method	MSU-Fumed	MSU-Fumed	MSU-Fumed	MSP-Fumed	MSP-Fumed	MSP-Fumed	Non-Fumed	Non-Fumed	Non-Fumed	Buccal

Table E14. Fusion profiles generated from spent cartridge casings loaded by individual WW.

Locus	38-1a	38-1b	38-1c	38-7a	38-7b	38-7c	38-2a	38-2b	38-2c	WW
Amel	Y	X	X				X	X,Y	X	X,X
D3	14,16	16					16,18	16,17	15,17	16,18
D1	11,14, 17.3	15		11			11	15		11,12
D2	11,14			14				11,14		11,14
D10		15,16						13,16	15	15,16
D13			10,12				8	11	9	8,9
Penta E	12			10				7,8		11,12
D16	12	11,12,13, †		12			12	12	12	12,12
D18		12		15	15		12	12,16,17	14,15	12,15
D2		18						18	18	17,21
CSF		11,12	†							11,12
Penta D								9,12	12	10,12
THO1	6,9.3	7,9.1,9.3	6	6,9.3	8,9.3		9.3	9.3	8,9.3	9.3,9.3
vWA		16,17			17*,18*		15,17	15,17		15,17
D21							28	28,32.2		28,30
D7				11				10		10,11
D5							13	11		13,13
TPOX							8	11		8,12
DYS391								11		N/A
D8	17	10			10,12,14	10	10	10,12		10,12
D12	21	18,23	17*,21		18	23	18,19.3	19.3,25		18,19.3
D19		15	14	14			14	14,15.2	13	13,14
FGA		23	21					23		20,24
D22			16							16,16
Fuming Method	MSU-Fumed	MSU-Fumed	MSU-Fumed	MSP-Fumed	MSP-Fumed	MSP-Fumed	Non-Fumed	Non-Fumed	Non-Fumed	Buccal

Table E15. Fusion profiles generated from spent cartridge casings loaded by individual SS.

Locus	**40-2a**	**40-2b**		**40-1a**	**40-1b**	**40-1c**	**40-3a**	**40-3b**	**40-3c**	**SS**
Amel	Y							X,Y		X,Y
D3						16	14,17	14,17		14,18
D1	17.3			12,17.3				15,18.3		14,17.3
D2		11		10			10	11		11,11
D10							13,14	13,15		14,14
D13							11			10,12
Penta E							7	14		7,19
D16							12	10,12		11,12
D18							10	16		10,12
D2		17		20			22	19		17,20
CSF							11,12	12		11,12
Penta D							9	9		9,12
THO1							8	7,8		8,8
vWA		17		15,17			18	15		17,17
D21				29,32.2		29	29	29,31		29,32.2
D7										12,12
D5		13						11		13,13
TPOX				8			8	8		9,9
DYS391				11			11			11
D8		12		13			†,10,11, 13	11,13		12,13
D12	15	15,23		15			15,20	18	†	15,24
D19						14		13,14		14,15
FGA									23.1	22,24
D22		16		18				13		16,16
Fuming Method	MSU-Fumed	MSU-Fumed	MSU-Fumed	MSP-Fumed	MSP-Fumed	MSP-Fumed	Non-Fumed	Non-Fumed	Non-Fumed	Buccal

Table E16. Fusion profiles generated from spent cartridge casings loaded by individual Y.

Locus	**41-3a**	**41-3b**	**41-3c**	**41-2a**	**41-2b**	**41-2c**	**41-4a**	**41-4b**	**41-4c**	**Y**
Amel	XY		X			X	Y	X,Y	X,Y	X,Y
D3		15,17				14,16,17		14	17	16,17
D1			14,15			14		16.3,17.3		12,14
D2					11	14		11.3	14	14,15
D10		15				14	14	15,16		14,15
D13						11		12	9,13	13,14
Penta E		7				14,18		5,14		5,14
D16			10			11	11	11,12	13	11,12
D18			12,20.2			17	16	16,17	17	17,17
D2	17					17		20	17,24	17,24
CSF			12,14		12			10		OL,12,14
Penta D								12	8	8,13
THO1	9.3		9.3		9.3		6	9,9.3	6,9.3	9,9.3
vWA	14	17	14,18		17	14		16,18	14	14,16
D21					33.3*	30.2		28,32.2	30.2	29,30.2
D7			8	10	10	8		11,12	10,12	8,10
D5				12				12		12,12
TPOX		8				8		8	11	8,8
DYS391								11		11
D8	12	12,13,15, 19	10,13,†		13		10,15	9,12	10,14	10,14
D12		24	20,21			21		21,23	17,20	17,21
D19			14			13		12,14	13,15.2	13,16.2
FGA		23,24				21,27		21.2,22	20,27	22,27
D22								11,16		11,16
Fuming Method	MSU-Fumed	MSU-Fumed	MSU-Fumed	MSP-Fumed	MSP-Fumed	MSP-Fumed	Non-Fumed	Non-Fumed	Non-Fumed	Buccal

Table E17. Fusion profiles generated from spent cartridge casings loaded by individual II.

Locus	**50-4a**	**50-4b**	**50-4c**	**50-3a**	**50-3b**	**50-3c**	**50-5a**	**50-5b**	**50-5c**	**II**
Amel		X	X	X	XY	XY	X,Y	Y	X,Y	X,Y
D3			16	15	15		17	17	17	17,17
D1		15,18.3					12,15	15,18.3		15,18.3
D2			11.3	14			11,11.3	10,11	10,11	10,11
D10			15				15	13	15	13,15
D13				12			12	11,12	11	11,12
Penta E								13,14		13,14
D16		12		11	12		11,12	11,12	11,12	12,12
D18				13				16	16	16,17
D2			23		25				19	19,21
CSF							10	12		12,12
Penta D										9,13
THO1		6,7,8		7,8,9.3			7,8,9.3	6,8,9,9.3	8	8,9.3
vWA		15	15	17		17	17,18	15,17	15,17	15,17
D21		29			31		29	31		29,31
D7								12		10,12
D5			12	12			11	12	12	11,12
TPOX				11			11	8		8,8
DYS391									11	11
D8		11		14	13	11,13	11,13,14, 16	11,13	11,13,15, 16	11,13
D12			18,20	20		23	18	18,20,†	18,19	18,20
D19			14	14	13,14		16.2	14	15.2	14,15.2
FGA		23			25		22,23	23,†	21,†	21,23
D22			12	17*		15		15,16		15,16
Fuming Method	MSU-Fumed	MSU-Fumed	MSU-Fumed	MSP-Fumed	MSP-Fumed	MSP-Fumed	Non-Fumed	Non-Fumed	Non-Fumed	Buccal

APPENDIX F. HANDLER AND NON-HANDLER ALLELES AMPLIFIED WITH FUSION FROM COLLECTION 1

Table F1. Summary of the number of handler (H) alleles, non-handler (NH) alleles, and percent profile produced using Fusion for all samples in Collection 1.

Sample	Treatment	Fusion % Profile	# H alleles (Fusion)	# NH alleles (Fusion)
2-1a	Fumed at MSP	17.9%	7	0
2-1b	Fumed at MSP	15.4%	6	2
2-1c	Fumed at MSP	30.8%	12	1
2-2a	Fumed at MSU	20.5%	8	10
2-2b	Fumed at MSU	7.7%	3	2
2-2c	Fumed at MSU	46.2%	18	25
2-3a	Non-Fumed	71.8%	28	3
2-3b	Non-Fumed	41.0%	16	1
2-3c	Non-Fumed	28.2%	11	3
3-2a	Fumed at MSP	2.4%	1	2
3-2b	Fumed at MSP	7.3%	3	0
3-2c	Fumed at MSP	4.9%	2	0
3-3a	Fumed at MSU	0.0%	0	0
3-3a	Fumed at MSU	9.8%	4	5
3-3b	Fumed at MSU	14.6%	6	16
3-4a	Non-Fumed	4.9%	2	3
3-4b	Non-Fumed	2.4%	1	3
3-4c	Non-Fumed	21.9%	9	4
8-3a	Fumed at MSP	37.5%	15	12
8-3b	Fumed at MSP	12.5%	5	1
8-3c	Fumed at MSP	37.5%	15	0
8-4a	Fumed at MSU	25.0%	10	10
8-4b	Fumed at MSU	-	-	-
8-4c	Fumed at MSU	45.0%	18	26
8-5a	Non-Fumed	31.7%	13	7
8-5b	Non-Fumed	48.8%	20	1
8-5c	Non-Fumed	34.1%	14	5
10-4a	Fumed at MSP	9.8%	4	6
10-4b	Fumed at MSP	24.4%	10	20
10-4c	Fumed at MSP	-	-	-
10-5a	Fumed at MSU	0.0%	0	0
10-5b	Fumed at MSU	2.4%	1	6
10-5c	Fumed at MSU	4.9%	2	7

Table F1 (cont'd)

10-6a	Non-Fumed	0.0%	0	1
10-6b	Non-Fumed	9.7%	4	2
10-6c	Non-Fumed	0.0%	0	0
13-5a	Fumed at MSP	47.7%	21	3
13-5b	Fumed at MSP	63.6%	28	6
13-5c	Fumed at MSP	43.2%	19	0
13-6a	Fumed at MSU	77.3%	34	1
13-6b	Fumed at MSU	84.1%	37	7
13-6c	Fumed at MSU	34.1%	15	11
13-7a	Non-Fumed	70.4%	31	2
13-7b	Non-Fumed	97.7%	43	0
13-7c	Non-Fumed	65.9%	29	4
15-1a	Non-Fumed	9.7%	4	3
15-1b	Non-Fumed	4.9%	2	2
15-1c	Non-Fumed	2.4%	1	1
15-6a	Fumed at MSP	4.9%	2	2
15-6b	Fumed at MSP	2.4%	1	7
15-6c	Fumed at MSP	0.0%	0	0
15-7a	Fumed at MSU	2.4%	1	3
15-7b	Fumed at MSU	22.0%	9	18
15-7c	Fumed at MSU	9.8%	4	10
23-1a	Fumed at MSU	23.7%	9	11
23-1a	Fumed at MSU	10.5%	4	8
23-1b	Fumed at MSU	13.2%	5	14
23-2a	Non-Fumed	57.9%	22	0
23-2b	Non-Fumed	60.5%	23	2
23-2c	Non-Fumed	31.6%	12	4
23-7a	Fumed at MSP	60.5%	23	2
23-7b	Fumed at MSP	60.5%	23	2
23-7c	Fumed at MSP	26.3%	10	1
24-4a	Fumed at MSP	0.0%	0	1
24-4b	Fumed at MSP	14.6%	6	23
24-4c	Fumed at MSP	4.9%	2	3
24-5a	Fumed at MSU	14.6%	6	7
24-5b	Fumed at MSU	7.3%	3	3
24-5c	Fumed at MSU	12.2%	5	8
24-6a	Non-Fumed	2.4%	1	2
24-6b	Non-Fumed	1220.0%	5	14
24-6c	Non-Fumed	12.2%	5	2
25-1a	Fumed at MSP	0.0%	0	1

Table F1 (cont'd)

25-1b	Fumed at MSP	7.3%	3	0
25-1c	Fumed at MSP	7.3%	3	1
25-2a	Fumed at MSU	0.0%	0	2
25-2b	Fumed at MSU	7.3%	3	6
25-2c	Fumed at MSU	0.0%	0	2
25-3a	Non-Fumed	12.2%	5	3
25-3b	Non-Fumed	17.1%	7	1
25-3c	Non-Fumed	7.3%	3	12
26-2a	Fumed at MSP	28.6%	12	4
26-2b	Fumed at MSP	35.7%	15	4
26-2c	Fumed at MSP	59.5%	25	7
26-3a	Fumed at MSU	23.8%	10	18
26-3b	Fumed at MSU	9.5%	4	12
26-3c	Fumed at MSU	31.0%	13	39
26-4a	Non-Fumed	52.4%	22	1
26-4b	Non-Fumed	52.4%	22	7
26-4c	Non-Fumed	57.1%	24	3
27-3a	Fumed at MSP	2.5%	1	4
27-3b	Fumed at MSP	15.0%	6	3
27-3c	Fumed at MSP	0.0%	0	1
27-4a	Fumed at MSU	7.5%	3	8
27-4b	Fumed at MSU	37.5%	15	24
27-4c	Fumed at MSU	5.0%	2	1
27-5a	Non-Fumed	22.5%	9	7
27-5b	Non-Fumed	30.0%	12	6
27-5c	Non-Fumed	37.5%	15	9
33-5a	Fumed at MSP	10.9%	5	3
33-5b	Fumed at MSP	10.9%	5	6
33-5c	Fumed at MSP	32.6%	15	11
33-6a	Fumed at MSU	30.4%	14	13
33-6b	Fumed at MSU	76.1%	35	21
33-6c	Fumed at MSU	26.1%	12	5
33-7a	Non-Fumed	50.0%	23	4
33-7b	Non-Fumed	34.8%	16	1
33-7c	Non-Fumed	26.1%	12	0
36-1a	Non-Fumed	27.3%	12	1
36-1b	Non-Fumed	6.8%	3	1
36-1c	Non-Fumed	11.4%	5	5
36-6a	Fumed at MSP	4.5%	2	2
36-6b	Fumed at MSP	6.8%	3	3

Table F1 (cont'd)

36-6c	Fumed at MSP	11.4%	5	3
36-7a	Fumed at MSU	2.3%	1	3
36-7b	Fumed at MSU	18.2%	8	5
36-7c	Fumed at MSU	4.5%	2	3
38-1a	Fumed at MSU	17.1%	7	7
38-1b	Fumed at MSU	29.3%	12	11
38-1c	Fumed at MSU	7.3%	3	7
38-2a	Non-Fumed	41.5%	17	0
38-2b	Non-Fumed	41.5%	17	18
38-2c	Non-Fumed	21.9%	9	4
38-7a	Fumed at MSP	17.1%	7	2
38-7b	Fumed at MSP	14.6%	6	3
38-7c	Fumed at MSP	2.4%	1	1
40-1a	Fumed at MSP	20.5%	8	6
40-1b	Fumed at MSP	0.0%	0	0
40-1c	Fumed at MSP	5.1%	2	1
40-2a	Fumed at MSU	7.7%	3	0
40-2b	Fumed at MSU	17.9%	7	1
40-2c	Fumed at MSU	-	-	-
40-3a	Non-Fumed	33.3%	13	10
40-3b	Non-Fumed	30.8%	12	18
40-3c	Non-Fumed	0.0%	0	1
41-2a	Fumed at MSP	4.5%	2	0
41-2b	Fumed at MSP	7.1%	3	4
41-2c	Fumed at MSP	40.5%	17	4
41-3a	Fumed at MSU	11.4%	5	1
41-3b	Fumed at MSU	6.8%	3	10
41-3c	Fumed at MSU	20.5%	9	9
41-4a	Non-Fumed	9.1%	4	3
41-4b	Non-Fumed	40.9%	18	21
41-4c	Non-Fumed	40.9%	18	8
50-3a	Fumed at MSP	18.6%	8	8
50-3b	Fumed at MSP	14.0%	6	4
50-3c	Fumed at MSP	14.0%	6	1
50-4a	Fumed at MSU	0.0%	0	0
50-4b	Fumed at MSU	20.9%	9	2
50-4c	Fumed at MSU	16.3%	7	4
50-5a	Non-Fumed	39.5%	17	11
50-5b	Non-Fumed	69.8%	30	4
50-5c	Non-Fumed	46.5%	20	4

APPENDIX G. DNA QUANTITIES RECOVERED FROM SPENT CARTRIDGE CASINGS FROM COLLECTION 2

Table G1. Quantitation results for individually swabbed 0.45 casings from Collection 2.

Sample	Volume (µL)	Concentration (pg/µL)	Yield (pg)
39-4.45a	27.50	4.57E-01	12.57
39-4.45b	34.00	8.16E-01	27.74
39-4.45c	31.80	1.49E+00	47.38
51-2.45a	34.00	2.42E-01	8.23
51-2.45b	26.70	4.08E-01	10.89
51-2.45c	22.00	9.96E-01	21.91
52-3.45a	27.40	1.06E+00	29.04
52-3.45b	30.70	1.71E+00	52.50
52-3.45c	28.60	7.27E-01	20.79
53-4.45a	33.20	2.56E+00	84.99
53-4.45b	25.30	1.67E+00	42.25
53-4.45c	28.50	1.17E+00	33.35
54-3.45a	34.20	2.91E-01	9.95
54-3.45b	31.10	1.57E-01	4.88
54-3.45c	35.00	2.40E-01	8.40
55-1.45a	30.30	2.76E-01	8.36
55-1.45b	30.20	2.79E-01	8.43
55-1.45c	29.20	4.20E-01	12.26
56-4.45a	29.40	2.95E-01	8.67
56-4.45b	29.00	5.00E-02	1.45
56-4.45c	33.80	4.61E-02	1.56
57-1.45a	36.00	4.35E-01	15.66
57-1.45b	31.70	3.48E-01	11.03
57-1.45c	33.00	1.34E-01	4.42
58-3.45a	21.00	1.51E+00	31.71
58-3.45b	28.40	2.05E+00	58.22
58-3.45c	27.20	1.30E+00	35.36
59-2.45a	30.00	8.70E-01	26.10
59-2.45b	30.90	5.77E-01	17.83
59-2.45c	33.30	3.83E-01	12.75
60-4.45a	30.20	8.37E-01	25.28
60-4.45b	31.20	1.07E+00	33.38
60-4.45c	34.80	3.74E+00	130.15
61-4.45a	28.90	1.78E-01	5.14
61-4.45b	29.50	5.13E-01	15.13

Table G1 (cont'd)

61-4.45c	25.50	8.66E-01	22.08
62-3.45a	28.80	3.93E-01	11.32
62-3.45b	27.50	4.24E+00	116.60
62-3.45c	30.50	2.76E+00	84.18
63-2.45a	24.60	1.22E+00	30.01
63-2.45b	34.00	1.12E+00	38.08
63-2.45c	28.40	1.17E+00	33.23
65-2.45a	29.60	4.94E-01	14.62
65-2.45b	26.50	1.37E+00	36.31
65-2.45c	32.50	7.95E-01	25.84
66-1.45a	28.80	1.11E+00	31.97
66-1.45b	31.70	1.02E+00	32.33
66-1.45c	30.70	2.42E-01	7.43
67-1.45a	29.50	1.09E+00	32.16
67-1.45b	34.50	2.09E+00	72.11
67-1.45c	34.20	4.41E-01	15.08
68-1.45a	30.50	9.75E-02	2.97
68-1.45b	36.00	3.60E-01	12.96
68-1.45c	30.00	1.79E-01	5.37
69-2.45a	28.90	3.02E-01	8.73
69-2.45b	30.00	1.43E-01	4.29
69-2.45c	29.80	6.39E-02	1.90
70-3.45a	33.40	4.46E-01	14.90
70-3.45b	33.10	5.57E-01	18.44
70-3.45c	30.30	1.33E+00	40.30

Table G2. Quantitation results of individually swabbed 0.22 casings from Collection 2.

Sample	Volume (µL)	Concentration (pg/µL)	Yield (pg)
39-4.22a	32.80	7.74E-01	25.39
39-4.22b	32.00	4.16E-01	13.31
51-2.22a	32.60	1.46E-01	4.76
51-2.22b	33.60	1.12E-01	3.76
51-2.22c	36.30	1.54E-01	5.59
52-3.22a	27.40	6.34E-02	1.74
52-3.22b	31.50	4.69E-01	14.77
52-3.22c	31.30	2.01E-01	6.29
53-4.22a	33.00	7.32E-01	24.16
53-4.22b	35.50	5.04E-01	17.89
53-4.22c	29.70	2.17E-01	6.44

Table G2 (cont'd)

54-3.22a	31.30	4.69E-01	14.68
54-3.22b	29.60	4.81E-01	14.24
54-3.22c	32.50	2.91E-01	9.46
55-1.22a	33.70	7.07E-01	23.83
55-1.22b	28.30	9.86E-01	27.90
55-1.22c	31.20	1.90E-01	5.93
56-4.22a	32.50	5.92E-01	19.24
56-4.22b	30.10	5.07E-01	15.26
56-4.22c	36.00	6.06E-01	21.82
57-1.22a	29.80	6.60E-02	1.97
57-1.22b	27.70	9.23E-02	2.56
57-1.22c	33.00	4.99E-02	1.65
58-3.22a	30.00	1.24E-01	3.72
58-3.22b	31.80	6.50E-02	2.07
58-3.22c	35.50	3.51E-01	12.46
59-2.22a	26.00	6.27E-01	16.30
59-2.22b	29.50	5.80E-01	17.11
59-2.22c	30.00	4.50E-01	13.50
60-4.22a	29.30	5.20E-01	15.24
60-4.22b	26.50	1.32E+00	34.98
60-4.22c	33.50	1.35E+00	45.23
61-4.22a	29.30	6.95E-02	2.04
61-4.22b	29.00	3.18E-02	0.92
61-4.22c	28.50	4.89E-02	1.39
62-3.22a	31.50	9.92E-01	31.25
62-3.22b	34.00	1.14E+00	38.76
62-3.22c	31.60	3.55E-01	11.22
63-2.22a	30.40	7.72E-01	23.47
63-2.22b	33.20	1.08E+00	35.86
63-2.22c	31.00	1.00E+00	31.00
65-2.22a	28.10	5.23E-01	14.70
65-2.22b	28.70	2.78E-01	7.98
65-2.22c	29.30	3.18E-01	9.32
66-1.22a	34.00	1.70E-01	5.78
66-1.22b	28.40	8.60E-02	2.44
66-1.22c	27.70	5.88E-02	1.63
67-1.22a	30.30	8.63E-01	26.15
67-1.22b	35.80	6.03E-01	21.59
67-1.22c	37.50	5.60E-01	21.00
68-1.22a	28.30	1.48E-01	4.19

Table G2 (cont'd)

68-1.22b	28.40	3.47E-02	0.99
68-1.22c	27.30	1.74E-01	4.75
69-2.22a	31.10	7.13E-02	2.22
69-2.22b	29.20	1.56E-01	4.56
69-2.22c	25.70	8.81E-02	2.26
70-3.22a	28.20	9.74E-01	27.47
70-3.22b	31.80	6.33E-01	20.13
70-3.22c	31.80	8.16E-01	25.95

Table G3. Quantitation results for cumulatively swabbed 0.45 casings from Collection 2.

Sample	Volume (µL)	Concentration (pg/µL)	Yield (pg)
39-1.45	29.70	1.45E+00	43.07
39-2.45	28.60	2.46E+00	70.36
39-3.45	31.70	2.05E+00	64.99
51-1.45	32.20	1.76E-01	5.67
51-3.45	32.50	5.62E-01	18.27
51-4.45	29.60	6.59E-01	19.51
52-1.45	35.40	1.28E+01	453.12
52-2.45	33.10	2.41E+00	79.77
52-4.45	35.30	2.90E+00	102.37
53-1.45	27.80	5.30E+00	147.34
53-2.45	27.70	2.55E+00	70.64
53-3.45	27.60	7.64E+00	210.86
54-1.45	31.30	7.70E+00	241.01
54-2.45	24.80	3.20E+00	79.36
54-4.45	26.60	2.50E+00	66.50
55-2.45	32.80	7.42E-01	24.34
55-3.45	35.20	1.85E+00	65.12
55-4.45	29.80	1.39E+00	41.42
56-1.45	31.50	1.08E+00	34.02
56-2.45	35.00	1.26E+00	44.10
56-3.45	34.10	2.25E+00	76.73
57-2.45	31.70	4.69E+00	148.67
57-3.45	33.50	2.88E-01	9.65
57-4.45	31.00	6.26E-01	19.41
58-1.45	28.20	1.57E+00	44.27
58-2.45	28.70	1.70E+00	48.79
58-4.45	28.30	1.58E+00	44.71
59-1.45	34.80	7.45E-01	25.93

Table G3 (cont'd)

59-3.45	32.30	3.05E-01	9.85
59-4.45	29.00	5.62E-01	16.30
60-1.45	32.80	1.28E+00	41.98
60-2.45	34.70	1.25E+00	43.38
60-3.45	35.70	2.78E+00	99.25
61-1.45	32.50	1.34E+00	43.55
61-2.45	32.50	1.48E+00	48.10
61-3.45	31.80	1.00E+00	31.80
62-1.45	32.00	2.77E+00	88.64
62-2.45	35.00	3.58E+00	125.30
62-4.45	34.00	3.15E+00	107.10
63-1.45	30.50	8.61E-01	26.26
63-3.45	30.30	1.05E+00	31.82
63-4.45	29.00	1.23E+00	35.67
65-1.45	32.40	3.57E+00	115.67
65-3.45	27.80	3.93E+00	109.25
65-4.45	28.50	3.78E+00	107.73
66-2.45	18.20	2.29E+00	41.68
66-3.45	33.50	5.22E+00	174.87
66-4.45	30.30	1.98E+00	59.99
67-2.45	27.50	4.95E-01	13.61
67-3.45	29.70	8.86E-01	26.31
67-4.45	28.50	5.11E+00	145.64
68-2.45	30.70	2.11E+00	64.78
68-3.45	32.00	4.79E-01	15.33
68-4.45	31.50	3.71E-01	11.69
69-1.45	29.40	4.08E-01	12.00
69-3.45	34.10	5.76E-01	19.64
69-4.45	32.20	9.05E-01	29.14
70-1.45	36.20	1.62E+00	58.64
70-2.45	34.50	1.94E+00	66.93
70-4.45	35.10	2.61E+00	91.61

Table G4. Quantitation results for cumulatively swabbed 0.22 casings from Collection 2.

Sample	Volume (µL)	Concentration (pg/µL)	Yield (pg)
39-1.22	30.30	1.51E+00	45.75
39-2.22	28.20	1.37E+00	38.63
39-3.22	33.30	1.42E+00	47.29
51-1.22	28.10	3.11E-01	8.74
51-3.22	26.50	6.58E-01	17.44
51-4.22	32.10	5.16E-01	16.56
52-1.22	32.30	6.61E-01	21.35
52-2.22	30.80	7.13E-01	21.96
52-4.22	29.20	2.59E-01	7.56
53-1.22	30.00	5.70E-01	17.10
53-2.22	32.00	5.15E-01	16.48
53-3.22	32.10	5.83E-01	18.71
54-1.22	29.80	1.86E+00	55.43
54-2.22	28.10	1.49E+00	41.87
54-4.22	29.30	1.16E+00	33.99
55-2.22	31.40	4.68E-01	14.70
55-3.22	33.50	2.99E-01	10.02
55-4.22	28.10	8.02E-02	2.25
56-1.22	31.80	1.20E+00	38.16
56-2.22	27.70	9.37E-01	25.95
56-3.22	31.50	5.69E-01	17.92
57-2.22	30.50	2.11E-02	0.64
57-3.22	30.10	9.34E-02	2.81
57-4.22	29.00	1.59E-01	4.61
58-1.22	33.40	5.43E-01	18.14
58-2.22	32.80	2.54E-01	8.33
58-4.22	33.30	1.92E-01	6.39
59-1.22	30.10	2.61E-02	0.79
59-3.22	32.20	5.33E+00	171.63
59-4.22	28.50	2.96E+00	84.36
60-1.22	29.80	4.92E+00	146.62
60-2.22	28.30	3.91E+00	110.65
60-3.22	34.00	2.76E+00	93.84
61-1.22	34.20	3.78E-01	12.93
61-2.22	33.00	5.04E-01	16.63
61-3.22	32.30	5.69E-01	18.38
62-1.22	26.30	1.64E+00	43.13
62-2.22	30.30	5.73E-01	17.36
62-4.22	34.10	5.39E-01	18.38

Table G4 (cont'd)

63-1.22	31.50	8.06E-01	25.39
63-3.22	33.50	7.29E-01	24.42
63-4.22	27.90	9.01E-01	25.14
65-1.22	31.40	2.97E-01	9.33
65-3.22	31.40	1.77E+00	55.58
65-4.22	32.60	1.25E+00	40.75
66-2.22	27.20	5.62E-01	15.29
66-3.22	32.10	6.28E-01	20.16
66-4.22	31.00	8.11E-02	2.51
67-2.22	33.00	7.89E-01	26.04
67-3.22	35.30	4.08E-01	14.40
67-4.22	35.20	3.11E-01	10.95
68-2.22	26.80	1.53E-01	4.10
68-3.22	34.30	1.65E+00	56.60
68-4.22	34.60	4.77E-01	16.50
69-1.22	34.50	2.52E-01	8.69
69-3.22	34.50	2.92E-01	10.07
69-4.22	29.70	5.01E-01	14.88
70-1.22	32.60	2.16E-01	7.04
70-2.22	28.90	3.74E-01	10.81
70-4.22	20.00	3.69E-01	7.38

APPENDIX H. FUSION STR PROFILES FROM COLLECTION 2

Red font: non-loader allele

**: allele was above the threshold using OSIRIS, but below the threshold using GeneMapper®.*

†: off-ladder allele

Blank cell: no alleles were amplified

N/A: not applicable

Table H1. Fusion profiles generated from spent cartridge casings loaded by individual SSS.

Locus	39-4.45a	39-4.45b	39-4.45c	39-4.22a	39-4.22b	SSS
Amel		X	X		X	X,X
D3	15	15,16		16	15	15,16
D1		18.3	12	12	16,18.3	12,18.3
D2	14	11	11	11,14		11,14
D10	15	14				14,15
D13			11	11		11,12
Penta E						13,17
D16		10,14	12,14	†,12,14	9,14	12,14
D18	12,15	12,21		12		12,12
D2						19,24
CSF	10		10			10,12
Penta D						9,14
THO1	9.3	9.3	9.3	9,9.3	6,9,9.3	9,9.3
vWA	19	19	18	18		18,19
D21	31.2	31.2				31.2
D7						9,10
D5						11,11
TPOX			8			8,8
DYS391						N/A
D8	13,15			13	10,13,15	13,15
D12		17,19				17,19
D19	13	13	13,14			13,14
FGA		25	25			21,25
D22				16		16,16

Table H1 (cont'd)

Locus	39-1.45	39-2.45	39-3.45	39-1.22	39-2.22	39-3.22	SSS
Amel	X	X,Y*	X,Y	X,Y	X	X	X,X
D3	15	15,16	14,15,16	15,16,18	14,15,16	14,15,16	15,16
D1	12	11,12	16.3,17.3	17.3*,18.3	17.3	11	12,18.3
D2	11,14	11,14	11,14	14		11	11,14
D10	14,15	14,15,16	15	14			14,15
D13		11,12	10,12		11,12		11,12
Penta E			14		13	13	13,17
D16	11,12,14	11,14	11,12,14	†,12,14	12	12,14	12,14
D18	12		12,15,16,17	15	12,16,17	-12	12,12
D2	24	19*,24	22		19,24	24	19,24
CSF	10	10	10,11,12	12	10	10	10,12
Penta D	9	14		14	9,14	14	9,14
THO1	7,8,9,9.3	6,9,9.3	9,9.3	9,9.3	6,9,9.3	6,9,9.3	9,9.3
vWA		18,19	18,19	18	17*	17	18,19
D21	31.2	30,31.2	28,31.2	31.2,32	31.2		31.2,31.2
D7	8		10,12		9,10		9,10
D5	11	11	12	11			11,11
TPOX	8	8	8				8,8
DYS391							N/A
D8	13,15	9,10,12,13,15	9,13,15	11,13	13,15,16	10,13,15	13,15
D12	17,19	17,19,20	17,19,25	17,19	17,18.3		17,19
D19	13,15.2	12,13	12,13,14	13,14	14	13	13,14
FGA	21	21,22	21.2,22,25		21	21	21,25
D22		16	11,16	16	16	16	16,16

Table H2. Fusion profiles generated from spent cartridge casings loaded by individual NN.

Locus	51-2.45a	51-2.45b	51-2.45c	51-2.22a	51-2.22b	51-2.22c	NN
Amel	X	X	X,Y		X	X	X,X
D3	16	14	14,16				14,16
D1	16	12	12,16			16	12,16
D2	10,11.3		11	11		10	10,11
D10		14,15	15		14	14	14,15
D13			8				8,12
Penta E							7,21
D16	12		12	12	12		12,12
D18	14.2	12,14	14,14.2				14,14.2
D2	17,23					17,23	17,23
CSF							12,13
Penta D				13			13,13
THO1	9,9.3	6	9.3	6,9		7,9,9.3	9,9.3
vWA	17	17	17		17	17	17,17
D21	29	31.2,33.2	29				29,31.2
D7			11			9	9,11
D5	9		9				9,10
TPOX							8,8
DYS391							N/A
D8	13,14,15	13,14,15	13,15	13	13,15		13,15
D12	18.3		22		18	22	18,22
D19		15.2	14,15.2				14,15.2
FGA							22,26
D22							11,11

Table H2 (cont'd)

Locus	51-1.45	51-3.45	51-4.45	51-1.22	51-3.22	51-4.22	NN
Amel	X	X,Y	X		X	Y	X,X
D3	14,15,16		14,16	18	14	18	14,16
D1	16,17.3	12		12			12,16
D2		11,14				11.3	10,11
D10				14,15			14,15
D13			12				8,12
Penta E		7,21					7,21
D16	11,12	12	12	12	12	12	12,12
D18		14	14,14.2	12	14	18	14,14.2
D2		23	23	17	17,23	17	17,23
CSF					13	12	12,13
Penta D				13	9		13,13
THO1	6,9.3	9	9,9.3	9.3	9	6,9	9,9.3
vWA	16,17	16,17	15	17	18	15,17	17,17
D21		29,31.2			31.2	29	29,31.2
D7		9				9	9,11
D5						9	9,10
TPOX				8			8,8
DYS391							N/A
D8	13,15	9,12,13,15	13			13	13,15
D12	17,18,22	18	18,22				18,22
D19		14	15.2			14	14,15.2
FGA			21.2,22			26	22,26
D22						15	11,11

Table H3. Fusion profiles generated from spent cartridge casings loaded by individual ZZZ.

Locus	52-3.45a	52-3.45b	52-3.45c	52-3.22a	52-3.22b	52-3.22c	ZZZ
Amel	X	X	X		X	X	X,X
D3	14	18	17		18		14,18
D1		15,17.3					15,17.3
D2		10,11	11				10,11
D10		12,14					12,14
D13		12					9,12
Penta E		13			12		9,13
D16	12,16	9,12	9,12	12			9,12
D18	16	10.2,14	16		16	16	16,20
D2	19	19,21	21		20,21		19,21
CSF		12					11,12
Penta D		13					12,13
THO1	6,9.3	9.3	6,9.3		6	6	6,9.3
vWA	17	17		17	17	17	17,17
D21	28.2	28,29					28,29
D7	15	12			12		10,12
D5	11	13			13		11,13
TPOX		8					8,11
DYS391							N/A
D8	10	10,12	10,12		10,12		10,12
D12	24	24,25			24	25	24,25
D19		14	14				13,14
FGA	19						19,26
D22			16				15,16

Table H3 (cont'd)

Locus	52-1.45	52-2.45	52-4.45	52-1.22	52-2.22	52-4.22	ZZZ
Amel	X,Y	X	X	X	X	X	X,X
D3	17	14,17	14,18	14,18	14,18	18	14,18
D1	15,18.3	15	15,17.3		17.3	15	15,17.3
D2	10,11		11	11	10,11	11	10,11
D10	15		14		14		12,14
D13	11,12	9	9,12		9	12	9,12
Penta E	14		13				9,13
D16	12	9,12	9	†,11,12	9	9,12	9,12
D18	16,17	16,20	16,20	16	16,20	16	16,20
D2	19,21,25	19	19		21	21	19,21
CSF	12		12	11			11,12
Penta D	9,13	12	13				12,13
THO1	6,8,9.3	6,9.3	6,9.3	6,9.3	6	6,9.3	6,9.3
vWA	15,17	17	17	17			17,17
D21	29	28,29	28,29	28,29		28	28,29
D7	10,12	12	10	10	12	10,12	10,12
D5	11,12	13	11			13	11,13
TPOX	8	8	11	11			8,11
DYS391	11						N/A
D8	11,12,13	10,12	10,12	10,12	10,12		10,12
D12	18,18.3,20	24	24	24	15,24,25	24	24,25
D19	14,15.2	14	13,14	13,14	13	13	13,14
FGA	21,23	19,26	19,26	26	26	19,25,26	19,26
D22	15,16		16				15,16

Table H4. Fusion profiles generated from spent cartridge casings loaded by individual B.

Locus	53-4.45a	53-4.45b	53-4.45c	53-4.22a	53-4.22b	53-4.22c	B
Amel	X	X				X	X,X
D3	18		18*				18,18
D1	11,12	12					12,15
D2	11.3						11,11.3
D10	15				14		13,15
D13	13	13					12,13
Penta E		12,13					12,13
D16	11	11	11				11,11
D18	16	12,16					12,16
D2	17	25					17,25
CSF				10			10,11
Penta D	13						10,13
THO1	6,9	6	6,9				6,9
vWA	17,18	17,18	17				17,18
D21			28				28,28
D7		10					10,10
D5	12						10,12
TPOX		11					8,11
DYS391							N/A
D8	13,16	13,16	16	9,13			13,16
D12	17,18,18.3	18	18,18.3	18,18.3			18,18.3
D19	15	15					13.2,15
FGA	22	23					22,23
D22							15,15

Table H4 (cont'd)

Locus	53-1.45	53-2.45	53-3.45	53-1.22	53-2.22	53-3.22	B
Amel	X	X	X		X	X	X,X
D3	18	18	18	14	16,18		18,18
D1	12,15,17	12,15	12,15		12	11	12,15
D2	11.3,14	11,11.3	11,11.3			11.3	11,11.3
D10	15	13	13,15				13,15
D13				10,12			12,13
Penta E	12,13,18		12,13				12,13
D16	11	11	11	11	11,12		11,11
D18	12,16	12,16,18	12,16	16,17	12		12,16
D2	23,25	17,25	17,25				17,25
CSF	10,12	10	10,11				10,11
Penta D		10	10,13	12	10		10,13
THO1	6,9	6,9	6,9	6,9		7,9	6,9
vWA	17,18	17,18	17,18			17	17,18
D21	28	28	28	32.2	28		28,28
D7	10	10	10		10		10,10
D5	10,12	10	10	14			10,12
TPOX		8	11				8,11
DYS391							N/A
D8	9,10,13,14,16	13,16	13,16		13	11,15,16	13,16
D12	17,18.3	18,18,3	18,18.3				18,18.3
D19	13.2,15	13.2,15	13.2,15				13.2,15
FGA	22	23	22,23	22		22	22,23
D22		15	15				15,15

Table H5. Fusion profiles generated from spent cartridge casings loaded by individual BBB.

Locus	54-3.45a	54-3.45b	54-3.45c	54-3.22a	54-3.22b	54-3.22c	BBB
Amel	X		X	X	X	X	X,X
D3	16	16			16,17	15	15,16
D1	12	12	12				11,12
D2	11	14					11,14
D10				13			14,14
D13			12		11		11,12
Penta E							13,16
D16	11,12,13	†,13	12		12	†,12	12,13
D18	14,16	14				14	14,16
D2	24			24		24	24,24
CSF	10	10					10,10
Penta D	9		9				9,9
THO1	8,9	8		9	9		8,9
vWA	17		17	17	18		17,17
D21		28	28		29		29,30
D7							7,8
D5				9			9,11
TPOX							8,8
DYS391							N/A
D8	14	14	8,9	14		14	14,14
D12	21	20	21	20		19	20,21
D19							12,13
FGA	22						20,22
D22							11,16

Table H5 (cont'd)

Locus	54-1.45	54-2.45	54-4.45	54-1.22	54-2.22	54-4.22	BBB = 54
Amel	X	X	X	X	X	X	X,X
D3	15,16	15,16	15,16	15	15,16	15	15,16
D1	11,12	11	11,12	12,15.3	11,12	12	11,12
D2	11,14	11	11.3	11,11.3	11		11,14
D10	14	14	14			14	14,14
D13	11,12		11	10		12	11,12
Penta E	13	16	13,14				13,16
D16	12,13	12,13	11,12	12,13*	12,13	12,13	12,13
D18	14,16	14,16	12,14,16,17	16		16	14,16
D2	19,21,24	24	24	18	24	22	24,24
CSF	10		10*	12	10		10,10
Penta D	9					9	9,9
THO1	8,9	8	8,9,9.3	6,9,9.3	8,9,9.3	7,8	8,9
vWA	17	17	17	17	17	17	17,17
D21	29	30	29,30	28,33.2			29,30
D7	7,8	6.3,7,9	7,8	10	7,8	8	7,8
D5	9,11	11	11	12	8,11	11,12	9,11
TPOX	8	8	8	12	8		8,8
DYS391							N/A
D8	14	14	14	13,14	13,14	14	14,14
D12	20,21		20,23	17,20	20	19.3,20,21	20,21
D19	12,13	13	12	12	13	12	12,13
FGA	20,22		22	21	20,22		20,22
D22	11						11,16

Table H6. Fusion profiles generated from spent cartridge casings loaded by individual C.

Locus	55-1.45a	55-1.45b	55-1.45c	55-1.22a	55-1.22b	55-1.22c	C
Amel			X				X,X
D3	18			15,18			15,18
D1							14,16
D2				10			11,11
D10		14					14,15
D13			12				11,12
Penta E							11,13
D16			†	11			11,12
D18				16,17			11,17
D2							17,25
CSF		10					10,11
Penta D							9,10
THO1		6				8	6,6
vWA							11,14
D21							28,29
D7							9,13
D5							11,12
TPOX				8			12,12
DYS391							N/A
D8		13,14					13,14
D12							17,19
D19							13,14
FGA							20,24
D22							15,17

Table H6 (cont'd)

Locus	55-2.45	55-3.45	55-4.45	55-2.22	55-3.22	55-4.22	C
Amel	X	X	X	X	X,Y	X,Y	X,X
D3	15,16	15,17,18	14,18	15			15,18
D1	11,12	14,15,15.3		11,14,15.3,16	14		14,16
D2		11	11				11,11
D10		15	15			15	14,15
D13		11,12	12				11,12
Penta E		7,11	5,14				11,13
D16	12,13	11,12	11,12	8.3*	12,14	11	11,12
D18	11,14,17	11	11	12,18			11,17
D2		17					17,25
CSF		10	10				10,11
Penta D		10	12				9,10
THO1	6	6	6	3,9,9.3	6	9.3	6,6
vWA		14,18	14,17	14,17		18	11,14
D21		28,29		29			28,29
D7		9	13	10			9,13
D5							11,12
TPOX		8,12				12	12,12
DYS391			11				N/A
D8	13,14	10,13,14	13,14	13,15	13,14	13,14	13,14
D12	17,19.3	17,18,19,19.3, 20	17,19,21	15,20	20	19,21	17,19
D19	8.2,14	13,14	12,13	13			13,14
FGA		18,24		20			20,24
D22		15,17					15,17

Table H7. Fusion profiles generated from spent cartridge casings loaded by individual AA.

Locus	56-4.45a	56-4.45b	56-4.45c	56-4.22a	56-4.22b	56-4.22c	AA
Amel	Y			X		X	X,Y
D3			9,17				15,15
D1		19.3					18.3,19.3
D2							11,11.3
D10							13,14
D13							9,12
Penta E							5,5
D16							9,12
D18			13				13,14
D2							19,21
CSF							10,11
Penta D							11,14
THO1	9.3						6,9.3
vWA							15,17
D21			28				28,30.2
D7	9						9,9
D5							12,13
TPOX							8,11
DYS391							10
D8		15					12,15
D12						20	19,22
D19					15	15	13,15
FGA							18,21
D22							11,17

Table H7 (cont'd)

Locus	56-1.45	56-2.45	56-3.45	56-1.22	56-2.22	56-3.22	AA
Amel	X	X,Y		X		X,Y	X,Y
D3	15	14,15	16	14		15	15,15
D1	18.3,19.3			14	15		18.3,19.3
D2	11.3	14				11	11,11.3
D10	13,16					14	13,14
D13	9		12				9,12
Penta E		15					5,5
D16	9	9	9,11	11,12	13	9	9,12
D18	14	11,17	14,16	16	14,15	13	13,14
D2		17		20		20	19,21
CSF					11		10,11
Penta D							11,14
THO1	6	7,9	7,9.3		8	6	6,9.3
vWA	15,17		14			18	15,17
D21			28	28*			28,30.2
D7							9,9
D5	11,13		13	11			12,13
TPOX	11		11	8			8,11
DYS391							10
D8	12	10	10,13	9,12	12	12	12,15
D12	22	21,23	17,22	21,23		18	19,22
D19		16.2	13		13.2		13,15
FGA	21		20,21	20			18,21
D22							11,17

Table H8. Fusion profiles generated from spent cartridge casings loaded by individual A.

Locus	57-1.45a	57-1.45b	57-1.45c	57-1.22a	57-1.22b	57-1.22c	A
Amel				X		X	X,X
D3	15						15,16
D1					14		11,14
D2							14,16
D10							15,16
D13	12						13,14
Penta E							7,12
D16	11,12	9					11,11
D18					11		12,17
D2					18		17,25
CSF					10		10,12
Penta D							12,12
THO1	7	6					7,9.3
vWA	16	15	17				17,18
D21	32.2						28,30
D7				10			10,12
D5						11	9,12
TPOX							8,11
DYS391							N/A
D8		9,15				13	12,15
D12	18.3				20		18,24
D19	19						13,15.2
FGA							23,24
D22					16		15,16

Table H8 (cont'd)

Locus	57-2.45	57-3.45	57-4.45	57-2.22	57-3.22	57-4.22	A
Amel	X	X	X		X	Y	X,X
D3	15,16	16	18	16	18		15,16
D1	11,14		14		14	12,17.3	11,14
D2	14,16						14,16
D10	15,16						15,16
D13	13,14		14				13,14
Penta E	7,12						7,12
D16	11	11	11		11		11,11
D18	12,17	17	12			17	12,17
D2	17,25		17,25				17,25
CSF	10,12		12			10	10,12
Penta D	12						12,12
THO1	7,9.3	7,9.3	7	6	6	8	7,9.3
vWA	17,18	16	18	14	18		17,18
D21	28,30,31		30	28			28,30
D7	10,12		10				10,12
D5	9,12		12				9,12
TPOX	8,11						8,11
DYS391							N/A
D8	12,15		13	12		13,14	12,15
D12	18,24	15	18,19.3,24		20		18,24
D19	13,15.2						13,15.2
FGA	23,24						23,24
D22	15,16						15,16

Table H9. Fusion profiles generated from spent cartridge casings loaded by individual J.

Locus	58-3.45a	58-3.45b	58-3.45c	58-3.22a	58-3.22b	58-3.22c	J
Amel		Y	X	X			X,Y
D3	15,16	16,18		16			15,16
D1		17.3					13,13
D2						13	11.3,13
D10							13,14
D13							11,11
Penta E		7				16	11,16
D16	12	†,9,11	9	13	11,12		12,13
D18	10	16					10,18
D2				18			17,18
CSF		12					
Penta D							8,9
THO1	6,8	9	10	6,7	6		6,8
vWA	18	15,17					18,18
D21		31	28				30.2,31.2
D7							12,12
D5							10,12
TPOX		10					8,10
DYS391							10
D8	12,13	10,13,14	11,12,13,16				11,12
D12		17,23			17,18		18,18
D19		14,15					14,15
FGA							20,22
D22							16,16

Table H9 (cont'd)

Locus	58-1.45	58-2.45	58-4.45	58-1.22	58-2.22	58-4.22	J
Amel	X,Y	X,Y	X,Y	X	X,Y	X	X,Y
D3		16,18	14,15,16		16	15,16,17	15,16
D1	12,13,15	11	11	14,17.3		12	13,13
D2		11.3					11.3,13
D10	13,15	13		14	14	14	13,14
D13	10	11	12	11			11,11
Penta E				12	16		11,16
D16	12,13	9,12,13	11,12,13	11		11,12,13,14	12,13
D18	16,18	10,12,14,18	10	17		10	10,18
D2	18			17		19	17,18
CSF		10			10	11	
Penta D	13				8		8,9
THO1	6,9,9.3	6,7,8	6,8,9.3	6,9.3	8	6,9,9.3	6,8
vWA	15,17,18	18	18,19	15		17,18	18,18
D21	28						30.2,31.2
D7			12				12,12
D5		10		12		12	10,12
TPOX						10,11	8,10
DYS391							10
D8	15	11,12,13	11,13	14	11,12	8,9,11,12,13, 15	11,12
D12	18,18.3,21	18,18.3	18,20	18		17,18,18.3,21	18,18
D19	13,13.2,14	15	14			13	14,15
FGA	21	20		23		20,24	20,22
D22	14,15			15			16,16

Table H10. Fusion profiles generated from spent cartridge casings loaded by individual KKK.

Locus	59-2.45a	59-2.45b	59-2.45c	59-2.22a	59-2.22b	59-2.22c	KKK
Amel	Y		X,Y	Y		X,Y	X,Y
D3			15	15		15	15,16
D1	17			11	15,16,18.3	16	11,16
D2	10	14		14	14	10	10,14
D10	15			14			14,18
D13							10,10
Penta E							5,7
D16	12	11,12	9,11	†,9,11	9	9	9,11
D18				18	14,18		16,18
D2							18,19
CSF	10						10,14
Penta D							9,14
THO1	6	8,9.3	8,9.3	8,9.3	8	8,9.3	8,9.3
vWA	15		17	14,17		14	14,17
D21	29			30		30	30,30
D7				10		8	8,10
D5		10			10		10,13
TPOX							8,8
DYS391							9
D8		13,14	11,14	11,12,13,14, 16	14	14,16	14,16
D12	15	15,20	20	15	15*,20	15,20	15,20
D19		13,15.2		13			13,14
FGA	23				18,21,22	24	21,24
D22							14,15

Table H10 (cont'd)

Locus	59-1.45	59-3.45	59-4.45	59-1.22	59-3.22	59-4.22	KKK
Amel	Y	X,Y		X,Y	X,Y	X,Y	X,Y
D3	15,18	16	15	16	15,16	15,16	15,16
D1	11,12,15	16	11,15,16	11,17.3	11,16	11	11,16
D2	11	14		14	14	10	10,14
D10	14,18				14,18	18	14,18
D13	12			10	10	10	10,10
Penta E	13			7	7,12		5,7
D16	9,11	9,13	9,11	11	9,11	9,11	9,11
D18	18			18	16,18	16,18	16,18
D2	18,20	18		19	18	18,19	18,19
CSF	10	14	10		10,14	14	10,14
Penta D	9,14	14			9,14	8	9,14
THO1	8	9.3	9.3	8,9.3	8,9.3	6,8,9,9.3	8,9.3
vWA	14,17		14	14	14,17	14,17	14,17
D21	28,29,30	28,29			30	30,31.2	30,30
D7			10		8,10	8	8,10
D5	10			13	10,13	10,13	10,13
TPOX					8	8	8,8
DYS391				9	9		9
D8	13,14,16	16	13,16	13,14,16	14,16	14,16	14,16
D12	15,17,19,20	20	15,19,20	20	15,18,20	15	15,20
D19					13,14	13	13,14
FGA	24				21,24	21	21,24
D22			14		14,15		14,15

Table H11. Fusion profiles generated from spent cartridge casings loaded by individual JJJ.

Locus	60-4.45a	60-4.45b	60-4.45c	60-4.22a	60-4.22b	60-4.22c	JJJ
Amel	X		X	X	X,Y	X	X,Y
D3	16,18		18		18	16	16,18
D1	16.3	16.3*	12,14,16.3	12	12,16.3	12	12,16.3
D2			11	11	11	11,11.3	11,11
D10	13,16		13				13,13
D13	12						12,12
Penta E			17	7			7,17
D16	12	12	12	12	12	12	12,12
D18	17		17		17	17	17,17
D2	19		20				20,22
CSF		10	12				10,12
Penta D	10						10,11
THO1	9,9.3	9	9,9.3	6,7,9,9.3	6,9,9.3	9,9.3	9,9.3
vWA	18	14,18	14,18	14,18		14,18	14,18
D21	28,30		29,30	30	30,30.2		30,30.2
D7			9,11	11	9		9,11
D5							12,13
TPOX		8					8,8
DYS391			10				10
D8	10,14	14	10,14	10,14	10	14	10,14
D12	20		19.3,20	20		19.3,20	19.3,20
D19		15.2	12			15.2	12,15.2
FGA	20	18	18		18,20		18,20
D22	11						11,15

Table H11 (cont'd)

Locus	60-1.45	60-2.45	60-3.45	60-1.22	60-2.22	60-3.22	JJJ
Amel	X,Y	X	X,Y	X,Y	X,Y	X,Y	X,Y
D3		16,17	15,18	16,18	16,18	16,17,18	16,18
D1	12,16.3	16.3	12,16.3	12,16.3	12	12,14,16.3	12,16.3
D2	11	11	11	11	11	11	11,11
D10			13	13	13		13,13
D13	12	12	12	12	12	12	12,12
Penta E	17		5,7,17	7,17	7	17	7,17
D16	†,12,13	12	12	12	12	12	12,12
D18		17	15,17	17	17	17	17,17
D2	20,22	20	19,20,21,22	20,22	22	20,22	20,22
CSF	10		10	10,12		10	10,12
Penta D	11	9,11	10	10,11	10		10,11
THO1	7,9,9.3	7,8,9,9.3	9,9.3	9,9.3	9,9.3	6,7,9,9.3	9,9.3
vWA	14,18	14,17,18	14,17,18	14,18	14,18	14,18	14,18
D21	30.2	28,30.2	30,30.2	30,30.2	30.2	29.2,30.2	30,30.2
D7			9,11	9,11	9,11		9,11
D5			12	12,13	12,13	13	12,13
TPOX			8	8		8	8,8
DYS391		10	10	10	10		10,10
D8	10,12,14	10,13,14	10,12,14,15	10,14	10,14	10,14	10,14
D12	19.3,20	19.3,21	19.3,20,22	19.3,20	20,21	19.3,20	19.3,20
D19		12,15.2	12,15.2	12,15.2	12,15.2	12	12,15.2
FGA	18,20	18	20,21,22	18,20	20		18,20
D22		16	15	11,15	11,15		11,15

Table H12. Fusion profiles generated from spent cartridge casings loaded by individual I.

Locus	61-4.45a	61-4.45b	61-4.45c	61-4.22a	61-4.22b	61-4.2c	I
Amel	X	X	X				X,X
D3	15			14*		16	16,16
D1							16,17.3
D2			11	11			11,11
D10	15						13,15
D13		12					13,13
Penta E							7,7
D16	4	9,11,13	11				11,11
D18	12,15	13		15			15,16
D2			17				17,17
CSF		11					12,13
Penta D							9,11
THO1	5,11	6,9.3	6,8		9.3		8,9.3
vWA	14,15,18,19			16			14,18
D21				27			27,30
D7							8,10
D5		13					11,12
TPOX					10		8,8
DYS391							N/A
D8	13	12,13		10		13,14,15	13,14
D12		22					18,20
D19		13,15,15.2	15		15	14	14,15
FGA							21,23
D22							15,16

Table H12 (cont'd)

Locus	61-1.45	61-2.45	61-3.45	61-1.22	61-2.22	61-3.22	I
Amel		X	X	X	X	X	X,X
D3			16			16	16,16
D1	15,17.3			15	17.3		16,17.3
D2	11	11					11,11
D10	13						13,15
D13							13,13
Penta E	9						7,7
D16	9	11,13	11				11,11
D18			15	15			15,16
D2	17	17	17		17		17,17
CSF			13				12,13
Penta D							9,11
THO1	6,9.3	8,9.3	8,9.3				8,9.3
vWA	14,18	16	17	18			14,18
D21	29	29,30	30		30		27,30
D7	12				8	9	8,10
D5	11						11,12
TPOX	8				8		8,8
DYS391							N/A
D8	11,13	13,14	13,14	13		10	13,14
D12	24,25		18			18	18,20
D19			13.2,14,15	13.2			14,15
FGA		21,23					21,23
D22							15,16

Table H13. Fusion profiles generated from spent cartridge casings loaded by individual YYY.

Locus	62-3.45a	62-3.45b	62-3.45c	62-3.22a	62-3.22b	62-3.22c	YYY
Amel		X	X	X	X	X	X,X
D3		17,18		16,17			17,18
D1	15,15.3	15,19.3	15.3	14			14,15
D2		10	10	10	10		10,10
D10		16	16				16,16
D13				9	13	12	9,13
Penta E				5			5,10
D16		13	13	12,14		13	13,14
D18		13,15	13,15	13	15	13,18	13,15
D2		20		21	18		18,20
CSF							11,12
Penta D							9,11
THO1	6,7	6,7	7,9.3	9,9.3	6,7,9.3	6,7	6,7
vWA	18	14	18	14*	14	14	14,18
D21	27	30	27			30	27,30
D7		10,12	12	9			10,12
D5			11	12			11,12
TPOX							8,11
DYS391				10			N/A
D8		13	13		13	13	13,13
D12	18	18,21	18,21	19	18,21		18,21
D19		15.2		13			14,15.2
FGA		24		21		24,26	20,24
D22				11			11,11

Table H13 (cont'd)

Locus	62-1.45	62-2.45	62-4.45	62-1.22	62-2.22	62-4.22	YYY
Amel	X	X		X	X	X	X,X
D3	17	15,17,18	17,18			17,18	17,18
D1	14,15	12,14,15	14,15,16			14	14,15
D2	10	10					10,10
D10	16	16	13,15				16,16
D13		9,13					9,13
Penta E	5,10	5,10	5				5,10
D16	13,14	13,14	12,13,14	13	13	†,14	13,14
D18	13,15	13,15	13,14,15			13	13,15
D2		18	18		18		18,20
CSF	11	12				11	11,12
Penta D		11					9,11
THO1	6,7	6,7	6,7,9.3	6	6	6,7	6,7
vWA	14,18	14,18	14,18			18	14,18
D21	27	27	27,30			27	27,30
D7	10,12	10			10	12	10,12
D5	12	12	10				11,12
TPOX	11	8,11	8				8,11
DYS391							N/A
D8	13	13	13	13	12,13	13	13,13
D12	18,21	17,18,21	18,21			18	18,21
D19	14,15.2	13,14,15.2	14,15.2		15.2	14	14,15.2
FGA	20,23*	20,24					20,24
D22	11						11,11

Table H14. Fusion profiles generated from spent cartridge casings loaded by individual EE.

Locus	**63-2.45a**	**63-2.45b**	**63-2.45c**	**63-2.22a**	**63-2.22b**	**63-2.22c**	**EE**
Amel			X			X	X,Y
D3			16				16,18
D1							16.3,17.3
D2	11						14,15
D10	13						13,15
D13							10,12
Penta E							7,18
D16	11,13		9				9,13
D18		16	14				13,15
D2							20,25
CSF							10,12
Penta D							12,13
THO1	9.3	8	9				8,9.3
vWA					18		17,18
D21	30.2		31.2			32.2	29,31
D7							9,12
D5							11,13
TPOX							8,8
DYS391							11,11
D8	8	8	8	16		8	8,13
D12							22,23
D19			15				13,15
FGA							21,23
D22	11						15,16

Table H14 (cont'd)

Locus	63-1.45	63-3.45	63-4.45	63-1.22	63-3.22	63-4.22	EE
Amel	X	X,Y	X,Y	X		X,Y	X,Y
D3	14,16	16,17				16,17	16,18
D1	11	16.3				17.3	16.3,17.3
D2		11.3,15		11,14,15		15	14,15
D10							13,15
D13	12		13				10,12
Penta E							7,18
D16	12,13	11,14		12		9	9,13
D18	12,13,15,17					13	13,15
D2				17,20		18	20,25
CSF			11			10	10,12
Penta D							12,13
THO1	7,8	9,9.3	6,8,9.3			8	8,9.3
vWA		18					17,18
D21	28					29	29,31
D7		10					9,12
D5							11,13
TPOX							8,8
DYS391							11,11
D8	8,9,13		10,13,14	8,9,15,16	8,10,13,16	13	8,13
D12	19.3	20,21	15			22	22,23
D19		13		13,15	13,15		13,15
FGA	21.2	21		21			21,23
D22							15,16

Table H15. Fusion profiles generated from spent cartridge casings loaded by individual JJ.

Locus	65-2.45a	65-2.45b	65-2.45c	65-2.22a	65-2.22b	65-2.22c	JJ
Amel	X	X			X	X	X
D3	14,17	17				16	14,17
D1		12,16				16	12,16
D2				11		14	11,14
D10	13						14,14
D13							11,12
Penta E	18					11	10,11
D16	10,11	11		12	11	11,12,13	11,11
D18		14,19			14		14,19
D2	18,19						18,19
CSF		11			11		11,13
Penta D							11,14
THO1	5,11	8,9.3		9	9	9.3	9,9.3
vWA	16	11,14		13,14		18	11,15
D21	31	29			30.2		30.2,31.2
D7					9	10	9,10
D5		11					12,13
TPOX	8						8,8
DYS391						10	N/A
D8	13	13	13	13		13	13,13
D12	20	16,21		15,20,21	19	15	15,20
D19							11,13.2
FGA	20						22,24
D22		14					11,14

Table H15 (cont'd)

Locus	65-1.45	65-3.45	65-4.45	65-1.22	65-3.22	65-4.22	JJ
Amel	X	X	X		X	X	X,X
D3	14,17,18	14,17	14,16,17,18	18	16,17	17	14,17
D1		12,16	12	12		16	12,16
D2	11,14	11,14	11,14		14	14	11,14
D10	14	14	14		14	14	14,14
D13		11,12	11,12				11,12
Penta E		10,11	10,12				10,11
D16	11	11	11	11	12	11	11,11
D18	14,17	14,19	16,19	14	19		14,19
D2	19	18,19	18,19		18		18,19
CSF		11,13		13	10,11,13		11,13
Penta D		11,14					11,14
THO1	9,9.3	9,9.3	6,9,9.3	6,9	9,9.3	9,9.3	9,9.3
vWA	14,18	14,18	14,15,17,18	18	14,18	14	11,15
D21	30.2,31.2	30.2,31.2	28,30.2,31.2	30.2			30.2,31.2
D7	10	9,10					9,10
D5	11,12	11,12	11		11		12,13
TPOX		8					8,8
DYS391							N/A
D8	13	13	12,13,15	13	13	13	13,13
D12	15,20	15,20	15,18,20	20		21	15,20
D19	11,13.2	11,13.2	14,15				11,13.2
FGA		22,24					22,24
D22		11,14					11,14

Table H16. Fusion profiles generated from spent cartridge casings loaded by individual DDD.

Locus	66-1.45a	66-1.45b	66-1.45c	66-1.22a	66-1.22b	66-1.22c	DDD
Amel	X,Y	X,Y	X	X	X	X	X,Y
D3	14	14,18	18	18			14,18
D1	14,17.3	14					14,17.3
D2	11			11			11,11
D10		14					14,14
D13							10,12
Penta E				19			7,19
D16	†,11,12	7				12	11,12
D18	12	17	10				10,12
D2	20						17,20
CSF							11,12
Penta D		9					9,12
THO1	8	8	8				8,8
vWA	17	17	16,17	17,18		17	17,17
D21							29,32.2
D7							12,12
D5	10						13,13
TPOX							9,9
DYS391							11
D8	12,13	12,13	13	16	12,14		12,13
D12	15,23,24	15		24		24	15,24
D19	13*,14	14		14,15			14,15
FGA	22	22		24			22,24
D22							16,16

Table H16 (cont'd)

Locus	66-2.45	66-3.45	66-4.45	66-2.22	66-3.22	66-4.22	DDD
Amel	X,Y	X,Y	X		Y		X,Y
D3	14,16,17,18	14,18	14	14,18	14	14	14,18
D1	14	14,17.3		14	17.3		14,17.3
D2		11	11,11.3	11	11		11,11
D10		14		14			14,14
D13	10	10,12					10,12
Penta E	7,13	7,19		7			7,19
D16	11,12	11,12	11,12		11,12		11,12
D18	12	10,12	10	10	10		10,12
D2	20	17,20		20			17,20
CSF	11	11,12		12			11,12
Penta D		12					9,12
THO1	8,9	8	6,7,8,9.3	6,8,9.3	7		8,8
vWA	14,17	17	16,17	17	14		17,17
D21	28,32.2	29,32.2			29		29,32.2
D7	12	12					12,12
D5	13	13			13		13,13
TPOX	9	9	9				9,9
DYS391	11	11					11
D8	12,13	12,13	11,12,13,14, 15,16			13	12,13
D12		15,24	15,18,23		15,21	15	15,24
D19	13,15	14,15		14,15	13.2		14,15
FGA	24	22,24					22,24
D22	16	16		16			16,16

Table H17. Fusion profiles generated from spent cartridge casings loaded by individual VVV.

Locus	67-1.45a	67-1.45b	67-1.45c	67-1.22a	67-1.22b	67-1.22c	VVV
Amel			X		X	X,Y	X,Y
D3		14	14,15			15	14,17
D1				17.3			15,17.3
D2			11				11,14
D10							12,13
D13	12		11				11,11
Penta E							7,8
D16					12		12,12
D18	12			17			12,16
D2							17,18
CSF							11,11
Penta D							9,12
THO1			9.3				9.3,9.3
vWA							17,17
D21							28,32.2
D7							10,11
D5							11,13
TPOX							11,11
DYS391							11
D8	8		16				8,12
D12		18.3	15		15	15,18.3	15,25
D19		15					14,15.2
FGA				21.2			22,23
D22							11,15

Table H17 (cont'd)

Locus	67-2.45	67-3.45	67-4.45	67-2.22	67-3.22	67-4.22	VVV
Amel	Y		X	X			X,Y
D3		17	15,17	16	15		14,17
D1	17.3		16,16.3,17.3				15,17.3
D2			11				11,14
D10			13	14			12,13
D13			9,12				11,11
Penta E			12,21				7,8
D16		12	11,13	11,12			12,12
D18	17		14,16				12,16
D2			17,24	19			17,18
CSF			10				11,11
Penta D			9,11	9			9,12
THO1	9.3		7,9.3	9		9,9.3	9.3,9.3
vWA		17	14,16				17,17
D21			30,30.2	28			28,32.2
D7			12		10		10,11
D5			13				11,13
TPOX			8,11				11,11
DYS391							11
D8	11,16		14	10	9,11,12		8,12
D12	23		17,22	15,18,18.3		18	15,25
D19			13,15,15.2			14	14,15.2
FGA			22,23		23	21	22,23
D22							11,15

Table H18. Fusion profiles generated from spent cartridge casings loaded by individual XXX.

Locus	68-1.45a	68-1.45b	68-1.45c	68-1.22a	68-1.22b	68-1.22c	XXX
Amel	X	X		X		Y	X,Y
D3							14,16
D1		15.3		17.3			12,16
D2	11						11.3,14
D10		11				14	14,16
D13							9,12
Penta E				14			7,7
D16	13	†*,13	13	†,14			12,13
D18				25		18	14,16
D2				20			18,25
CSF							10,12
Penta D						10	9,11
THO1				13.3			7,9.3
vWA		23					18,18
D21	28.2		30				28,30
D7		5					10,10
D5			11,12				11,11
TPOX				8			8,8
DYS391							10
D8	14	15		9,15		10,16	14,15
D12				21			19,21
D19				14		15	14,15
FGA						23	23.2,25
D22	16						14,16

Table H18 (cont'd)

Locus	68-2.45	68-3.45	68-4.45	68-2.22	68-3.22	68-4.22	XXX
Amel	X	X	X				X,Y
D3	14,18*	18			15	18	14,16
D1		16	12,16,17.3		17	12,17	12,16
D2	11*	11				11	11.3,14
D10					14	16	14,16
D13					12		9,12
Penta E							7,7
D16	9,13	12	13		9,13		12,13
D18	16,20		14			12	14,16
D2	18		17		25	25	18,25
CSF		10					10,12
Penta D		13	11				9,11
THO1	6,7,9		7	9.3	6	7,9.3	7,9.3
vWA	18		18		15,16		18,18
D21				30.2	32.2		28,30
D7		10					10,10
D5		12					11,11
TPOX						8	8,8
DYS391							10
D8	11,12,13		13,14		13,14	10,16	14,15
D12					17,23		19,21
D19		15			14		14,15
FGA				22	25		23.2,25
D22					16		14,16

Table H19. Fusion profiles generated from spent cartridge casings loaded by individual R.

Locus	69-2.45a	69-2.45b	69-2.45c	69-2.22a	69-2.22b	69-2.22c	R
Amel	X	X			X	X	X,X
D3		16					16,17
D1	15	15				12	12,18.3
D2							11,11
D10							13,14
D13							12,12
Penta E							10,14
D16	9,11,16						12,14
D18					18		17,18
D2							20,23
CSF							10,13
Penta D							12,13
THO1	9	6			9	9.3	6,9
vWA		17			15		15,17
D21				29			30,30.2
D7	11						10,10
D5							10,11
TPOX							8,11
DYS391							N/A
D8	7,11		13,16	13	10		10,12
D12	19				20		18,21
D19	13					14	14,15.2
FGA							21,25
D22							15,15

Table H19 (cont'd)

Locus	69-1.45	69-3.45	69-4.45	69-1.22	69-3.22	69-4.22	R
Amel	X		X	X		X	X,X
D3		16	16				16,17
D1			12		17.3		12,18.3
D2		11		11.3			11,11
D10							13,14
D13							12,12
Penta E						14	10,14
D16							12,14
D18		17					17,18
D2							20,23
CSF						10	10,13
Penta D							12,13
THO1	6	6,9	6	9	8		6,9
vWA	18				17		15,17
D21							30,30.2
D7							10,10
D5				11			10,11
TPOX						11	8,11
DYS391							N/A
D8	10		12	10	10	10	10,12
D12					19		18,21
D19	14				15		14,15.2
FGA							21,25
D22							15,15

Table H20. Fusion profiles generated from spent cartridge casings loaded by individual OOO.

Locus	70-3.45a	70-3.45b	70-3.45c	70-3.22a	70-3.22b	70-3.22c	OOO
Amel		Y	X	X			X,Y
D3	14		14	14			14,14
D1							16.3,17.3
D2							11,11.3
D10			15	16			15,16
D13	12		12	10			10,12
Penta E							5,14
D16			12				11,12
D18							16,17
D2			20				20,22
CSF							10,11
Penta D							11,12
THO1	9		9,9.3,11	9	9.3		9,9.3
vWA	16				20	12	16,18
D21	32.2						28,32.2
D7	11		12				11,12
D5							12,12
TPOX							8,8
DYS391							11
D8			12,13	9			9,12
D12			21				21,23
D19							12,14
FGA		22		22			21.2,22
D22							11,16

Table H20 (cont'd)

Locus	70-1.45	70-2.45	70-4.45	70-1.22	70-2.22	70-4.22	OOO
Amel	X,Y	Y	X,Y	X	Y	X	X,Y
D3		14,16	14,17			14	14,14
D1	11,16.3	16.3,17.3	16.3,17.3				16.3,17.3
D2	10	11,11.3	11,11.3				11,11.3
D10	16		16				15,16
D13	10	10					10,12
Penta E	5,14						5,14
D16	9,11	11,12,13	11,12			11,12	11,12
D18	16	16,17	17			16	16,17
D2		20	20		22		20,22
CSF		10,11	11				10,11
Penta D					12		11,12
THO1	9.3	9,9.3	9,9.3		9	9.3	9,9.3
vWA	16,18	16,18	15,16,18		16	18	16,18
D21		28,32.2	32.2				28,32.2
D7	12	12					11,12
D5		12	12				12,12
TPOX			8				8,8
DYS391		11	11				11
D8	12	9,12	9,12	9	12	12,13,16	9,12
D12	23	23	21,22,23	21		21,23	21,23
D19	12,15		12		12,13		12,14
FGA	21.2	21.2	21.2,22		22		21.2,22
D22	16						11,16

APPENDIX I. HANDLER AND NON-HANDLER ALLELES AMPLIFIED WITH FUSION FROM COLLECTION 2

Table I1. Summary of number of handler (H) alleles, non-handler (NH) alleles, and percent profile produced using Fusion from the individually swabbed 0.45 casings from Collection 2.

Sample	# H Alleles	# NH Alleles	% Profile
62-3.45b	21	1	51.2
60-4.45c	25	2	61.0
62-3.45c	14	2	34.1
53-4.45a	21	2	52.5
67-1.45b	1	2	2.4
58-3.45b	5	17	12.8
52-3.45b	28	2	63.6
53-4.45b	19	0	47.5
58-3.45a	8	1	20.5
39-4.45c	13	0	32.5
65-2.45b	11	7	26.8
70-3.45c	11	2	25.0
58-3.45c	3	5	7.7
63-2.45a	4	4	8.7
53-4.45c	9	0	22.5
63-2.45c	6	2	13.0
63-2.45b	2	1	4.3
66-1.45a	18	4	46.2
67-1.45a	2	1	4.9
60-4.45b	10	0	24.4
52-3.45a	12	3	27.3
66-1.45b	15	1	38.5
51-2.45c	21	1	51.2
59-2.45a	4	7	9.1
61-4.45c	6	1	15.8
60-4.45a	18	3	43.9
39-4.45b	15	2	37.5
65-2.45c	1	0	2.4
52-3.45c	12	1	27.3
59-2.45b	9	3	20.5
70-3.45b	2	0	4.5
61-4.45b	5	11	13.2
65-2.45a	9	8	22.0
39-4.45a	11	1	27.5

Table I1 (cont'd)

70-3.45a	6	0	13.6
67-1.45c	6	2	14.6
57-1.45a	3	6	7.0
55-1.45c	2	1	4.8
51-2.45b	11	4	26.8
62-3.45a	6	1	14.6
59-2.45c	10	1	22.7
68-1.45b	3	5	7.1
57-1.45b	1	4	2.3
69-2.45a	2	9	4.9
56-4.45a	3	0	6.8
54-3.45a	17	1	44.7
55-1.45b	5	0	11.9
55-1.45a	1	0	2.4
51-2.45a	15	3	36.6
66-1.45c	6	1	15.4
54-3.45c	7	3	18.4
68-1.45c	3	1	7.1
61-4.45a	6	7	15.8
54-3.45b	9	2	23.7
69-2.45b	4	1	9.8
57-1.45c	1	0	2.3
68-1.45a	4	2	9.5
69-2.45c	0	2	0.0
56-4.45b	2	0	4.5
56-4.45c	2	2	4.5

Table I2. Summary of number of handler (H) alleles, non-handler (NH) alleles, and percent profile produced using Fusion from the individually swabbed 0.22 casings from Collection 2.

Sample	# H Alleles	# NH Alleles	% Profile
60-4.22c	14	1	34.1
60-4.22b	16	1	39.0
62-3.22b	11	1	26.8
63-2.22b	1	0	2.2
63-2.22c	2	1	4.3
62-3.22a	11	10	26.8
55-1.22b	0	0	0.0
70-3.22a	7	0	15.9
67-1.22a	1	2	2.4
70-3.22c	0	1	0.0
39-4.22a	13	1	32.5
63-2.22a	0	1	0.0
53-4.22a	4	1	10.0
55-1.22a	4	3	9.5
70-3.22b	1	1	2.3
59-2.22a	18	4	40.9
56-4.22c	2	1	4.5
67-1.22b	3	0	7.3
56-4.22a	1	0	2.3
59-2.22b	10	5	22.7
67-1.22c	3	2	7.3
65-2.22a	5	4	12.2
60-4.22b	14	1	34.1
56-4.22b	1	0	2.3
53-4.22b	0	1	0.0
54-3.22b	6	2	15.8
52-3.22b	11	2	25.0
54-3.22a	7	1	18.4
59-2.22c	16	0	36.4
39-4.22b	8	4	20.0
62-3.22c	9	3	22.0
58-3.22c	2	0	5.1
65-2.22c	9	5	22.0
54-3.22c	6	2	15.8
65-2.22b	7	1	17.1
53-4.22c	1	0	2.5
52-3.22c	5	0	11.4

Table I2 (cont'd)

55-1.22c	0	1	0.0
68-1.22c	3	5	7.1
66-1.22a	9	2	23.1
69-2.22b	5	1	12.2
51-2.22c	11	1	26.8
68-1.22a	5	8	11.9
51-2.22a	5	1	12.2
58-3.22a	5	1	12.8
51-2.22b	7	0	17.1
57-1.22b	3	3	7.0
69-2.22c	3	1	7.3
66-1.22b	2	1	5.1
69-2.22a	0	2	0.0
61-4.22a	3	3	7.9
57-1.22a	2	0	4.7
58-3.22b	3	2	7.7
52-3.22a	2	0	4.5
66-1.22c	4	0	10.3
57-1.22c	1	2	2.3
61-4.22c	4	1	10.5
68-1.22b	0	0	0.0
61-4.22b	2	1	5.3

Table I3. Summary of number of handler (H) alleles, non-handler (NH) alleles, and percent profile produced using Fusion from the cumulatively swabbed 0.45 casings from Collection 2.

Sample	# H Alleles	# NH Alleles	% Profile
52-1.45	24	20	54.5
54-1.45	35	2	92.1
53-3.45	36	0	90.0
53-1.45	27	9	67.5
66-3.45	38	0	97.4
67-4.45	14	25	34.1
57-2.45	43	1	100.0
65-3.45	39	2	95.1
65-4.45	22	15	53.7
62-2.45	34	4	82.9
65-1.45	20	5	48.8
54-2.45	20	2	52.6
62-4.45	22	7	53.7
52-4.45	32	0	72.7
60-3.45	35	11	85.4
62-1.45	29	1	70.7
70-4.45	28	3	63.6
53-2.45	30	1	75.0
54-4.45	26	7	68.4
39-2.45	29	12	72.5
52-2.45	24	1	54.5
66-2.45	24	7	61.5
56-3.45	10	9	22.7
68-2.45	7	9	16.7
39-3.45	28	20	70.0
66-4.45	12	11	30.8
70-2.45	27	2	61.4
55-3.45	30	11	71.4
58-2.45	19	9	48.7
70-1.45	18	4	40.9
58-4.45	14	8	35.9
58-1.45	12	19	30.8
61-2.45	11	3	28.9
39-1.45	24	5	60.0
55-4.45	17	8	40.5
61-1.45	10	9	26.3

Table I3 (cont'd)

60-1.45	23	4	56.1
56-2.45	4	12	9.1
60-2.45	22	8	53.7
63-4.45	5	6	10.9
56-1.45	17	2	41.5
63-3.45	9	8	19.6
61-3.45	14	2	36.8
69-4.45	5	0	12.2
67-3.45	3	0	7.3
63-1.45	9	10	19.6
59-1.45	22	12	50.0
55-2.45	10	7	23.8
51-4.45	15	2	36.6
57-4.45	15	3	34.9
69-3.45	5	0	12.2
51-3.45	17	5	41.5
59-4.45	13	3	29.5
67-2.45	3	4	7.3
68-3.45	6	4	14.3
69-1.45	4	1	9.8
68-4.45	9	3	21.4
59-3.45	12	3	27.3
57-3.45	6	2	14.0
51-1.45	11	6	26.8

Table I4. Summary of number of handler (H) alleles, non-handler (NH) alleles, and percent profile produced using Fusion from the cumulatively swabbed 0.22 casings from Collection 2.

Sample	# H Alleles	# NH Alleles	% Profile
59-3.22	41	2	93.2
60-1.22	41	0	100.0
60-2.22	31	1	75.6
59-4.22	30	3	68.2
60-3.22	26	5	63.4
54-1.22	11	16	28.9
65-3.22	11	6	26.8
68-3.22	8	10	19.0
62-1.22	4	0	9.8
39-1.22	21	7	52.5
54-2.22	22	3	57.9
39-3.22	18	5	45.0
39-2.22	24	8	60.0
65-4.22	9	2	22.0
56-1.22	5	10	11.4
54-4.22	17	4	44.7
56-2.22	3	5	6.8
63-4.22	12	2	26.1
63-1.22	8	6	17.4
67-2.22	5	8	12.2
63-3.22	4	2	8.7
52-2.22	20	1	45.5
52-1.22	20	2	45.5
51-3.22	9	2	22.0
66-3.22	10	4	25.6
53-3.22	6	4	15.0
62-2.22	7	1	17.1
53-1.22	6	6	15.0
56-3.22	9	3	20.5
61-3.22	3	2	7.9
66-2.22	14	2	35.9
58-1.22	7	10	17.9
62-4.22	15	1	36.6
51-4.22	13	5	31.7
53-2.22	9	2	22.5
61-2.22	6	0	15.8

Table I4 (cont'd)

69-4.22	5	0	12.2
68-4.22	7	5	17.1
55-2.22	9	13	21.4
67-3.22	3	3	7.3
61-1.22	4	2	10.5
70-2.22	8	1	18.2
70-4.22	10	2	22.7
51-1.22	10	1	24.4
67-4.22	3	2	7.3
55-3.22	6	3	14.3
65-1.22	8	3	19.5
69-3.22	2	4	4.9
52-4.22	19	1	43.2
58-2.22	9	1	23.1
69-1.22	4	1	9.8
70-1.22	3	0	6.8
58-4.22	15	19	38.5
57-4.22	2	6	4.7
68-2.22	1	2	2.4
57-3.22	4	3	9.3
66-4.22	3	0	7.7
55-4.22	7	4	16.7
59-1.22	18	2	40.9
57-2.22	3	2	7.0

APPENDIX J. MTDNA PROFILES GENERATED FROM SPENT CARTRIDGE CASINGS

Red font: polymorphism not consistent with handler

Blank: no polymorphisms

A: adenine

T: thymine

C: cytosine

G: guanine

Y: mixture between cytosine and thymine

R: mixture between adenine and guanine

M: mixture between adenine and cytosine

S: mixture between cytosine and guanine

Table J1. MtDNA profiles generated from spent cartridge casings loaded by individual NN.

Sample	HV1	HV2	mtDNA Classification	Quantification Level
NN	16293G,16311T	195C, 263G, 309.1C, 315.1C	------	------
51-1.45	16293G, 16311T	*(73 not sequenced)* 195C, 263G, 309.1C, 315.1C	Consistent	Low
51-4.22	16069T, 16126C, 16160G	73G, 185A, 263G, 295T, 315.1C, 462T	Inconsistent	Medium

Table J2. MtDNA profiles generated from spent cartridge casings loaded by individual ZZZ.

Sample	HV1	HV2	mtDNA Classification	Quantification Level
ZZZ	16126C, 16294T, 16296T, 16304C	73G, 263G, 315.1C	------	------
52-1.45	16256T	204C, 263G, 315.1C	Inconsistent	High
52-3.22a	16126C, 16294T, 16296T, 16304C	73G, 263G, 315.1C	Consistent	Low
52-3.22b	16185T, 16223T, 16355A, 16362C	73G, 263G, 315.1C	Inconsistent	Medium
52-3.45b	16126C, 16294T, 16296T, 16304C	73G, 263G, 315.1C	Consistent	High
52-3.45c	16126C, 16294T, 16296T, 16304C	73R, 263G *(315.1 not sequenced)*	Mixed-Consistent	Medium

Table J3. MtDNA profiles generated from spent cartridge casings loaded by individual B.

Sample	HV1	HV2	mtDNA Classification	Quantification Level
B	16069T, 16126C, 16160G, 16222T	73G, 185A, 263G, 295T, 315.1C, 462T	------	------
53-1.22	16069Y, 16126Y, 16160R, 16222Y	73G, 185A, 263G, 295T, 315.1C, 462T	Mixed-Consistent	Medium
53-1.45	16069Y, 16126C, 16160R, 16222Y	73G, 185A, 263G, 295T, 315.1C, 462T	Mixed-Consistent	High
53-3.45	16069T, 16126C, 16160G, 16222T	73G, 185A, 263G, 295T, 315.1C, 462T	Consistent	High
53-4.45a	16069T, 16126C, 16160G, 16222T	73G, 185A, 263G, 295T, 315.1C, 462T	Consistent	High
53-4.45b	16069T, 16126C, 16160G, 16222T	73G, 185A, 263G, 295T, 315.1C *(462 not sequenced)*	Consistent	High

Table J4. MtDNA profiles generated from spent cartridge casings loaded by individual BBB.

Sample	HV1	HV2	mtDNA Classification	Quantification Level
BBB		66T, 152C, 263G, 315.1C	------	------
54-1.22		263G, 315.1C	Inconsistent	High
54-1.45		66T, 152C, 263G, 315.1C	Consistent	High
54-3.22a		66T, 152C, 263G, 315.1C	Consistent	Medium
54-3.22c		66T, 152C, 263G, 315.1C	Consistent	Medium
54-3.45b		66T, 152C, 263G, 315.1C	Consistent	Low

Table J5. MtDNA profiles generated from spent cartridge casings loaded by individual C.

Sample	HV1	HV2	mtDNA Classification	Quantification Level
C	16192T, 16256T, 16270T	73G, 263G, 315.1C	------	------
55-1.22b		263G, 315.1C	Inconsistent	High
55-3.45	16192T, 16256T, 16270T	*(73 not sequenced)* 263G, 315.1C	Consistent	Medium
55-4.22		263G, 315.1C	Inconsistent	Low

Table J6. MtDNA profiles generated from spent cartridge casings loaded by individual AA.

Sample	HV1	HV2	mtDNA Classification	Quantification Level
AA	16104T, 16126C, 16294T, 16304C	73G, 152C, 263G, 315.1C	------	------
56-3.22	16104Y, 16126Y, 16294Y, 16304Y	73G, 152Y, 263G, 315.1C	Mixed-Consistent	Medium
56-4.45b	16069Y, 16126Y, 16160R, 16222Y	73G, 152C, 263G, 315.1C	Mixed-Inconsistent	Low
56-4.45c	16104T, 16126C, 16294T, 16304C	73G, 152C, 263G, 315.1C	Consistent	Low

Table J7. MtDNA profiles generated from spent cartridge casings loaded by individual A.

Sample	HV1	HV2	mtDNA Classification	Quantification Level
A	16051G, 16129C, 16183C, 16189C	73G, 152C, 217C, 263G, 315.1C	------	------
57-1.22a	16051G, 16162G	263G, 315.1C	Inconsistent	Low
57-1.22c	16093C, 16189C	73G, 263G *(315 not sequenced)*	Inconsistent	Low
57-1.45c	16051R, 16126Y, 16129S, 16183M, 16189Y, 16294Y, 16296Y	73G, 152Y, 217Y, 263G, 315.1C	Mixed-Consistent	Low
57-2.22	16051R, 16126Y, 16129S, 16183M, 16189C, 16294Y, 16296Y	73G, 152C, 217Y, 263G, 315.1C	Mixed-Consistent	Low
57-2.45	16051G, 16129C, 16183C, 16189C	73G, 152C, 217C, 263G, 315.1C	Consistent	High
57-3.22	16051R, 16126Y, 16129S, 16183M, 16189Y, 16294Y, 16296Y	73G, 185R, 263G, 295Y, 315.1C	Mixed-Consistent	Low
57-3.45	16051G, 16126Y, 16129S, 16183C, 16189Y	73G, 152C, 263G, 315.1C	Mixed-Consistent	Low
57-4.22	16051R, 16126Y, 16129S, 16183M, 16189Y, 16294Y, 16296Y	73G, 152Y, 217Y, 263G, 315.1C	Mixed-Consistent	Low

Table J8. MtDNA profiles generated from spent cartridge casings loaded by individual J.

Sample	HV1	HV2	mtDNA Classification	Quantification Level
J		263G, 309.2C, 315.1C	------	------
58-1.22		263G *(309, 315 not sequenced)*	Consistent	Medium
58-1.45	16069Y, 16126Y, 16160R, 16222Y	73R, 185R, 263G *(309, 315 not sequenced)*	Mixed-Consistent	Medium
58-2.45	16126Y, 16222Y	263G, 309.2C, 315.1C	Mixed-Consistent	Medium
58-3.22b		263G, 309.2C, 315.1C	Consistent	Low
58-3.22c		*not sequenced*	Consistent	Medium
58-3.45b		263G *(309, 315 not sequenced)*	Consistent	High
58-4.22		73G, 185A, 263G, 295T, 315.1C, 462T	Inconsistent	Low
58-4.45		73R, 152Y, 263G *(309, 315 not sequenced)*	Mixed-Consistent	Medium

Table J9. MtDNA profiles generated from spent cartridge casings loaded by individual KKK.

Sample	HV1	HV2	mtDNA Classification	Quantification Level
KKK	16311C	93G, 263G, 315.1C	------	------
59-1.22	16311C	93R, 263G, 315.1C	Mixed-Consistent	Low
59-2.22c	16311C	93G, 263G, 315.1C	Consistent	Medium
59-2.45b	16311C	73R, 93R, 263G, 315.1C	Mixed-Consistent	Medium
59-3.22	16311C	93G, 263G, 315.1C	Consistent	High
59-3.45	16311C	73R, 93R, 263G, 295Y, 315.1C	Mixed-Consistent	Low
59-4.22	16311C	93G, 263G, 315.1C	Consistent	High
59-4.45	16311C	73R, 93R, 263G, 315.1C	Mixed-Consistent	Low

Table J10. MtDNA profiles generated from spent cartridge casings loaded by individual JJJ.

Sample	HV1	HV2	mtDNA Classification	Quantification Level
JJJ	16126C, 16294T, 16296T	73G, 263G, 315.1C	------	------
60-1.22	16126C, 16294T, 16296T	73G, 263G, 315.1C	Consistent	High
60-2.22	16126C, 16294T, 16296T	73G, 263G, 315.1C	Consistent	High
60-3.22	16126C, 16294T, 16296T	73G, 263G, 315.1C	Consistent	High
60-4.22b	16126C, 16294T, 16296T	73G, 263G, 315.1C	Consistent	High
60-4.22c	16126C, 16294T, 16296T	73G, 263G, 315.1C	Consistent	High
60-4.45c	16126C, 16294T, 16296T	73G, 263G, 315.1C	Consistent	High

Table J11. MtDNA profiles generated from spent cartridge casings loaded by individual I.

Sample	HV1	HV2	mtDNA Classification	Quantification Level
I	16192T, 16256T, 16270T, 16291T	73G, 263G, 315.1C	------	------
61-2.45	16192T, 16256T, 16270T, 16291T	73G, 263G, 315.1C	Consistent	Medium
61-3.22	16192T, 16246T, 16270T, 16291T	73G, 263G, 315.1C	Consistent	Medium
61-4.22c	16192T, 16256T, 16270T, 16291T	73G, 263G, 315.1C	Consistent	Low
61-4.45a	16192T, 16256T, 16270T, 16291T	73G, 263G, 315.1C	Consistent	Low
61-4.45b	16104Y, 16126Y, 16192Y, 16256Y, 16270Y, 16291Y, 16294Y, 16304Y	73G, 263G, 315.1C	Mixed-Consistent	Medium

Table J12. MtDNA profiles generated from spent cartridge casings loaded by individual YYY.

Sample	HV1	HV2	mtDNA Classification	Quantification Level
YYY	16126C, 16294T, 16296T, 16304C	73G, 263G, 309.1C, 315.1C, 458T	------	------
62-2.22	16069Y, 16126Y, 16160R, 16162R, 16222Y, 16294T, 16296T, 16304C	73G, 263G, 309.1C, 315.1C, 458T	Mixed-Consistent	Medium
62-3.22a	16093C, 16104T, 16126C, 16294T	73G, 152C, 263G, 315.1C	Inconsistent	High
62-3.22b	16126C, 16294T, 16296T, 16304C	73G, 263G, 309.1C, 315.1C, 458T	Consistent	High
62-3.22c	16126C, 16294T, 16296T, 16304C	73G, 263G, 309.1C, 315.1C, 458T	Consistent	Medium
62-3.45b	16126C, 16294T, 16296T, 16304C	73G, 263G, 309.1C, 315.1C, 458T	Consistent	High
62-3.45c	16051R, 16126Y, 16126R, 16294T, 16296T, 16304C	73G, 263G, 309.1C, 315.1C, 458T	Mixed-Consistent	High
62-4.22	16093C, 16192T, 16294T, 16296T, 16304C	73G, 263G, 315.1C	Inconsistent	Medium

Table J13. MtDNA profiles generated from spent cartridge casings loaded by individual EE.

Sample	HV1	HV2	mtDNA Classification	Quantification Level
EE	16126C, 16189C, 16294T, 16296T, 16298C	73G, 195C, 263G, 315.1C	------	------
63-2.22b	16126Y, 16189Y, 16294Y, 16296Y, 16304Y	73R, 195Y, 263G *(315 not sequenced)*	Mixed-Consistent	High
63-2.22c	16126Y, 16294Y, 16296Y	73R, 263G, 315.1C	Mixed-Inconsistent	High

Table J14. MtDNA profiles generated from spent cartridge casings loaded by individual JJ.

Sample	HV1	HV2	mtDNA Classification	Quantification Level
JJ	16147T	263G, 309.2C, 315.1C	------	------
65-2.22c	16147T	263G *(309, 315 not sequenced)*	Consistent	Medium
65-2.45a	16126Y, 16222Y	73G, 242Y, 263G, 295Y, 315.1C	Mixed-Inconsistent	Medium
65-2.45c	16147T	263G, 309.2C, 315.1C	Consistent	Medium
65-3.22	16126Y, 16147Y, 16294Y	73R, 263G, 309.1Y, 315.1C	Mixed-Consistent	High
65-3.45	16147T	263G, 309.2C, 315.1C	Consistent	High

Table J15. MtDNA profiles generated from spent cartridge casings loaded by individual DDD.

Sample	HV1	HV2	mtDNA Classification	Quantification Level
DDD	16051G, 16162G	73G, 263G, 315.1C	------	------
66-1.22c	16051G, 16162G	73G, 263G, 315.1C	Consistent	Low
66-2.22	16051G, 16162G	73G, 263G, 315.1C	Consistent	Medium
66-3.45	16051G, 16162G,	73G, 263G, 315.1C	Consistent	High
66-4.22	16051G, 16162G	73R, 263G, 315.1C	Mixed-Consistent	Low

Table J16. MtDNA profiles generated from spent cartridge casings loaded by individual VVV.

Sample	HV1	HV2	mtDNA Classification	Quantification Level
VVV	16189C	73G, 150T, 263G, 315.1C	------	------
67-1.45b	16189C	73G, 150T, 263G, 315.1C	Consistent	High
67-2.45	16189Y	73G, 150Y, 242Y, 263G, 295Y, 315.1C	Mixed-Consistent	Low
67-4.45	16179T, 16242T	73G, 150T, 195Y, 263G, 315.1C	Inconsistent	High

Table J17. MtDNA profiles generated from spent cartridge casings loaded by individual XXX.

Sample	HV1	HV2	mtDNA Classification	Quantification Level
XXX	16093C, 16189C	263G, 315.1C	------	------
68-1.45a	16093C, 16189C	263G, 315.1C	Consistent	Low
68-1.22b	16051G, 16129C, 16183C, 16189C,	73G, 152C, 217C, 263G, 315.1C	Inconsistent	Low
68-2.22	16093T/Y, 16189T/Y	263G, 315.1C	Mixed-Consistent	Low
68-3.22		263G, 315.1C	Inconsistent	High
68-3.45	16069T, 16126C, 16160G, 16222T	73G, 185A, 263G, 295T, 315.1C, 462T	Inconsistent	Low
68-4.45	16093Y, 16189Y	263G, 315.1C	Mixed-Consistent	Low

Table J18. MtDNA profiles generated from spent cartridge casings loaded by individual R.

Sample	HV1	HV2	mtDNA Classification	Quantification Level
R	16093C, 16192T, 16256T, 16270T, 16291T	73G, 263G, 315.1C	------	------
69-1.45	16104T, 16126C, 16294T, 16304C	73G, 152C, 263G, 315.1C	Inconsistent	Low
69-2.45b	16093C, 16192T, 16256T, 16270T, 16291T	73G, 263G, 315.1C	Consistent	Low
69-2.45c	16256Y	73G, 152C, 195C, 263G, 309.1C, 315.1C	Inconsistent	Low

Table J19. MtDNA profiles generated from spent cartridge casings loaded by individual OOO.

Sample	HV1	HV2	mtDNA Classification	Quantification Level
OOO		263G, 309.1C, 315.1C	------	------
70-1.45		263G, 309.1C, 315.1C	Consistent	Medium
70-2.45		263G, 309.1C, 315.1C	Consistent	Medium
70-3.22a		263G, 309.1C, 315.1C	Consistent	High
70-3.45b	16051G, 16129C, 16183C, 16189C	73G, 152C, 217C, 263G, 315.1C	Inconsistent	Medium

APPENDIX K. COMPARISON OF HANDLER AND NON-HANDLER ALLELES AMPLIFIED WITH FUSION AND MTDNA PROFILE CLASSIFICATIONS

Table K1. Number of alleles both consistent with (H) and not consistent with (NH) the handler and the corresponding mtDNA profile result.

Sample	Quantitation Level	mtDNA Result	#H Alleles	#NH Alleles
52-1.45	High	Inconsistent	24	20
54-1.45	High	Consistent	35	4
53-3.45	High	Consistent	36	2
53-1.45	High	Mixed-Consistent	27	9
66-3.45	High	Consistent	38	0
67-4.45	High	Inconsistent	14	27
57-2.45	High	Consistent	43	1
65-3.45	High	Consistent	39	2
59-3.22	High	Consistent	41	4
60-1.22	High	Consistent	41	0
60-2.22	High	Consistent	31	2
59-4.22	High	Consistent	30	4
60-3.22	High	Consistent	26	5
54-1.22	High	Inconsistent	11	17
65-3.22	High	Mixed-Consistent	11	8
68-3.22	High	Inconsistent	8	11
62-3.45b	High	Consistent	21	3
60-4.45c	High	Consistent	25	4
62-3.45c	High	Mixed-Consistent	13	3
53-4.45a	High	Consistent	21	3
67-1.45b	High	Consistent	1	3
58-3.45b	High	Consistent	5	17
52-3.45b	High	Consistent	28	2
53-4.45b	High	Consistent	20	4
60-4.22c	High	Consistent	14	3
60-4.22b	High	Consistent	17	1
62-3.22b	High	Consistent	11	2
63-2.22b	High	Mixed-Consistent	1	3
63-2.22c	High	Mixed-Inconsistent	2	3
62-3.22a	High	Inconsistent	11	10
55-1.22b	High	Inconsistent	2	4
70-3.22a	High	Consistent	7	1
70-2.45	Medium	Consistent	27	2

Table K1 (cont'd)

55-3.45	Medium	Consistent	30	14
58-2.45	Medium	Mixed-Consistent	19	9
70-1.45	Medium	Consistent	18	4
58-4.45	Medium	Mixed-Consistent	14	10
58-1.45	Medium	Mixed-Consistent	12	19
61-2.45	Medium	Consistent	11	5
39-1.45	Medium	Consistent	24	5
62-2.22	Medium	Mixed-Consistent	7	1
53-1.22	Medium	Mixed-Consistent	6	7
56-3.22	Medium	Mixed-Consistent	9	3
61-3.22	Medium	Consistent	3	3
66-2.22	Medium	Consistent	14	2
58-1.22	Medium	Consistent	7	10
62-4.22	Medium	Inconsistent	15	2
51-4.22	Medium	Inconsistent	13	6
39-4.45b	Medium	Consistent	15	5
65-2.45c	Medium	Consistent	0	4
52-3.45c	Medium	Mixed-Consistent	12	3
59-2.45b	Medium	Mixed-Consistent	9	3
70-3.45b	Medium	Inconsistent	2	1
61-4.45b	Medium	Mixed-Consistent	5	12
65-2.45a	Medium	Mixed-Inconsistent	9	9
39-4.45a	Medium	Consistent	11	2
52-3.22b	Medium	Inconsistent	11	3
54-3.22a	Medium	Consistent	7	2
59-2.22c	Medium	Consistent	16	0
39-4.22b	Medium	Consistent	8	4
62-3.22c	Medium	Consistent	9	4
58-3.22c	Medium	Consistent	2	3
65-2.22c	Medium	Consistent	9	5
54-3.22c	Medium	Consistent	6	5
59-4.45	Low	Mixed-Consistent	12	3
67-2.45	Low	Mixed-Consistent	3	5
68-3.45	Low	Inconsistent	6	7
69-1.45	Low	Inconsistent	4	3
68-4.45	Low	Mixed-Consistent	9	5
59-3.45	Low	Mixed-Consistent	12	6
57-3.45	Low	Mixed-Consistent	6	2
51-1.45	Low	Consistent	11	7

Table K1 (cont'd)

58-4.22	Low	Inconsistent	15	19
57-4.22	Low	Mixed-Consistent	2	8
68-2.22	Low	Mixed-Consistent	1	2
57-3.22	Low	Mixed-Consistent	4	4
66-4.22	Low	Mixed-Consistent	3	4
55-4.22	Low	Inconsistent	7	4
59-1.22	Low	Mixed-Consistent	18	4
57-2.22	Low	Mixed-Consistent	3	3
61-4.45a	Low	Consistent	6	11
54-3.45b	Low	Consistent	9	7
69-2.45b	Low	Mixed-Consistent	4	2
57-1.45c	Low	Consistent	1	6
68-1.45a	Low	Consistent	4	5
69-2.45c	Low	Inconsistent	0	5
56-4.45b	Low	Mixed-Inconsistent	2	2
56-4.45c	Low	Consistent	2	5
57-1.22a	Low	Inconsistent	2	3
58-3.22b	Low	Consistent	3	2
52-3.22a	Low	Consistent	2	3
66-1.22c	Low	Consistent	4	2
57-1.22c	Low	Inconsistent	1	8
61-4.22c	Low	Consistent	4	2
68-1.22b	Low	Inconsistent	0	2
61-4.22b	Low	Consistent	2	5

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REFERENCES

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