This is to certify that the
thesis entitled

INVESTIGATIVE STUDIES INTO THE RECOVERY OF DNA FROM IMPROVISED EXPLOSIVE DEVICE CONTAINERS

presented by

Shane Gregory Phillip Hoffmann

has been accepted towards fulfillment
of the requirements for the

M.S. degree in Forensic Science

Major Professor's Signature

6/30/08

Date

INVESTIGATIVE STUDIES INTO THE RECOVERY OF DNA FROM IMPROVISED EXPLOSIVE DEVICE CONTAINERS

By

Shane Gregory Phillip Hoffmann

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Forensic Science

2008

ABSTRACT

INVESTIGATIVE STUDIES INTO THE RECOVERY OF DNA FROM IMPROVISED EXPLOSIVE DEVICE CONTAINERS

By

Shane Gregory Phillip Hoffmann

Combating improvised explosive devices (IEDs) and apprehending those responsible have become national priorities due to their use in the Middle East and the threat they pose domestically. IEDs are often concealed in containers (e.g., a backpack, box, or briefcase), as was demonstrated in the Centennial Olympic Park and Madrid train bombings. The goal of this research was to identify the person(s) responsible for an IED through post-blast DNA recovery from IED containers. Eight study participants were asked to use backpacks in everyday activities for eleven days, after which they served as containers for pipe bombs. Regions likely to be handled by the study participants were swabbed, and DNA recovered was amplified and typed using miniSTRs. Handler profiles were called blindly using data from all swabs. Profiles compiled for seven of eight backpacks matched the handler's at all nine loci, with DNA recovered from all swabs producing at least the handler's partial profile. Overall, higher yields of DNA and more loci with handler alleles were obtained from the straps, top handle, neck region, and front middle. Recovering DNA from IED containers is a practical approach that that can easily be implemented in IED investigations and vastly improves upon the discriminatory power achieved in previous studies that concentrated on collecting DNA from IEDs themselves.

ACKNOWLEDGEMENTS

My sincere appreciation is extended to my committee members for the time they have devoted to assisting me throughout the research and writing process. To my advisor, Dr. David Foran, I would like to thank you for three semesters of financial assistance and for the continuous support of my research. Also, thank you for the valuable insight you contributed to the project and your many hours of revisions. My gratitude is extended to First Lt. Shawn Stallworth, who, along with other members of the bomb squad, put their lives in harms way to make this research possible. Finally, I would like to thank Dr. Sheila Maxwell for volunteering to serve on the committee of a student she had just recently met.

My thanks extend to my peers who directly assisted in the project. To Ms. Stefanie Kremer, whose patience and guidance was instrumental in familiarizing me with the lab and my research. To Ms. Kamila Gomez, for her assistance in collecting and separating post-blast fragments. Also, to the study participants who graciously volunteered to carry around the backpacks for a week and a half.

I would further like to thank my classmates, friends, and teachers who have all contributed to making my experience at Michigan State one that I will hold fond memories of. I came here for an education, but am leaving with so much more.

Finally, my gratitude is extended to my family for all the years of unwavering support and always believing in my abilities despite some difficult times.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Images in this thesis are presented in color.

INTRODUCTION

Improvised explosive devices (IEDs) have gained publicity due to their use in ongoing conflicts in the Middle East as they have become the weapon of choice for terrorists. As of November 2007, IEDs have been responsible for 70% of American combat casualties in Iraq and 50% of combat casualties in Afghanistan (Wilson 2007). Their effectiveness in the Middle East has caused concern over their implementation world-wide, especially in the United States. In October 2007, Department of Homeland Security (DHS) Secretary Michael Chertoff addressed the Center for Strategic and International Studies regarding IEDs. He emphasized the Department's focus by saying, "Our office is 100 percent committed to protecting the people of the United States from IEDs. All of our counterterrorism efforts focus directly or indirectly on bombing prevention..." (Chertoff 2007). This concern is well justified as the USA has shown vulnerability to attacks by IEDs in the past.

An IED is "an explosive device that is placed or fabricated in an improvised manner; incorporates destructive, lethal, noxious, pyrotechnic, or incendiary chemicals; and is designed to destroy, incapacitate, harass, or distract" (National Research Council 2007). Counterterrorism consultant David Williams defined IEDs based on their components and stated that an IED is "any collection of components that are compiled in conjunction with a power source, an initiator, and an energetic material used as a main charge" (personal communication). The definitions are rather broad, but they are reflective of the fact that IEDs can take any form and are only limited by the ingenuity of the developer.

Besides the flexibility in design that IEDs allow, they have become a popular weapon for a variety of other reasons. The Counterterrorist Handbook (Bolz et al. 2005) lists the following reasons as to why IEDs are so appealing:

1. The media flocks to events involving IEDs, drawing attention to the administering group and their cause.
2. The actual bombing can be accomplished by a limited number of people.
3. There is minimal risk of the bomber being detected.
4. IEDs are inexpensive and cost effective compared to other tactics (kidnapping, etc.).
5. Random bombings have a considerable psychological impact on the population.
6. Materials are often readily available and IEDs can be constructed through the use of legitimately purchased products.
7. IED components can be brought to the target and assembled on-site.
8. IEDs can be readily concealed and easily transported and delivered.

As is indicated in the last point, IEDs are often concealed and delivered in a secondary container such as a backpack, purse, or suitcase, as these items do not draw attention (Burke 2000). Some of the more notable IED events in recent history involved the use of backpacks to conceal IEDs. For instance, on July 27, 1996, during the Olympic Games in Atlanta, Georgia, a suspicious green backpack was noticed underneath a bench in Centennial Park. Authorities observed wires and pipes in the bag and assumed it was a bomb. As the area was being cleared a bomb threat was called in that warned of a device like the one that was found. Shortly after 1 a.m. the bomb inside the backpack went off, killing two people and injuring 111. The backpack contained three pipe bombs along with nails and screws to increase the shrapnel (Noe 2007). Another highly publicized IED attack in which the devices were concealed in backpacks occurred in Madrid on March 11, 2004. Thirteen IEDs were placed on four commuter trains during morning rush hour. Ten of these exploded, resulting in 191 deaths and over

1,800 injuries. The three IEDs that did not go off were detonated by police in a controlled environment (Timeline: Madrid investigation 2004).

The flexibility permitted in IED design allows terrorists to continually adapt to countermeasures. For example, jammers have been developed to block the signals of radio controlled IED detonators such as cell phones (Wilson 2007). Two such jammers, the IED Countermeasures Equipment (ICE) and the Warlock, hamper radio controlled explosive detonators using low power radio frequency energy. Terrorists responded to these countermeasure by simply making bombs with more powerful radio triggers or by using infrared triggers, rendering the jammers less effective (Atkinson 2007a). Complicating matters more is the fact that the time it takes terrorists to adapt is usually shorter than the time involved in implementing new countermeasures (National Research Council 2007). It will be up to the nation's science and research community to continue to develop new countermeasures that can detect the most recent IED advancements (Chertoff 2007).

In an ideal world, all IED events would be impeded before activation of the device. Consequently, the majority of US research and funding has been targeted for developing preventive measures against IED attacks (Wilson 2007). However, the continuous evolution of IEDs, combined with the unlimited number of targets, makes complete IED prevention impossible. If an IED is activated, focus must turn to mitigating the effects and apprehending those responsible. As mentioned, IEDs have become popular weapons among terrorists because there is very little risk of the perpetrator being detected. Many IEDs can be set to detonate via a timing device, or their detonation can be controlled from a remote location. Since terrorists are outnumbered by

the forces they are up against it is critical to the longevity of their campaign that they remain elusive. This is especially true for terrorists who possess bomb making knowledge (Atkinson 2007a). Not capturing terrorists leads to fear of more attacks among the targeted population, and gives justification to the success of the attack in the minds of the terrorists, prolonging the campaign. On the other hand, the capture of one terrorist can drastically alter a group's campaign as it often leads to the arrests of other group members (Sageman 2004). With this in mind, there has been an increased focus on the forensic analysis of recovered IEDs (Atkinson 2007b). As of September 2007, FBI director Robert Mueller said that 2,500 latent prints had been developed from non-detonated IED components recovered in the combat theaters, resulting in 60 identifications and more than 1,000 forensic matches among IEDs (Mueller III 2007). To further aid in identifying IED perpetrators, forensic scientists have sought alternative methods for identifying the manufacturers of IEDs.

Past research in identifying IED handlers using DNA

Van Oorschot and Jones (1997) found that brief contact between a person and an object is sometimes all that is needed to recover the handler's DNA profile from the object. Since then, scientists have examined the feasibility of recovering DNA from a variety of objects ranging from shoe insoles (Bright and Petricevic 2004) to deflagrated pipe bomb components (Esslinger et al. 2004; Gehring 2004; Kremer and Foran 2008), the latter which directly pertains to the work presented here. The ability to obtain and type DNA from pipe bombs is largely dependent on two factors, the number of shed epithelial cells deposited on the bombs, and the level of DNA degradation. Research has

shown that individuals vary in their tendency to shed epithelial cells (Lowe et al. 2002), hence, a 'good' shedder may leave enough cells to produce a full profile, while a 'poor' shedder will leave behind enough cells to produce a partial or no profile. When it comes to the integrity of the DNA, it is very likely that DNA from the shed cells has already experienced some level of degradation depending upon the conditions to which it has been exposed (temperature, moisture, etc.). DNA on an IED is prone to further degradation due to the heat produced during the explosion, which is problematic when performing polymerase chain reaction (PCR) based assays, as the fragmented DNA prevents recovery of larger amplicons.

The initial attempt to obtain DNA profiles from exploded pipe bombs investigated the prospect of typing recovered DNA using short tandem repeat (STR) methodology (Esslinger et al. 2004). This study had minimal success as only one full profile (10 loci) was obtained after typing DNA from twenty pipe bombs using Profiler Plus™ (Applied Biosystems, Foster City, CA). A likely explanation for the results was that not enough nuclear DNA was recovered, given that DNA quantitation using Quantiblot® indicated that DNA samples from all twenty bombs were below the lowest quantitation standard (.03125 ng/μL). Figuring the DNA in the samples was too dilute, they were concentrated from 80 – 140 μL to 10 μL. This led to the one full profile observed, however, the rest of samples still resulted in partial or no profiles.

Attempts to obtain DNA profiles from deflagrated pipe bombs turned to the analysis of mitochondrial DNA (mtDNA) (Gehring 2004). Features of mtDNA, including high copy number and resistance to degradation (Foran 2006; Holland and Parsons 1999), made it a promising alternative to nuclear DNA testing. Robin and Wong

(1988) estimated the number of mitochondria per cell in mammals ranges from 80 – 680 depending upon cell type, and that the total number of mtDNAs can range from 200 – 1700 per cell. Foran (2006) examined features of mtDNA that make it a better alternative than nuclear DNA when working with forensic samples that are likely to contain degraded DNA. He found that mtDNA's cellular location plays a substantial role in slowing the rate of degradation, and that the transcriptional activity of the loci being assayed may also influence their rate of degradation. Using mtDNA to identify IED handlers allowed for 18 out of 38 bombs to be correctly assigned to a single donor (Gehring 2004). Further, seven bombs were correctly assigned to a subset of donors, while twelve and one bomb(s) were not assignable or incorrectly assigned, respectively. These results were further supported by mtDNA work performed by Kremer and Foran (2008) where 11 and 10 bombs (n = 34) were correctly assigned to a single donor or subset of donors, respectively.

Despite improved success in identifying IED handlers through analysis of mtDNA recovered from deflagrated pipe bombs, the results were not as individualizing as those obtained using STRs. To try and enhance the discrimination power, Kremer and Foran (2008) used two sets of 'miniSTRs' (see below), NCO1 (Coble and Butler 2005) and miniSGM (STRBase), to obtain handler's profiles from deflagrated pipe bombs. In this way, eight bombs were correctly assigned to a single donor while nine bombs were correctly assigned to a subset of donors. The other 17 bombs in the study were either incorrectly assigned (14) or not assigned (3). Their results shadowed those obtained using mtDNA in regards to correctly assigning handlers, as no significant difference was observed when comparing handler assignments using mtDNA and miniSTRs.

miniSTRs

Since their introduction into the field of forensic biology in the early 1990's STRs have developed into the standard marker for human identity testing (Butler 2006). In 1998, the FBI selected thirteen STR loci to be used in their Combined DNA Index System (CODIS), a national database dedicated to storing DNA profiles from convicted offenders and forensic casework (Budowle et al. 1999). By incorporating multiply dyes and carefully designing primers, commercial multiplexes containing as many as 16 loci (including the 13 CODIS loci) have since been developed (Collins et al. 2004; Krenke et al. 2002). These multiplexes perform well for DNA typing of non-compromised samples, however, their performance is hindered with degraded DNA (Butler et al. 2003; Coble and Butler 2005). Fragmented DNA often results in the inability to amplify larger STR amplicons, preventing a full profile from being obtained due to allele and/or locus drop-out (Whitaker et al. 1995). Researchers found that redesigning primers so they were closer to the core repeat resulted in increased success in typing compromised DNA samples (Ricci et al. 1999; Wiegand and Kleiber 2001; Yoshida et al. 1997). The reduced sized amplicons became known as miniSTRs (Butler et al. 2003).

The flexibility in designing primers that allows for creation of 16 locus multiplexes is lost when working with miniSTRs (Butler et al. 2003). However, researchers were still able to create "miniplexes"—combinations of primers for amplifying multiply miniSTR loci at one time (Butler et al. 2003). Using redesigned primers for 16 loci (including 12 CODIS loci) they created five miniplexes (miniplex 1 – 5) consisting of three loci, and one miniplex (Big Mini) containing 6 loci (Table 1). The different combinations of primers were successfully used to amplify the targeted loci and

produced allele calls that were concordant with those obtained using commercial STR kits. They also proved effective in amplifying degraded DNA samples. Additional miniplexes consisting mainly of CODIS loci have since been described including a 6 locus miniplex (Grubwieser et al. 2006) and 5 – 7 loci miniplexes (Parsons et al. 2007) (Table 1). In 2007, Applied Biosystems released the largest miniplex to date, Minifiler™, a miniSTR kit consisting of eight autosomal loci (seven of which are CODIS loci) and the sex determining locus amelogenin (Table 1). The kit was marketed for amplification and typing of compromised (degraded and inhibited) DNA samples.

Despite progress in multiplexing miniSTR loci, the discriminatory power obtained with commercial STR kits was still lacking. In order to increase the power of discrimination, Coble and Butler (2005) created two more miniplexes (mini01 and mini02), each consisting of three non-CODIS loci (Table 1). An additional 20 non-CODIS miniSTR loci have recently been described in hopes of creating larger multiplexes (Hill et al. 2008).

Table 1. A review of reported miniplexes.

Name	Number of loci	Loci included	Source(s)
Miniplex 1	3	CSF1PO, THO1, TPOX	Butler et al. (2003)
Miniplex 2	3	D5S818, D8S1179, D16S539	"
Miniplex 3	3	FGA, D7S820, D21S11	"
Miniplex 4	3	VWA, D13S317, D18S51	"
Miniplex 5	3	**PentaD, PentaE, D2S1338**	"
Big Mini	6	CSF1PO, THO1, TPOX, FGA, D7S820, D21S11	"
Mini01 (NCO1)	3	**D10S1248, D14S134, D22S1045**	Coble and Butler (2005); STRBase
Mini02 (NCO2)	3	**D1S1677, D2S441, D4S2364**	"
MiniSGM	6	THO1, Amelogenin, **D2S1338**, D18S51, D16S539, FGA	STRBase
MiniSTR-multiplex	6	THO1, Amelogenin, **D2S1338**, D18S51, D16S539, FGA	Grubwieser et al. (2006)
MP1	7	THO1, D21S11, D18S51, D16S539, Amelogenin, FGA, **PentaD**	Parsons et al. (2007)
MP2	6	D21S11, D13S317, D7S820, CSF1PO, D8S1179, VWA	"
MP3	5	FGA, CSF1PO, D21S11, **PentaD, PentaE**	"
Minifiler™	9	D13S317, D7S820, **D2S1338**, D21S11, D16S539, D18S51, CSF1PO, FGA, Amelogenin	Applied Biosystems (2007)

Bold indicates a non-CODIS locus. The miniSGM miniplex and the miniSTR-multiplex consist of the same loci, however, some primer sequences varied.

Analysis of trace and low copy number DNA

There are other concerns with recovering and analyzing DNA from IEDs besides degraded DNA, including working with trace amounts of DNA, PCR inhibition, and mixtures. Isolating DNA from shed epithelial cells usually results in the recovery of small quantities of DNA, referred to as trace DNA (Wickenheiser 2002). The term low copy number (LCN) has been coined to describe analysis of less than 100 pg of DNA (Gill et al. 2000), which is ten times less than the 1 ng of input DNA that many

multiplexes call for (Gill 2001). Hence, working with and analyzing LCN DNA has proven to be problematic as analyses can lead to increased allele drop-out due to stochastic sampling effects, increased stutter, heterozygote peak imbalance, and are more prone to sporadic contamination (Budowle et al. 2001; Gill 2001).

Researchers have found ways to combat problems associated with the analysis of small quantities of DNA leading to increased sensitivity of STR assays (Budowle et al. 2001). By reducing the overall PCR volume while maintaining the amount of input DNA, the amplification product is more concentrated, resulting in greater peak heights. Sensitivity can also be increased by filtrating the product after STR amplification, which removes ions and other small molecules that may be preferentially injected into the capillary during electrophoresis, interfering with the injection of the STRs. Using deionized formamide (required to keep the DNA denatured during capillary electrophoresis) also helps ensure that DNA will be preferentially injected. Two additional means of increasing sensitivity with LCN DNA samples include adding more amplified product to the formamide and increasing the injection time. It is also advised that replicate analyses be performed when working with minute quantities of DNA in order to confirm allele calls (Gill et al. 2000).

PCR inhibition and DNA quantitation

PCR inhibition is another potential problem when working with DNA recovered from deflagrated pipe bombs. It is not uncommon for forensic samples to include substances that co-extract with DNA and inhibit amplification (reviewed by Bessetti 2007). Heme and humic acid, found in blood and soil respectively, have been shown to

cause inhibition (Akane et al. 1994; Watson and Blackwell 2000), and could be sources of inhibition in the analysis of deflagrated IEDs. Although not documented, another potential source of inhibition when dealing with IEDs is residue resulting from the explosive material.

STR electropherograms with small or absent peaks may be indicative of PCR inhibition. One method to identify PCR inhibition involves spiking the potentially inhibited sample with high quality DNA before performing PCR (e.g., Shutler et al. 1999). If a PCR product is not obtained it can be concluded that the sample was inhibited. If product is observed the assumption can be made that the initial PCR contained insufficient template, meaning that the DNA was of poor quality (degraded) or that there was no DNA. Building on this approach, a method to recognize PCR inhibition has been developed using an internal positive control (IPC) incorporated into DNA quantitation via real-time PCR (Green et al. 2005).

Quantifiler™ (Applied Biosystems) is a standard kit for the quantification of human DNA using real-time PCR. Quantifying DNA is a routine and integral step in the processing of DNA samples, and is usually performed before STR analysis so the optimal amount of DNA can be added to the PCR reactions. In addition to quantifying DNA, the kit also contains a synthetic strand of DNA as an IPC. Failure of the IPC to amplify is an indication of the presence of inhibitors.

If inhibition is detected, various measures have proven successful in overcoming it. Supplementing the PCR reaction with adjuvants such as bovine serum albumin (BSA) or betaine has been effective in alleviating inhibition caused by heme (Al-Sound and Rådström 2000; Kreader 1996). Bourke et al. (1999) found that purifying DNA in the

presence of sodium hydroxide leads to improved amplification which is likely due to neutralizing inhibitors of *Taq* polymerase. Further, adding aluminum ammonium sulfate during the extraction of DNA from soil samples was shown to reduce the co-purification of inhibitors (Braid et al. 2003).

There are also numerous ways to overcome PCR inhibition that do not involve additives (reviewed by Rådström et al. 2004). Performing PCR using a diluted DNA sample results in increased amplification efficiency due to a reduction in inhibitors. However, this remedy is limited by the amount of DNA recovered, as diluting the sample too far may lead to an insufficient amount of template. A common method for reducing the effects of inhibitors is to purify (or repurify) the sample. Some purification options include spin columns, gel filtration, and DNA binding beads. Oftentimes multiple measures are utilized to decrease the chances of PCR inhibition (Rådström et al. 2004).

Analysis of mixtures

A DNA mixture occurs when two or more genotypes are present in an electropherogram. Mixtures complicate the analysis of both mitochondrial and nuclear DNA markers, leading to results with lower discriminatory power, or in some cases, no evidentiary value. The first indication of a mixture in an STR profile is the observation of three or more alleles at a locus (Clayton et al. 1998). However, there are a variety of circumstances that can lead to extra peaks in an electropherogram besides the presence of multiple individual's DNA (Clayton et al. 1998). Stutter is the most common cause for extra peaks, which is caused by slippage of the polymerase during replication of the STR. Stutter of tetranucleotide STRs results in peaks that are four nucleotides smaller (most

common) or larger than the true allele. The height of stutter peaks is usually less than 15% of the height of the main peak, however, when working with LCN DNA stutter peaks have even been reported to be larger than the peak from the true allele (Gill et al. 2000). 'N' peaks, which are generated when *Taq* polymerase adds an adenine residue to the terminal end of a newly synthesized DNA molecule during PCR, are another source of additional peaks. Incomplete addition of the nucleotide gives rise to peaks that are separated by one base, however, both peaks represent the same allele. A further cause of extra peaks stems from non-specific priming of the DNA. Such artifacts can usually be easily distinguished as they tend to be off ladder, are not reproducible, and have low intensity. Sporadic contamination also results in superfluous peaks and is exacerbated when working with LCN DNA. The additional alleles produced have been denoted 'drop- in'.

It is crucial to account for sources of additional peaks when analyzing STR data to ensure the proper labeling of a sample as a mixture or non-mixture. Mixture analysis can be a tedious and time consuming task. To help streamline the process Clayton et al. (1998) proposed six steps that should be implemented when interpreting mixtures.

1. Identify the presence of a mixture.
2. Designate allele peaks
3. Identify the number of potential contributors
4. Estimate the relative ratio of individuals contributing to the mixture
5. Consider all possible genotype combinations
6. Compare reference samples

Additionally, if mixtures are anticipated, steps can be taken to reduce their likelihood during the processing of the evidence. For example, it has been proposed that portions of evidence be swabbed independently (reviewed by Wickenheiser 2002), which reduces the chances of obtaining mixtures and increases the likelihood of deciphering the profiles of

the perpetrator(s) and/or victim(s). This methodology aided in the recovery of perpetrator profiles from a hot dog used in a sexual assault and an electrical cord used in a strangulation (Wickenheiser 2002).

A study involving mixture analysis from trace DNA produced an interesting finding that has proved beneficial in the interpretation of mixtures. Van Oorschot and Jones (1997) showed that when swabbing a surface that had been touched by two people, a mixture was obtained, but the major profile was that of the second person. This finding was supported by an observation made in a criminal case where a swab taken from the steering wheel of the victim's car produced a mixture of the victim's and suspect's DNA, with the suspect being the major contributor (Wickenheiser 2002). However, despite success in analyzing mixtures from trace DNA, caution is still urged. When quantities of DNA approach LCN status, mixture analysis is increasingly difficult due to the characteristics of LCN DNA outlined above.

Study aims: A new approach to identifying those involved in IED campaigns

Past approaches aimed at obtaining handler DNA profiles from deflagrated pipe bombs have been moderately successful, but they have lacked the discriminatory power desirable from forensic DNA analyses. To improve the success of identifying those responsible for IEDs, a new approach was sought. In discussions held with First Lieutenant Shawn Stallworth of the Michigan State Police Bomb Squad, he noted that IEDs the Bomb Squad encounters are often concealed in some type of container (personal communication). It was thought that obtaining handler DNA profiles could be enhanced if DNA recovery was targeted on these containers, and four reasons were hypothesized as

to why this might be a better alternative. First, a perpetrator is likely to have extended contact with the IED container, allowing ample opportunity to deposit shed epithelial cells. Second, based on work done by Kisilevsky and Wickenheiser (1999), porous surfaces of IED containers may retain shed cells better than the smoother surfaces of pipe bombs. Third, DNA on the container might be less degraded than the DNA on bomb components leading to improved amplification. Finally, as opposed to the small size of IED fragments recovered in previous studies (Gehring 2004; Kremer and Foran 2008), fragments of the container might to be larger and easier to collect.

Potential complications that needed to be considered included the possibility of encountering LCN DNA, mixtures, degraded DNA, and PCR inhibition. LCN difficulties can potentially be lessened by implementing LCN procedures described by Budowle et al. (2001; detailed above). The chance of mixtures can be reduced by independently swabbing multiple areas on a container instead of using a single swab for all regions. Processing multiple swabs from the same IED container can also be viewed as running samples in 'replicates', fulfilling a recommendation for working with LCN DNA. Degraded DNA and PCR inhibition can potentially be addressed by using Applied Biosystem's Minifiler™ kit. The kit was specifically designed for amplification and typing of degraded DNA, while the buffer (proprietary) was formulated to help overcome common PCR inhibitors such as heme and indigo (Applied Biosystems 2006).

In the research presented, study participants were asked to use backpacks in everyday activities for a period of one to two weeks. Backpacks were collected and served as containers for pipe bombs that were deflagrated in a controlled environment by members of the Michigan State Police Bomb Squad. All fragments of the backpack were

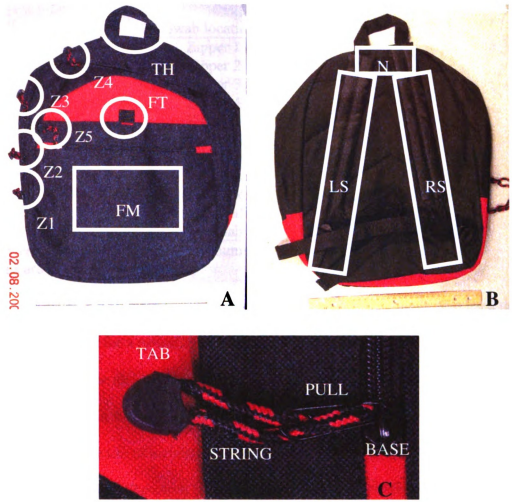
brought to the Michigan State University Forensic Biology Laboratory for DNA isolation. DNA was extracted from swabs and subsequently amplified, quantified, and typed. Handler genotypes were predicted blindly and checked for concordance with participant's known STR profiles to see if the correct handlers of the backpacks could be identified.

MATERIALS AND METHODS

Backpack preparation and distribution

Ten backpacks (LEED'S, Pittsburgh, PA) designated BP3 – BP12 (BP1 and BP2 were used in preliminary studies for feasibility and optimization purposes) were autoclaved for 45 minutes at 135°C, followed by a 45 minute drying time at 100°C. The backpacks were placed in a Spectrolinker XL-1500 UV Crosslinker (Spectronics Corporation, Westbury, NY) for 15 minutes per side (approximately 7.5 J/cm^2). Plastic shaft, cotton swabs (860PPC, Puritan Medical Products Co. LLC, Guilford, ME) were used to swab backpacks in areas that were targeted for post-blast DNA recovery. The double swab technique (Sweet et al. 1997) was incorporated in which a swab moistened with 150 µL of digestion buffer (20mM Tris, 50mM EDTA, 0.1% SDS, pH 7.5) was thoroughly passed over the targeted region, followed by a dry swab that was immediately applied to the same section. Eleven areas were swabbed (Figure 1) including the five zippers (comprised of the base, metal pull, string, and plastic tab), the top handle, the left and right strap, the neck region, the front middle region, and the front tab. Swabs were labeled with the backpack number followed by an identifier for the location swabbed (Table 2). These swabs served as controls and were stored at -20°C, however, the need did not arise for analysis.

Figure 1. Regions of the backpacks targeted for DNA collection.



Eleven areas of the backpacks were targeted for post-blast DNA recovery. Identifiers are detailed in Table 2. (A) Front view showing the five zippers, top handle, front tab, and front middle region. (B) Rear view showing the left and right straps and the neck region. (C) An intact zipper with all four components labeled.

Table 2. Identifiers for labeling swabs.

Swab location	Identifier
Zipper 1	Z1
Zipper 2	Z2
Zipper 3	Z3
Zipper 4	Z4
Zipper 5	Z5
Top handle	TH
Left strap	LS
Right strap	RS
Neck region	N
Front middle	FM
Front tab	FT

Swabs were labeled with the backpack number followed by the identifier. Swab locations are illustrated in Figure 1.

Backpacks 3 – 10 were randomly distributed to eight participants who used them in everyday activities for a period of 11 days. BP11 acted as a positive control in which a participant handled the eleven areas in Table 2 three times a day for three days, alternating the order in which the regions were handled each time. BP12 served as a negative control. The use of human subjects followed guidelines established by the University Committee on Research Involving Human Subjects (IRB # 07-577).

Pipe bomb preparation

Ten pipe bombs (five galvanized steel and five PVC) were assembled. Bombs were 1 foot in length, one inch in diameter, and had a pair of end caps, one of which had a ¼ inch hole drilled in the center for fuse placement. Pipes and end caps were soaked for 1 hour in 10% bleach, rinsed with distilled water, and were placed in a UV crosslinker for 10 minutes, turning half way through. ELIMINase® (Decon Laboratories, Inc., Bryn Mawr, PA) was applied to all surfaces according to the manufacturer's instructions and

rinsed twice with sterile water. Pieces were dried in a laminar flow hood. End caps without the hole were affixed to PVC pipe bombs using PVC cement. Steel end caps were not fastened. Pipe bombs were individually placed in new paper bags. Steel bombs were assigned numbers 3 – 7, while PVC bombs were assigned numbers 8 – 12, corresponding to the numbers given to the backpacks.

Backpack collection and bomb handling

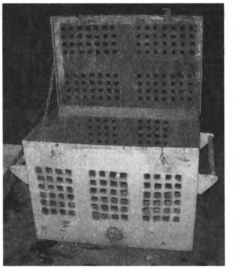
Upon collection of the backpacks, participants were asked to close all the zippers so the tabs were to the left when looking at the backpack from the front. The eight participants were randomly assigned a letter (A, B, E – J) that was not known by the main investigator. A second individual recorded the backpack numbers of the participants. Subjects were asked to mock assemble, for 30 seconds, the pipe bomb that corresponded to their backpack number. Participants who handled the steel pipe bombs were instructed to securely fasten the end caps without the hole. Buccal swabs were obtained from the participants for DNA reference samples.

Bomb deflagration and collection

Backpacks and pipe bombs were transported to the Lansing Fire Fighting Training Facility (Lansing, MI), and deflagrations were conducted in the facility's smoke room. Immediately preceding deflagration a member of the Michigan State Police Bomb Squad filled the pipes with 1.5 ounces of Green Dot Smokeless Shotshell Powder (Alliant Powder Co., Radford, VA) and affixed the end cap with a hole to the device. A fuse was inserted in the hole and the pipe bomb was placed inside the main pocket of the

corresponding backpack with only the fuse showing. The same bomb squad member placed the backpack bomb inside a steel crate (Figure 2) and lit the fuse via the circular hole in the front of the crate. After deflagration, bomb and backpack fragments were collected and placed together in a new paper bag. Between deflagrations the steel crate and the smoke room were swept to remove any uncollected debris. Upon returning to the lab, pipe bomb fragments were separated from the backpack fragments for use in other studies. All investigators involved in the deflagration process wore sleeves, facemasks, and gloves.

Figure 2. Crate used to retain backpack and IED fragments.



The crate was designed to limit the dispersal of IED and bomb fragments. Walls were constructed of steel with holes cut in them to relieve pressure from the blast; the floor was made of wood.

DNA recovery and extraction

Backpacks were processed separately to minimize the chance of cross contamination. Areas targeted for DNA recovery were individually swabbed in a laminar

flow hood that was thoroughly wiped down with 10% bleach and UV irradiated for 10 minutes. Potential DNA contamination of the swabs themselves (860PPC, the initial swabs utilized), discovered during the research, resulted in backpacks 3 – 6, 8, and 9 being swabbed with different cotton swabs (25-806 2PC, Puritan Medical Products Co.). These were designated in the same manner as control swabs (above), except that a 'P' was added to the end. Also, if the original location of a zipper was unidentifiable, a 'U' was incorporated (e.g., the first unidentifiable zipper from BP3 was labeled 3Z1UP). Both swabs (wet and dry) were placed in the same 1.5 mL tube and stored at -20°C until extractions were performed. A reagent blank consisting of two unused swabs was also created (designated by backpack number and the identifier RBSWAB).

DNA extractions were performed by adding 350 µL of digestion buffer (total volume of 500 µL including the 150 µL previously added to the swabs) and 6 µL of proteinase K (20 mg/mL) to tubes containing the swabs, which were vortexed and incubated overnight at 55°C. A second set of reagent blanks was initiated (designated by backpack number and identifier RB). After incubation, spin baskets were inserted into 2.0 mL tubes. One swab per extraction was centrifuged for 1 minute at 13,000 revolutions per minute (rpm) and discarded. The process was repeated with the second swabs using the same spin baskets, after which the baskets were removed and the liquid was pipetted back into the original tubes. An equal volume of phenol (500 µL) was added to the samples, vortexed, and centrifuged at 13,000 rpm for 6 minutes. The aqueous layers were transferred to new 1.5 mL tubes and equal volumes of chloroform were added. The tubes were vortexed and centrifuged for 6 minutes at 13,000 rpm. The aqueous layers were transferred to Microcon® YM-30 spin columns (Millipore

Corporation, Billerica, MA), and 100 µL of TE (10 mM Tris, 1 mM EDTA, pH 7.5) were added. The columns were centrifuged for 12 minutes at 14,000 x g and the flowthrough was discarded. The columns were washed with 200 µL TE, with the centrifugation time reduced to 8 minutes. Twenty microliters of TE were added to the column membranes and left for 5 minutes. Columns were inverted into new tubes and centrifuged for 3 minutes at 1000 x g. DNA samples were stored at -20°C. Reference buccal swabs were extracted using a ChargeSwitch® Forensic DNA Purification Kit (Invitrogen, Carlsbad, CA) as per the manufacturer's protocol, and stored at -20°C.

Amplification of miniSTRs using the Minifiler™ kit

DNAs extracted from the backpack swabs were amplified using an AmpF*l*STR® MiniFiler™ PCR Amplification Kit (Applied Biosystems). Reactions were carried out in 10 µL volumes, including 2 µL of the MiniFiler™ primer set, 4 µL of the MiniFiler™ Master Mix, and 4 µL of DNA template. Reactions of reference samples contained 1 µL of DNA diluted 1:100 in TE, and 3 µL TE, while positive controls had 3 µL of 007 control DNA (0.1 ng/µL) and 1 µL of TE. All reagents were briefly vortexed and centrifuged before use. Amplifications were performed using the following thermal cycling conditions: an 11 minute incubation at 95°C, followed by 30 cycles consisting of a 20 second denaturation at 94°C, 2 minutes of annealing at 59°C, and a 1 minute extension at 72°C, followed by a 45 minute final extension at 60°C.

Capillary electrophoresis and miniSTR analysis

Two microliters of amplified product (1.5 µL for reference samples) were combined with 24.5 µL of deionized formamide and 0.5 µL of GeneScan™ 500 LIZ® Size Standard (Applied Biosystems) in 0.5 mL tubes. Allelic ladders contained the same volumes of formamide and size standard in addition to 1.5 µL of the Minifiler™ allelic ladder DNA. Tubes were incubated at 95°C for 3 minutes and then placed on ice. Caps were cut off and one drop of mineral oil was added.

Electrophoresis was performed on an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems) using the GS STR POP4 (1ml) G5 v2.md5 run module. Runs were conducted using POP4 (performance optimized polymer 4; Applied Biosystems) and 1X buffer with EDTA (Applied Biosystems). Parameters included a 5 second injection at 15 kV, a 28 minute run time at 15 kV, and a temperature of 60°C. Data were analyzed using GeneMapper® ID software v3.2.1 (Applied Biosystems). Panels and bins were downloaded and imported into GeneMapper from www.appliedbiosystems.com (Support > Software Downloads > GeneMapper® ID Software v3.2 >Updaters & Patches). The analysis method was MiniFiler_GS500_HID_v1, the panel was MiniFiler_GS500_v1, and the matrix was DS-33 Matrix 7-12-07.

Electropherograms were manually reviewed and callable alleles were recorded. A threshold of 50 relative fluorescence units (RFUs) was used. Handler profiles were compiled blindly using the complete set of swabs from a backpack, and checked for concordance with the known (buccal) swabs by a second individual.

After the handler's profiles were known, results for each locus were placed in one of six categories:

1. The locus contained only the handler's correct alleles.
2. The locus had multiple allele calls, but the handler's alleles constituted the major profile.
3. The locus had multiple allele calls, but the handler's alleles could not be distinguished as the major profile.
4. The locus had at least one of the handler's correct alleles. This involved instances where there may have only been one allele called, or there may have been multiple alleles called, but only one matched the handler's profile.
5. None of the alleles matched the handler's profile.
6. No alleles were called at the locus.

The number of loci from each swab that contained the handler's correct alleles was counted; a locus met this criterion if it was in one of the first three categories described above. Swabs were then classified as producing all 9, 8 or 7, 6 or 5, or less than five loci with the handler's correct alleles. The number of swabs in each category was compared among backpacks. Additionally, the average number of loci with the handler's correct alleles was calculated per region swabbed. Loci in categories two or three above (excluding amelogenin) were reviewed to see if increased stutter could have accounted for the extra allele calls; if extra peaks were one repeat unit before or after the peaks attributed to the handler's alleles, the locus met this condition. Finally, electropherograms with six or more loci that had allele calls in addition to the handler's were designated mixtures.

DNA quantification using real-time PCR

DNAs were quantified using a Quantifiler™ Human DNA Quantification Kit (Applied Biosystems). Amplification and detection were performed on an iCycler™ thermal cycler and an iQ5 multi-color real-time PCR detection system (Bio-Rad, Hercules, CA), respectively. Dye calibrations were completed for VIC and FAM as instructed in the iQ™5 Optical System Instruction Manual (Bio-Rad). The VIC

calibrator consisted of a labeled oligonucleotide (VIC-CATTTCCTTC) (Applied Biosystems) diluted to 300 nM. The FAM solution was from the calibration kit (Bio-Rad). Calibrations were performed using 15 µL volumes in 0.2 mL dome cap tubes (Dot Scientific, Burton, MI) and 96 well, half skirted, Thermowell® Gold PCR plates (Corning Inc., Corning, NY) sealed with microseal® 'B' film (Bio-Rad).

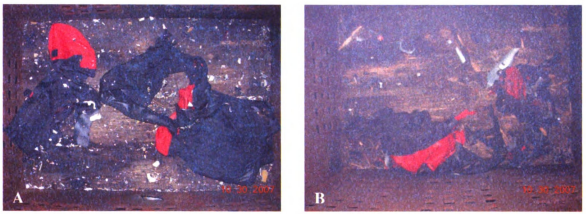
The 200 ng/µL human DNA stock solution supplied with the Quantifiler™ kit was serially diluted as per the manufacturer's protocol providing eight standards ranging from 50 ng/µL to 0.023 ng/µL. PCR reactions were carried out in 15 µL volumes consisting of 6.3 µL primer, 7.5 µL reaction mix, and 1.2 µL DNA. Reactions were set up using 96 well plates and then sealed. Standards were run in duplicate and unknowns in triplicate. Thermal cycling parameters included a 10 minute incubation at 95°C followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. Real-time data were analyzed using software generated thresholds for each fluorophore (PCR base line subtracted curve fit mode). Threshold cycles (C_T) for IPCs were reviewed and any values above 30 were noted. The software created a standard curve used to determine DNA quantities and calculated average DNA concentrations and standard deviations for all replicates.

RESULTS

Deflagration observations

Backpacks serving as containers for steel pipe bombs suffered more damage than those concealing PVC pipe bombs (Figure 3)—evident by an average of 4.7 zippers, or pieces thereof, recovered from backpacks subjected to PVC pipe bombs as opposed to 2.8 for backpacks containing steel pipe bombs. The majority of backpack fragments (zippers being the exception) were retained within the crate. Areas of the backpacks that were stitched or made of stronger material, such as the top handle, withstood the blast better than others. Backpack 8 briefly caught on fire after the pipe bomb was deflagrated, with flames shaken out by a member of the bomb squad.

Figure 3. Backpack fragmentation.



Examples of post-blast backpacks after serving as containers for PVC (A) and steel (B) pipe bombs.

Swabbing backpacks to recover shed cells

The number of regions swabbed per backpack ranged from seven on backpack 7 to eleven on backpack 10, with most of the variability caused by the failure to retain all the zippers. Twenty-eight of the forty zippers were recovered, of which only four were intact. The remaining 24 zippers included: seven that were missing the tab, four that were lacking the base, six that were missing the base and tab, four that had only a tab, and three consisting of only a string. The original locations were identified for 7 of the 28 zippers collected due to many becoming detached from the backpack during deflagration. The front middle region of backpack 3 was the only non-zipper portion not swabbed as it was unable to be identified.

Isolating DNA from recovered cells

A total of 75 swabs was obtained from the eight backpacks handled by participants. However, DNA from swab 8FMP was not included in the study due to a malfunction in the spin column during processing. Some swabs were soiled with residue from the explosives resulting in discoloration of the digestion buffer after incubation, most of which disappeared during the extraction process. Initially, a second chloroform treatment was administered for those samples in which discoloration persisted (performed on backpack 11 swabs), however, this was not beneficial and all subsequent samples were subjected to only one chloroform treatment.

Analysis of STR electropherograms

Handler profiles were compiled for backpacks 3 – 10 after electropherograms from all the samples were reviewed and allele calls were made (Table 3; Appendix A). Seven of eight profiles matched a participant's reference sample at all nine loci (Table 3); the lone exception was backpack 9, which matched a reference profile at eight loci. This stemmed from the inability to distinguish between a 30, 31 and a 30 homozygote at the D21 locus, where thirty was the sole allele in three samples, and 30 and 31 were present in six samples. In only one instance were 30 and 31 the only two alleles present. The correct call was later found to be 30, 31.

Reagent blanks that were processed with backpack 3 – 10 produced a total of 7 callable alleles, with peaks all below 150 RFUs (Appendix A). The swab 'reagent blank' for backpack 10 had peaks matching alleles 12 and 48.2 at CSF and FGA, respectively, as well as alleles X and Y at amelogenin, and a 10 called at CSF. The other two alleles in reagent blanks came from backpacks 8 (12 at D13) and 4 (11 at D7).

Table 3. Consensus handler profiles obtained from backpacks and the study participant to which they corresponded.

	Backpack							
Locus	3	4	5	6	7	8	9	10
D13	8,12	8,11	11,12	12	11,12	8,12	8,14	11,12
D7	10,11	9,10	11	10,11	8,12	8,11	8,10	9,12
Amel.	X	X	X	X	X	X	X	X
D2	23,24	20,25	23,24	20,24	20,21	20,21	17,20	19,25
D21	27,30	27,30	29,32	31,31.2	31,**OL**	28,30	*	29,30
D16	11,13	12,13	10	9,11	9,11	9,11	12,13	11
D18	15,17	13,16	16	13,14	17	15,16	13,16	13,16
CSF	11	12,13	11,12	11,12	10,11	11,13	12	12,13
FGA	21,24	21,25	21	20,25	22,23.2	18,24	20,22	22.23
Participant	B	E	G	I	J	F	A	H

A consensus handler profile was determined for each backpack using allele calls from the complete set of swabs (see Appendix A). *—could not distinguish between a 30 homozygote and a 30, 31. (**OL**)—the off ladder allele was 33.1.

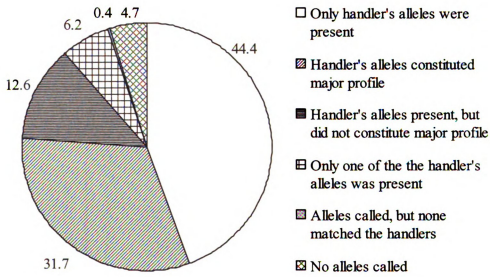
The ten swabs from positive control backpack 11 led to a profile that matched the handler despite two samples (11Z2UP and 11Z4UP) experiencing a high level of drop-out (Appendix A). Negative control backpack 12 had nine swabs that were analyzed. One full profile (nine loci) was obtained from sample 12Z1UP that could not be matched to anyone directly involved in the study (researchers or participants). The rest of the samples from backpack 12 produced a total of ten callable alleles, all of low intensity (Appendix A).

Categorizing loci based on the presence of handler alleles

All 666 loci reviewed were placed in one of six categories (see Materials and Methods) based upon the extent to which they contained the handler's alleles (Figure 4; Appendix A). Examples of four categories can be found in Figure 5. Five hundred and

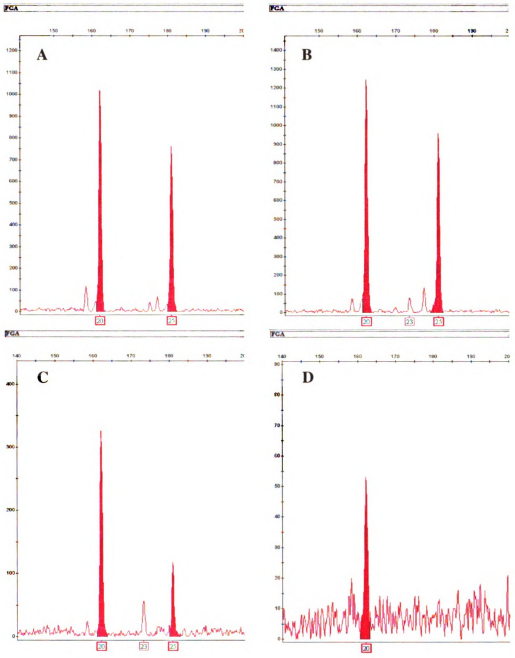
ninety-one loci (88.7%) contained the handler's actual alleles. Of these, 295 (49.9%) had callable alleles besides those of the handler. One of the handler's alleles (for heterozygotes) was present at 41 loci. For 27 of these the correct allele was the only one present, while 14 had additional, incorrect alleles. Thirty-one loci did not have any callable alleles, of which 16 occurred at the D7 locus. Three loci contained only alleles that did not match the handler's.

Figure 4. Percentage of loci placed in each of the six locus classifications.



The first three categories represent loci in which the handler's alleles were present in their entirety (88.7%). The second and third categories indicate loci that had callable alleles besides the handlers (44.3%). The last three categories represent the lack of recovery of handler alleles (11.3%).

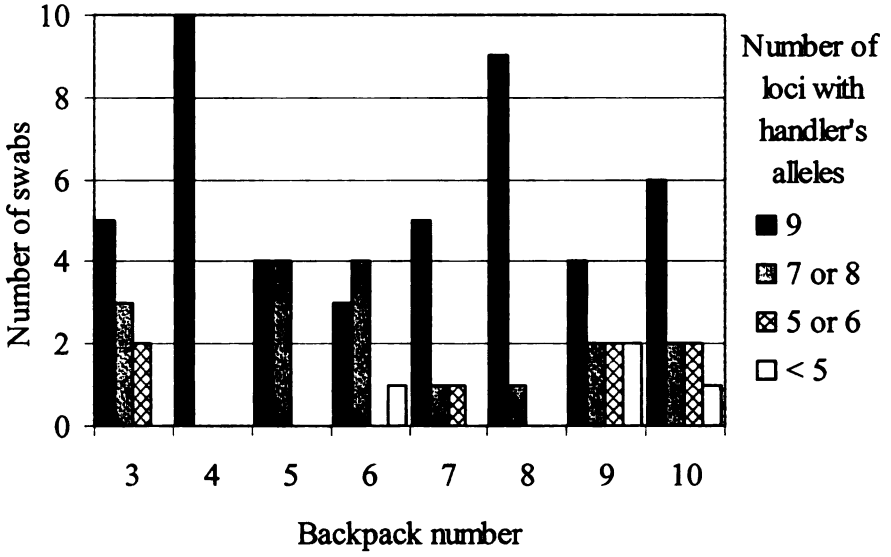
Figure 5. Examples of four locus classifications from backpack 6 samples.



FGA locus from four backpack 6 samples. The handler's correct alleles are 20 and 25 (shaded). (A) Only handler's alleles present (B) Handler's alleles as a major profile. (C) Handler's alleles present, but did not constitute a major profile. (D) Only one of the handler's alleles present (drop-out). The other two categories (alleles called but none matched the handler's, and no callable alleles) represent cases of complete drop-out (not shown).

Swabs were categorized based upon the number of loci that contained the handler's alleles, and then further analyzed on a per backpack basis. Forty-six of 74 swabs had the handler's alleles at all 9 loci including ten from backpack 4 and nine from backpack 8 (Figure 6). Backpack 6 had the lowest number of swabs with the handler's alleles at all loci (three), but four swabs had the correct alleles at seven or eight loci. Eleven swabs produced the handler's alleles at six or fewer loci, with seven coming from backpacks 9 and 10.

Figure 6. Number of loci containing handler's alleles from backpack swabs.



The majority of swabs (63) had the handler's alleles present at a minimum of seven loci (black and gray bars). The highest percentage of swabs with the correct handler alleles at all loci came from backpacks 4 (100%) and 8 (90%). Eleven swabs had the handler's alleles at six or fewer loci (striped and white bars) with seven coming from backpacks 9 and 10.

Peaks attributed to increased stutter and the occurrence of mixtures

Eighty-two of 193 loci (42.5%) classified as containing the handler's alleles as the major profile (excluding amelogenin) had their extra peaks at positions indicative of increased stutter. The same analysis performed on loci classified as containing the handler's alleles, but not as the major profile, found that 36 of 79 (49.4%) loci had their extra peaks at stutter locations. Eighteen samples were classified as mixtures based on at least six loci containing more than two alleles (Appendix A). The most mixtures came from backpacks 6 and 8 as they had four and five, respectively.

Comparing regions based on the number of loci with the handler's alleles

The top handle proved to be the most effective area for recovering a handler's alleles as all swabs produced the handler's full profile (Table 4). Other non-zipper regions ranged from an average of 8.88 loci (neck region) to 7 loci (front tab). Overall, swabs of the zippers averaged the handler's alleles at 7.35 loci, with intact zippers producing 8.75 loci and zippers missing both the base and the tab having 5.3 loci.

Table 4. Number of loci with handler alleles broken down by backpack region.

	Number of loci with handler alleles				
Swab location	9	7 or 8	5 or 6	<5	Average
Zippers					
Intact	3	1	-	-	8.75
no tab	3	2	2	-	7.43
only tab	2	2	-	-	8
only string	1	2	-	-	7.67
no base	3	-	1	-	8
no base or tab	2	1	1	2	5.3
Top handle	8	-	-	-	9
Left strap	6	2	-	-	8.75
Right strap	4	2	1	1	7.75
Neck region	7	1	-	-	8.88
Front middle	5	1	-	-	8.83
Front tab	2	3	2	1	7
Total	46	17	7	4	7.97

The number of loci containing the handler's alleles varied based upon the components of the zippers that were recovered. Intact zippers averaged the most loci with the handler alleles, while zippers missing the base and tab averaged the fewest. Less variation was seen in swabs of non-zippers as the top handle averaged 9 loci with the handler's alleles while the front tab averaged the fewest (7).

DNA quantification

DNA quantities were determined by taking the average of three real-time PCR replicates, and ranged from 1.41 pg/μL (10FTP) to 1.25 ng/μL (4NP) (Table 5; Appendix B). Fifty-three of 74 samples (71.6%) had DNA quantities less than 0.2 ng/μL, while 11 had quantities above 0.4 ng/μL with nine coming from backpacks 4, 7, and 8. The majority of samples from these backpacks had DNA quantities above 100 pg/μL, with only three samples below this value (7FTP, 8Z4P, and 8FTP). In comparison, no samples from backpacks 6, 9, and 10 had a DNA quantity in excess of 100 pg/μL.

The top handle, straps, and neck region had the highest average DNA quantities (approximately 300 pg/μL), while the front tab averaged the lowest of the non-zipper

regions (66.9 pg/μL). DNA amounts recovered from zippers averaged 91.3 pg/μL. The most DNA was obtained from zippers missing just a base (162.3 pg/μL) and having only a tab (150.5 pg/μL), while swabbing merely the string produced 41.0 pg/μL. The four zippers that were recovered intact averaged 61.5 pg/μL.

Table 5. Quantities of DNA recovered from backpack swabs.

	Backpack								
Swab	3	4	5	6	7	8	9	10	Average
Z1UP	0.0991	0.165	0.00933	0.00857	0.104	0.165	0.0180	0.0256	*
Z2UP	0.0686	0.393	0.00955	0.00230	-	0.242	0.0107	0.0125	*
Z3UP	0.0476	0.149	-	-	-	0.149	0.00700	-	*
Z4UP	-	0.251	-	-	-	0.0916	-	-	*
Z3P	-	-	-	-	-	-	-	0.0286	0.0286
Z4P	0.109	-	-	-	-	-	0.0268	0.0303	0.0553
Z5P	0.0246	-	-	-	-	0.296	-	0.0132	0.111
THP	0.215	0.732	0.324	0.0570	0.307	0.566	0.0302	0.0804	0.288
LSP	0.224	0.892	0.182	0.0401	0.189	0.805	0.0534	0.0658	0.306
RSP	0.418	1.233	0.0488	0.0529	0.213	0.271	0.0356	0.0573	0.291
NP	0.292	1.250	0.0169	0.0641	0.192	0.534	0.0579	0.0586	0.308
FMP	-	0.848	0.0435	0.0325	0.507	-	0.0359	0.0159	0.247
FTP	0.0279	0.385	0.0158	0.00810	0.0637	0.0158	0.0172	0.00141	0.0669

Quantities represent the average of three replicates. All quantities are given as ng/μL. (-)—indicates that the given backpack did not have a swab from that location. Averages for zippers from unidentified locations (*) are not given.

Amounts of DNA added to PCR reactions

Once DNA quantities per µL were known the values were multiplied by four to obtain the amount of DNA that was added to PCR reactions. Five of 74 reactions had DNA template quantities that were within the recommended range of 0.5 – 0.75 ng for the Minifiler™ kit (Applied Biosystems 2006a) (Table 6; Figure 7). The majority of reactions (45) had input amounts below 0.5 ng.

Table 6. DNA quantity added to PCR reactions.

Total DNA added to PCR reactions	Number of reactions
<.1*	17
0.1 – 0.3	23
.0.3 – 0.5	5
0.5 – 0.75**	5
0.75 – 1	6
1 – 1.5	6
>1.5	12

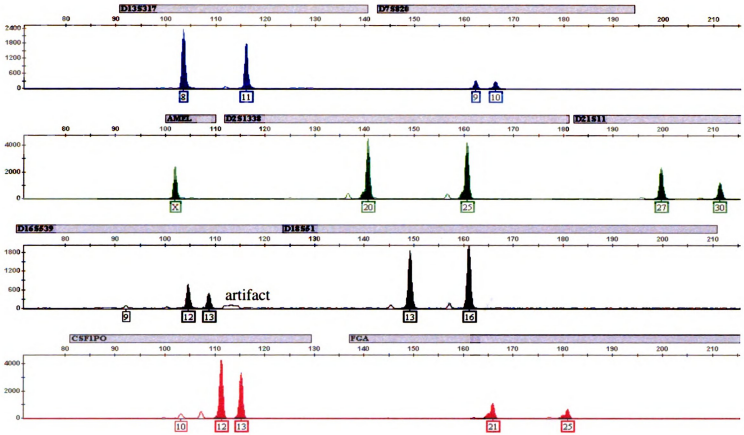
DNA quantities are given in ng. Total DNA amounts were obtained by multiplying the amount of DNA per sample by the number of microliters added to the reactions (4).
*— indicates low copy number. **— indicates the recommended range of input DNA.

Seventeen reactions were designated as LCN, accounting for 49 of the 75 (65.3%) loci that were categorized as having complete or partial drop-out. Twenty-one of the remaining 26 cases of drop-out were from reactions in which DNA input averaged 169.6 pg. Three LCN samples did not experience drop-out, producing full handler profiles.

Twelve reactions contained more than 1.5 ng of input DNA, or 2 – 3 times greater than the advised amount. Ten of these were from backpacks 4 and 8. Electropherograms from all 12 reactions produced the handler's profile. Excluding amelogenin, greater than half (55.2%) of the loci had more than two callable alleles.

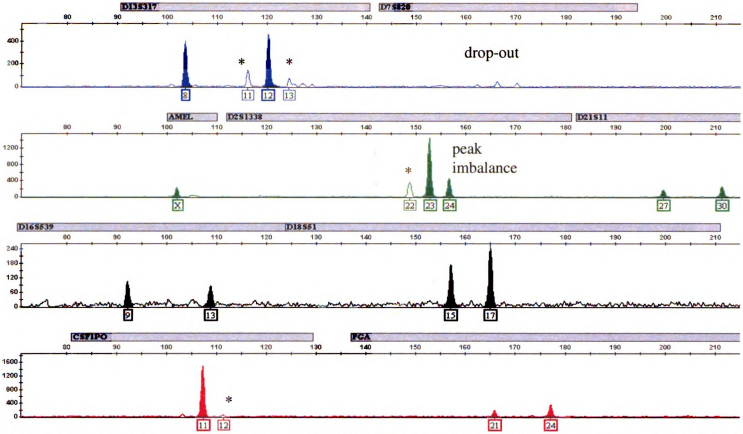
Full profiles were obtained from 42 of the 69 (60.9%) PCR reactions where input DNA was outside the recommended range. However, as the amount of DNA added to the reactions deviated from this range electropherogram quality was compromised. Electropherograms from reactions with less than the suggested quantity of DNA were characterized by increased stutter, allele drop-out/drop-in, heterozygote peak imbalance, and peaks of low intensity (e.g., Figure 8). Electropherograms produced from reactions in which too much DNA was added had features such as split peaks caused by incomplete 'A' addition, baseline noise, pull-up, and increased stutter (e.g., Figure 9).

Figure 7. Electropherogram from 4Z1UP, representing the recommended amount of input DNA for typing.



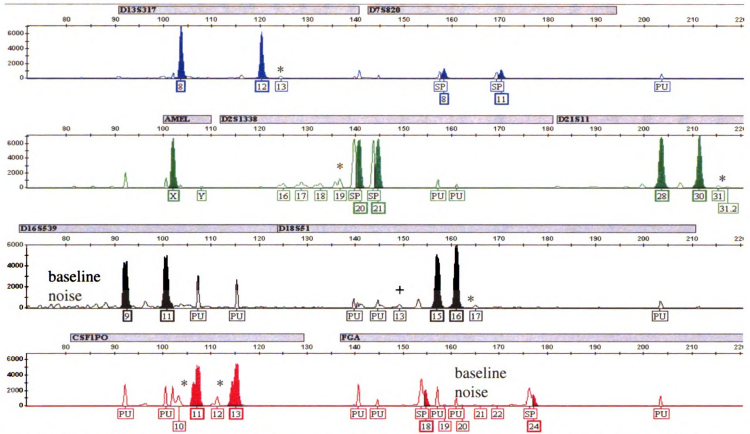
Sample 4Z1UP had 660 pg of DNA added to the PCR reaction. Handler alleles are shaded. When the recommended amount of DNA was added (0.5 – 0.75 ng) to PCR reactions electropherograms had more balanced peaks, higher RFU values, and fewer superfluous alleles. The two extra alleles in this electropherogram (9 at D16 and 10 at CSF) can possibly be attributed to drop-in. Peaks that are shown but nut numbered represent stutter, except for the artifact at D16.

Figure 8. Electropherogram from 3Z5P, representing LCN typing.



Sample 3Z5P had 98.4 pg of DNA added to the PCR reaction. Handler alleles are shaded. Electropherograms from LCN typing were characterized by increased stutter (*), heterozygote peak imbalance (locus D2), allele drop-out (locus D7), and low peak intensities. Note that peaks may seem larger than they are, but RFU values on the Y axis have been adjusted so peaks are visible (for reference, review RFU values in Figures 7 and 9).

Figure 9. Electropherogram from 8LSP, representing too much DNA being added to the PCR reaction.

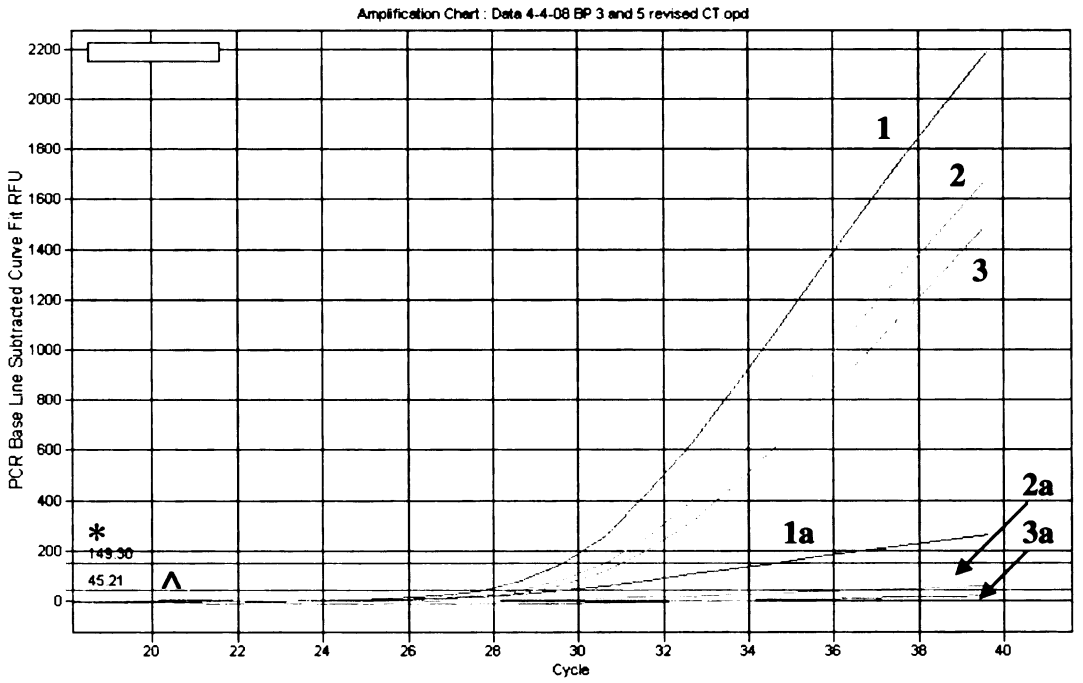


Sample 8LSP had 3.22 ng of DNA added to the PCR reaction. Handler alleles are shaded. Electropherograms were characterized by increased stutter (*), split peaks (SP), pull-up (PU), baseline noise (leading to false alleles), peaks with high RFU values (note RFU values on Y axis), and secondary stutter (+).

Detection of PCR inhibition via the internal positive control

The majority of IPCs (all but 5) had threshold cycle values within 20 – 30, indicating that the PCR reactions were minimally or not inhibited. The IPC threshold cycle for 3RSP replicates 2 (37.31) and 3 (threshold not reached in 40 cycles) were outside of this range, and replicate 1 also showed poor amplification, indicating partial inhibition (Figure 10). The IPCs failed to amplify for all three replicates from 7RSP, however, human DNA amplification was detected.

Figure 10. Quantitation results for 3RSP.



Amplification of human DNA and IPCs for replicates of 3RSP. The threshold for the DNA (149.30) is represented by the top horizontal line (*) and the IPC threshold (45.21) is represented by the lower horizontal line (^). Human DNA amplification is represented by the top three lines (1, 2, and 3), and the corresponding IPCs are denoted 1a, 2a, and 3a. All replicates show signs of PCR inhibition.

DISCUSSION

The goal of this study was to investigate the feasibility of recovering DNA from post-blast IED containers, and determine if DNA typing using miniSTRs could be used to correctly identify the handler. This approach stemmed from earlier research aimed at recovering and typing DNA from IED components themselves. Although a direct comparison to previous studies cannot be made due to different targets of DNA recovery, the results showed an improvement in the ability to correctly identify the handler. Correct handler profiles were obtained at all nine loci for seven of the eight backpacks, with the remaining backpack having a single ambiguous allele.

Discriminatory power was also enhanced compared to previous studies. The most successful study to date that identified handlers from recovered IED components used mtDNA (Gehring 2004), but mtDNA polymorphisms are linked and passed along maternal lineages, which limits statements concerning uniqueness. On the other hand, the selected STR loci sort independently, allowing individual allele frequencies to be multiplied, resulting in random match probabilities that often exceed one in a trillion. The Minifiler™ kit is reported to have a probability of identity of 8.21×10^{-11} for U.S. Caucasians (Applied Biosystems 2007a), which can be further improved if supplemented with results from standard STR kits. Identifiler™, a 16 locus multiplex from Applied Biosystems, has a probability of identity of 6.10×10^{-8} for U.S. Caucasians (excluding the eight STR loci in Minifiler™). A full profile created using both kits gives a probability of identity of 5.01×10^{-18} (Applied Biosystems 2006), representative of the

discrimination that is desired from forensic analyses and an immense improvement over mtDNA analyses.

There are a variety of possible reasons as to why more success was achieved in recovering and typing DNA from containers rather than the IEDs themselves, the main ones of which were portrayed in four hypotheses formulated prior to the research. First, it was proposed that the perpetrator (handler) would have extended contact with the container, with ample opportunities to deposit shed cells. All participants who carried the backpacks indicated that they used them for 7 – 10 days. However, depositing cells also depends on an individuals' shedder status, as was demonstrated in studies performed by Lowe et al. (2002). Quantifying the DNA from backpack samples revealed that participants who handled backpacks 3, 4, 7, and 8 were likely better shedders as these samples consistently had DNA quantities in the hundreds of picograms across all locations, while the other backpacks had lower values. Despite indications that some handlers were poor shedders, the period of handling proved sufficient to deposit cells, as every area swabbed resulted in at least a partial profile of the handler. Based on the results, it is likely that possessing the backpacks for fewer days would have been adequate to successfully identify the handler.

The second hypothesis was that the more textured surfaces of the backpacks would retain shed cells better than the smoother surfaces of the pipe bombs. Critical to all studies involving touch DNA is the transfer of cells from the handler to the object, and their persistence. Cells on smoother surfaces, such as pipe bombs, are likely to adhere loosely, making them more susceptible to removal upon subsequent contact. On the other hand, cells deposited on more textured substrates are retained better (Kisilevsky and

Wickenheiser 1999). All backpack regions targeted for DNA recovery were more textured than smooth except for the zipper tabs. The improved success in recovering DNA from backpacks compared to pipe bombs suggests that collection efforts should first focus on textured surfaces when conducting analyses involving touch DNA. However, caution should be taken as it may result in more mixtures as cells from previous handlers are also likely retained. This idea is supported by the observation that of the six zipper samples that were classified as mixtures, none were from those in which only the tabs were swabbed, but instead stemmed from zippers in which the textured strings were recovered and swabbed,. The possibility that smoother surfaces might not retain cells well could potentially be advantageous based on the thought that cells from previous handlers would be removed and only those of the last person to touch the area would remain.

The third hypothesis was that DNA on the containers would be less degraded than that recovered from IEDs. An assay was not performed that specifically addressed DNA degradation, however, results from this study were vastly improved compared to previous research in which STR analyses were performed on DNA recovered from pipe bombs (Esslinger et al. 2004; Kremer and Foran 2008). This improvement may have resulted from DNA on the containers being less degraded, the use of miniSTRs, or a combination of both. A reduction in DNA degradation is possible if the containers were not exposed to the same extreme temperatures as the pipes, thus better preserving the integrity of the DNA. However, it is unlikely that DNA on the containers did not experience any degradation, in which case the incorporation of miniSTRs may have been instrumental in obtaining better results, as they are specifically designed to amplify degraded DNA. An

assessment of DNA degradation could be made by analyzing backpack samples with a conventional STR kit, such as Profiler Plus™ used by Esslinger et al. (2004). If full handler profiles were still obtained it would support the hypothesis that DNA from the backpacks is less degraded, as Esslinger et al. (2004) only obtained one full profile.

The final hypothesis was that pieces of the container would be larger than the IED fragments recovered in previous studies, making them easier to collect and swab. This was substantiated by the fact that of the non-zipper regions targeted for swabbing only one was not recovered. Zippers were not retrieved with as much success since they were susceptible to being dismantled during deflagration, however, 70% of zippers were still collected. The ease of recovery enabled multiple areas of a backpack to be swabbed, which served as 'replicate' analyses. Rather than processing a single swab from a backpack, as was done with pipe bombs in previous studies, multiple swabs from different backpack regions were processed. Confidence was gained in calling handler profiles when identical alleles repeatedly showed up at a locus. It seems likely that identifying handlers would have been less successful if only one swab was taken from the backpacks due to difficulties in differentiating handler alleles from peaks caused by mixtures and artifacts. Also, instances of allele drop-out would have been hard to assess with only a single swab.

Recovering DNA from multiple areas of a backpack requires making a decision as to how many samples need to be analyzed in order to confidently call the handler's profile. Eleven regions were targeted for DNA recovery, but all eleven areas were swabbed for only two backpacks due to the inability to recover some regions. However, it was found that calling a handler's profile depended more on the quality of the

electropherograms than on the number of regions swabbed. For instance, seven samples were obtained from backpack 7, but the handler's alleles were readily determined because five electropherograms had full, clean, profiles. On the other hand, ten samples were not sufficient to call the handler's alleles at the D21 locus of backpack 9 due to allele drop-out in four samples and at least three callable alleles in five. If electropherograms are of good quality, swabbing three to five regions may be sufficient to confidently call the handler's profile, and additional areas can be swabbed and analyzed as needed.

The quality of electropherograms was often compromised by allelic drop-out, which in most cases was attributed to low levels of DNA being added to the PCR reactions, as 24 of 27 (88.9%) experiencing drop-out had DNA quantities below 275 pg. The three reactions that experienced drop-out with more than 275 pg of DNA (3NP, 5LSP, and 7RSP) had a total of five loci with drop-out, two each at D16 and D7, and one at FGA which was attributed to a split speak preventing the 50 RFU threshold from being obtained. Drop-out at three loci in sample 7RSP likely stemmed from partial inhibition as the IPC failed to amplify in all three replicates. However, as with degraded DNA, larger loci are more susceptible to drop-out stemming from PCR inhibition (Bessetti 2007) which does not fit the pattern of drop-out that was observed. Locus D7 is just the fourth largest in the Minifiler™ kit and only amelogenin is smaller than D16 based on size of the largest possible alleles. Of all the loci that had drop-out of the handler's alleles, 29.3% came from D7 and 13.3% were from D16. FGA, D21, and D18, the three largest loci in the kit, contributed to 13.3%, 12%, and 5.3% of total drop-out, respectively.

To the author's knowledge there has not been any information published concerning differential amplification success among loci in the Minifiler™ kit. The increased prevalence of drop-out at D7 compared to the other loci may be a consequence of sub-optimal primer annealing conditions. Different primers have ideal conditions (temperature, concentration, etc.) in which they achieve maximum amplification efficiency. When a variety of primers are incorporated into multiplexes, optimal conditions for individual primers are usually compromised (Butler 2005). An interesting observation was that samples in which the D7 locus did not experience drop-out had relatively low RFU values at the locus. This was especially evident in reactions that had more than 1.5 ng of DNA added, where RFU values for alleles at D7 typically ranged from 500 – 1000, while approaching 6000 RFUs for D13 (e.g., Figure 9).

Drop-out was more prevalent in samples with low quantities of DNA, although, three LCN samples did not experience drop-out at any locus. Two of these were from backpack 5 (5NP and 5FTP) which was used by participant G, but this subject was homozygous at five of the nine loci, meaning it might be more difficult to detect allele drop-out. The other LCN sample, 10FMP, had 63.6 pg of DNA added to the PCR reaction. However, as with the quantitation of other samples containing low amounts of DNA, there is little confidence in the precision and accuracy of this quantity due to stochastic sampling effects (i.e., there may have been more DNA). For example, DNA amounts for 10FMP ranged from 0.0 pg/μL to 32.6 pg/uL (Appendix B). Pipetting low quantities of DNA increases the chances of unequal sampling, and must be taken into account when quantifying small amounts of DNA. Quantifying samples in triplicate and calculating average DNA values is advised to circumvent this phenomenon.

Electropherogram quality was also affected by extra peaks as approximately 45% of the loci contained callable alleles in addition to the handler's. The majority of the extra peaks were attributed to stutter and mixtures. The presence of a peak one repeat unit before, and sometimes after, the true allele is indicative of stutter (e.g., Figure 7). Software used to analyze electropherograms can be programmed to not call peaks from this artifact, however, when input DNA deviates from the recommended range (0.5 – 0.75 ng for Minifiler), stutter peaks are known to increase (Applied Biosystems 2007a; Budowle et al. 2001), causing the software to call them. A rough assessment of the prevalence of increased stutter showed that 118 of the 272 (43.4%) loci categorized as having extra peaks (amelogenin was excluded because it is not an STR) could be re-categorized as containing only the handler's alleles after possible stutter was accounted for. Many of these peaks were likely a result of increased stutter as peak heights were 15 – 20% that of the true allele. However, it is unlikely that peaks in all 118 loci were the result of stutter. For example, sample 8Z1UP had three loci with extra peaks that could be attributed to stutter, however, this reaction was classified as a mixture as the peaks were one repeat unit greater than the true allele, a rare location for stutter.

Eighteen samples, including 8Z1UP, were classified as mixtures based on the presence of callable alleles besides the handler's at six or more loci. A possible explanation is that there were multiple handlers of the backpack, but all participants in the study said that they were the only user of their backpack. Nonetheless, casual contact is all that is needed to transfer shed epithelial cells (Van Oorschot and Jones 1997). Brief contact with a passerby, or a classmate simply moving the backpack, might have resulted in cells being deposited without the handler's knowledge.

Mixtures could have also been caused by contamination from researchers. Specific measures were implemented to avoid contamination knowing that it had caused problems in previous studies involving recovering DNA from IEDs (e.g., Gehring 2004; Kremer and Foran 2008). No direct sources of contamination were found when DNA profiles of researchers involved in this study were compared with mixtures, although it cannot be concluded that no researcher contamination occurred. For example, sample 8Z4UP had 15 of the main investigator's 18 alleles called. It is possible that the three alleles that were not called were masked by un-called stutter from the handler's alleles, as all missing alleles fell one repeat unit shorter than a handler's allele. It is also conceivable that the main investigator's DNA was such a minor component of the mixture that the three alleles not present in the profile dropped out. Sample 3Z1UP was the only other mixture where there was a strong indication that the main investigator was a contributor (15 of 18 alleles called). Five additional mixtures had indications that the main investigator may have been a contributor, but there were not as many of the investigator's alleles called as were seen in the previous two cases. Comparing mixture profiles with profiles of other investigators involved in the study did not result in any indications of their contamination.

Contamination from researchers could have occurred at any one of numerous steps ranging from backpack preparation to PCR amplification. However, the step most likely vulnerable to contamination was swabbing the backpacks, at which time they were handled extensively. In particular, the zippers required the most handling during swabbing because of their small size. Zipper samples accounted for 6 (33%) mixtures, including both cases where there was a strong indication that contamination stemmed

from the main investigator. The straps accounted for 7 mixtures, three of which had allele calls that suggested the main investigator was the contributor. On the other hand, no mixtures were obtained from front tab samples, and DNA recovered from the top handles only resulted in one mixture.

A study being conducted concurrently in the lab revealed another potential source of contamination. Mitochondrial DNA analyses performed on 'clean' swabs (860PPC, Puritan Medical Products Co.) showed the presence of mtDNA, which resulted in switching to a different type of swab (25-806 2PC, Puritan Medical Products Co.). However, the five STR analyses of the swab reagent blanks (backpacks 7, 9, and 10 – 12) made using the potentially contaminated swabs produced a total of two callable alleles, while the three reagent blanks made using the new swabs produced one (no swab reagent banks were processed with backpacks 3 and 4). The three callable peaks were attributed to sporadic contamination, and the concern over swab contamination was lessened given that nuclear DNA contamination was not detected. This is likely attributable to the increased sensitivity of mtDNA analyses.

Results from backpack 12 (the negative control) exposed another possible source of extra peaks in electropherograms—exogenous DNA remaining on the backpacks after the decontamination process. Analysis of 12Z1UP produced a full male profile (Appendix A) that could not be linked to anyone directly involved in the study (researchers or participants). Electropherograms from the remaining eight backpack 12 samples produced a total of ten callable alleles, six of which matched alleles from 12Z1UP. This suggested that all areas were not sufficiently exposed to UV irradiation, and DNA deposited on the backpack during manufacturing and/or delivery persisted.

Processing the control swabs that were taken before backpacks were distributed to participants (see Material and Methods) would have assisted in shedding light on the extent to which DNA remained on the backpacks after decontamination, however, this was deemed unnecessary as the peaks stemming from the negative control backpack samples were mostly of low RFU values.

In a forensic setting, backpacks would not be decontaminated in advance, and could easily harbor DNA from more than one individual. Even in the current research where decontamination procedures were implemented, mixtures and loci with more than two callable alleles were prevalent. However, as noted above, this problem was overcome by swabbing multiple backpack regions and using information from all the swabs to create a consensus profile.

Swabbing different backpack regions also revealed that some were better targets for DNA recovery than others. The top handle produced the handler's alleles at all nine loci for every backpack, while DNA recovered from swabs of the left strap, front middle, and neck region producing similarly good results (Table 4). Conversely, the right strap, zippers, and front tab averaged less than eight loci with the handler's correct alleles. Recovering alleles may have been dependent on the surface area of the region swabbed, given that the small zippers and front tab produced the poorest results. The right strap was an exception as it averaged one locus less than the left strap, however this number was skewed due to two right strap samples, 7RSP and 9RSP, which had 6 and 4 correct loci, respectively. As previously mentioned, problems involving 7RSP were likely due to PCR inhibition, while an insufficient amount of template DNA probably led to drop-out at 9RSP.

Recovering alleles was also likely influenced by the frequency of which participants utilized different regions of the backpacks. The overall level of contact is unknown of course, however, normal backpack use involves the straps being handled in order to wear it, providing ample opportunities for DNA to be deposited via grabbing the straps and putting the wearer's arms through them. Top handles are also commonly used on backpacks. The success in recovering alleles from the neck region could have stemmed from its location between the top of the straps and the top handle, making it susceptible to shed cells when either region was utilized. Further, this region was prone to contact with the handler's neck and hair. In this regard, it is recommended that DNA recovery efforts focus on regions that are likely to be handled or contacted by the user, and although swabbing multiple areas was shown to be a very successful approach to identifying handlers, larger regions may be advantageous for recovering a satisfactory number of cells for analysis.

Quantitating the DNA provided another means of comparing the different regions targeted for DNA recovery. Quantitation results showed all of the non-zipper regions, except for the front tab, averaged close to 300 pg/μL of DNA, supporting the hypothesis that textured regions and/or surface area are important factors in the recovery of cells. However, it is also possible that certain parts of the backpack, such as the front tab, were simply not utilized by the handler. Lower quantities of DNA were also recovered from the zippers, but there was variation in DNA amounts depending on the components that were swabbed. The average amount of DNA from zippers that retained tabs (124.76 pg/μL) was greater than those that did not have them (45.2 pg/μL). This disparity among zippers might have been caused by the ease of swabbing the plastic surface of tabs

compared to the strings, which were often singed. Even if DNA persisted on the singed strings, difficulties in swabbing them, such as the cotton tip of the swabs becoming unwound, likely hindered recovery. Strings that were not singed also presented problems when swabbing as they were small and intertwined with the pull. Like the tabs, the metal pulls were easy to swab, but the zippers were constructed in a manner that favored the user gripping the tab or the string, hence these areas likely harbored more DNA

Using Quantifiler™ to quantitate the DNA also provided a means of assaying PCR inhibition. Along with sample 7RSP, 3RSP showed signs of inhibition based upon poor amplification of the IPCs, however, in both cases genomic DNA was still detected Sample 3RSP was likely subjected to partial inhibition as, unlike 7RSP, a full handler's profile was obtained. It has been reported that when the IPC does not amplify, but human DNA does, the IPC results should be disregarded, however, this is only valid for high amounts of DNA (>10 ng) as competition between the genomic DNA and the IPC leads to the suppression of IPC amplification (Applied Biosystems 2006a; Katz 2007). Initially, inhibition concerns centered on post-blast residue, but inhibition most likely would have been more prevalent if that was the cause. Inhibition in samples from right straps suggests that there was some feature about the straps that hindered PCR. Straps were made from a different material than the rest of the backpack, and pre-blast control swabs of straps sometimes turned black. This substance may have been deleterious to PCR, although the left strap samples did not show any signs of inhibition. The exact reasons for PCR inhibition were not resolved, but its low occurrence in general resulted in minimal impact on the study. However, this does not mean PCR inhibition will be absent in all analyses of IED containers. Variation in the composition of containers (e.g.,

cardboard, fabric, wood, or metal), and the elements to which the containers have been exposed to before and during deflagration (e.g., soil, blood, water), provide many diverse situations, each with the potential of producing different PCR inhibitors.

CONCLUSIONS

The impact that IEDs have had in the Middle East has caused growing concern that these devices will become more prominent domestically, with the potential to have profound implications on American society. Two experts, counterterrorism consultant David Williams and Michigan State Police Bomb Squad Commander Lt. Shawn Stallworth, both say that they have already seen an increase in IED usage in the United States (personal communications). Even with the lack of a large scale domestic IED event since 9/11, IEDs are implemented daily throughout the United States.

Results of this study show that post-blast DNA recovered from IED containers can be used to correctly identify the handler. Analyzing multiple samples from the same backpack and creating a consensus profile proved to be an effective approach to identifying handlers and was necessary to circumvent drop-out and mixtures. All areas targeted for swabbing produced a handler's full profile from at least one backpack, but overall, regions that were larger and likely used more often resulted in higher quantities of DNA and more loci with the handler's alleles. With this in mind, it is recommended that swabbing first be directed towards areas that are likely to be handled, particularly larger areas initially and then smaller regions (e.g., zippers), as need, resources, and time permit. However, if only small pieces of a container are available, these should not be deemed irrelevant because they can still produce informative data.

The research presented offers a feasible and practical approach towards national efforts aimed at countering IEDs, and answers DHS Secretary Chertoff's call to the scientific community to develop means to counter IEDs (Chertoff 2007). Although much

of the focus of countering IEDs has been on developing measures to prevent them from detonating, the fact that this research focused on post-blast analysis does not take away from its value, as it is unlikely that IED countermeasures will ever thwart all IED events. This study demonstrated an effective way to identify those responsible for IEDs, and aids in their apprehension, which will most likely prevent future attacks and allow citizens to feel more secure. By disseminating the findings of the research to law enforcement personnel and emergency responders, the importance of properly collecting post-blast fragments can be stressed, and DNA analyses of IED containers can become a valuable tool in IED investigations.

APPENDIX A. Allele calls and loci categorizations

The tables show allele calls made for the backpacks involved in the study. Tables for the control backpacks (11 and 12) are included, however, they served solely as references and their data were not incorporated into analyses. Loci from each sample are colored to signify the category they were placed in (see Results). DNA profiles 'Call' were blindly made based upon the review of all loci, and checked for concordance with reference profiles from study participants 'Subject'. The following key is for interpreting all tables.

Symbol	Description
	The locus contained only the handler's correct alleles.
	The locus had multiple allele calls, but the handler's alleles constituted the major profile.
	The locus had multiple allele calls, but the handler's alleles could not be distinguished as the major profile.
	The locus had at least one of the handler's correct alleles.
	None of the alleles matched the handler's profile.
	No alleles were called at the locus.
BOLD	Peak was between 100 and 149.9 RFUs
*	Peak was between 50 and 99.9 RFUs
+	Classified as a mixture

Backpack 3

Swab	Locus								
	D13	D7	AMEL	D2	D21	D16	D18	CSF	FGA
3Z1UP+	8,12	10,11	X,Y*	23,24;18,20	27,30	11,13;10*,12*	15,17;12,16	11,12	21,24;20
3Z2UP	8,12		X	23,24	27,30		15,17	10,11	
3Z3UP	8,12		X	23,24;**17**	27,30	12*	15,17	11	21*,24*
3Z4P	8,12;**11**	**10**,11*	X	23,24;**25**	27,30	9*,11*,13*	15,17;13*	11	21,24
3Z5P	8,12;**11**,13*		X	22,23,24	27,30	9,13*	15,17	11,12*	21,24
3THP	8,12;**13**	10,11	X,**Y**	23,24;19,20	27,30	11,13;9	15,17	10,11,**12**,**14**	21,24
3LSP	8,12	10,11	X,**Y**	23,24;18*	27,30	11,13	15,17	10,11,**12**	21,24
3RSP+	8,12	10,11;**7**	X	23,24;20	27,30;28	8,9,11,13	15,17;18*	10,11	21,24;18.2
3NP	8,12	10,**11**	X	23,24;25*	27,30		15,17	10,11,12*	21,24
3FTP	12		X	23*,24	27*, 30	11*,13*	15,17	11	21
3RB									
Call	8,12	10,11	X	23,24	27,30	11,13	15,17	11	21,24
Subject B	8,12	10,11	X	23,24	27,30	11,13	15,17	11	21,24

Backpack 4

	Locus								
Swab	D13	D7	AMEL	D2	D21	D16	D18	CSF	FGA
4Z1UP	8,11	9,10	X	20,25	27,30	12,13;9*	13,16	12,13;10	21,25
4Z2UP	8,11	9,10	X	20,25	27,30;28*	12,13	13,16	12,13;10,11	21,25
4Z3UP	8,11	9,10	X,Y*	20,25	27,30	12,13;**9**	13,16	12,13;9*,10*	17,21,25
4Z4UP	8,11	9,10	X,Y*	20,25	27,30	12,13	13,16	12,13;**10**	21,25
4THP	8,11	9,10	X	20,25	27,30;**28**	12,13;9	13,16	12,13;11	21,25
4LSP	8,11	9,10	X	20,25	27,30;**28**	12,13;**9**	13,16	12,13;10,11	21,25
4RSP	8,11	9,10	X	20,25	27,30;28	12,13;8	13,16;**14**	12,13;10,11	21,25
4NP	8,11	9,10	X,Y*	20,25	27,30;28	12,13;8	13,16;**14**	12,13;10,11	21,25
4FMP	8,11;**12**	9,10	X	20,25	27,30;28	12,13;9	13,16;14,15	12,13;11	21,25
4FTP	8,11;**12**	9,10	X	20,25	27,30;**28**	12,13;8	13,16	12,13;10	21,25
4RB		11*							
Call	8,11	9,10	X	20,25	27,30	12,13	13,16	12,13	21,25
Subject E	8,11	9,10	X	20,25	27,30	12,13	13,16	12,13	21,25

Backpack 5

	Locus								
Swab	D13	D7	AMEL	D2	D21	D16	D18	CSF	FGA
5Z1UP	11*,12		X	23,24		10	16	11,12	21,23.2*
5Z2UP	11*,12*		X*	23,24;17	32	10	16	10,11,12	21
5THP	11,12	11	X	23,24;18	29,32;30	10	15,16	11,12	21
5LSP	11,12		X	23,24;20	29,32	10	16	11,12	21
5RSP	11,12;8*		X	23,24	29,32*	10	16	11,12	21
5NP	11,12;13*	11;8*	X	23,24;16,17,19,26	29,32;30	10	16	11,12;13	21
5FMP	11,12,13*	11	X	23,24	29,32	10	16	11,12;13	21,25*
5FTP	11,12	11	X	23,24	29,32	10	16	11,12	21
5RBSWAB									
5RB									
Call	11,12	11	X	23,24	29,32	10	16	11,12	21
Subject G	11,12	11	X	23,24	29,32	10	16	11,12	21

Backpack 6

	Locus								
Swab	D13	D7	AMEL	D2	D21	D16	D18	CSF	FGA
6Z1UP	12		**X**	20,24	31*,31.2	**9,11**	**13**,14*,17*	11,**12**,13	20*
6Z2UP	12*			17*,19,**24**	31.2*	9		12	25*
6THP	12	10,11	X	20,24;26	28,**30**,31,31.2,33*, **33.2**	9,11	13,14	11,12;10	20,25
6LSP+	8*,**10**,12	9*,**10**	X,Y*	20,24;17, **21**	28,**29**,**30**,31,31.2, **32.2**	9,11;10	13,14;12,16*	11,12;10	20,**21**,22*,23*, 25
6RSP+	8*,**10**,11, 12	8*,9*,10, 11	X,**Y**	20,21,23, 24,25	31,31.2;27*,28*,30	9,11;12*	13,14;12,**15**,**16**, 17*	11,12;10, 13	20,**23**,24*25
6NP+	8,12	10,11	X,**Y**	20,24;23	31,31.2	9,11;**12**	13,14;15,16*	11,12;10	20,25;23
6FMP+	11,12	10*,11*	X,**Y**	18,20,22, 24	27,28,**31**,31.2,**32.2**	9,11;12*, 13*	13,14;12,**15**,16*	10,11,12	20,25;21,22*,23*
6FTP	11*,12	10*,11*	X,**Y**	20,24	27*,28*,31,**31.2**	**9**,11,12*	**12**,**13**	11,12	20,23*,**25**
6RBSWAB									
6RB									
Call	12	10,11	X	20,24	31,31.2	9,11	13,14	11,12	20,25
Subject I	12	10,11	X	20,24	31,31.2	9,11	13,14	11,12	20,25

Backpack 7

	Locus								
Swab	D13	D7	AMEL	D2	D21	D16	D18	CSF	FGA
7Z1UP+	11,12;13	8,12;9*	X,Y*	17,20;18,22,**25**	31,OL;28,29,30	9,11	**7**,13*,14,17	10,11;**12**	22,23.2;24*
7THP	11,12	8,12	X	17,20	31,OL; 29	9,11*	12,16,17,18	10,11;**12**	22,23.2;**20**
7LSP	11,12;**13**	8,**12**	X	17,20;18,23*,24	31,OL;29*	9,11*	**12**,**15**,17	10,11;12	22, 23.2
7RSP	11,12		X	17,20;25	31,OL	9	12*,14*,15,17	10,11;12,**13**	22*
7NP	11,12	8,12	X,Y*	17,20;23*	31,OL; 29	9,11	16,17	10,11;12	22,23.2
7FMP+	11,12;8	8,12;11	X,Y*	17,20;22,23	31,OL;31.2	9,11	14,17	10,11;13	22,23.2;19*,20,25
7FTP	11,12;8*		X	17,20;22,23	31,OL	9,11*;12	17	10,11	**22**,23.2
7RBSWAB									
7RB									
Call	11,12	8,12	X	17,20	31,OL**	9,**11**	17	10,11	22,23.2
Subject J	11,12	8,12	X	17,20	31,OL**	9,**11**	17	10,11	22,23.2

**Allele consistently showed up between the bins for alleles 33 and 33.2, hence, the off ladder allele was concluded to be 33.1.

Backpack 8

	Locus								
Swab	D13	D7	AMEL	D2	D21	D16	D18	CSF	FGA
8Z1UP+	8,12;10*	8,11	X,Y*	20,21;17	28,30;31	9,11;12*	15,16;**13**	11,13	18,24;**19**
8Z2UP	8,12	8,11	X	20,21;**16,18**,24*	28,30	9,11	15,16;13*,17*	11,13;9	18,24;20
8Z3UP+	8,12;9*,11	8,11	X	20,21;18,25,26	28,30;**31**	9,11;12*, 13	15,16;12,13*, 17	11,12,13	18,24;20,21*,22
8Z4UP+	8,9*,11,12	8,9,11	X,Y	18,20,21,23,25	28,30	9,11,**12**, 13	12,14,15,16, 18	10,11,12, 13	18,20,21,23,24
8Z5P	8,12;13*	8,11	X	20,21;**22**,23,26	28,30	9,11	15,16;17*	11,13	18,24;20
8THP+	8,12;**13**	8,11	X;Y	20,21;16,17,18, 22*	28,30;**31.2, 32.2**	9,11	15,16;**17**	11,13;10	18,24;19*,20*
8LSP+	8,12;13	8,11	X,**Y**	20,21;16,17,18,19	28,30;31,**31.2**	9,11	15,16;13,17	11,13;10,12	18,24;21*,22*
8RSP	8,12;13*	8,11	X	20,21;**17**,25	28,30	9,11	15,16;12*	11,13;12	18,24;19*,20, 21*,22
8NP	8,12	8,11	X,**Y**	20,21;16,17,18	28,30;**31**	9,11	15,16	11,13	18,24
8FTP		**8,11**	X	14,21	28*,30	9,11*	15,16	10,11,13	**18**,19*,**21**,24
8RBSWAB 8RB	12*								
Call	8,12	8,11	X	20,21	28,30	9,11	15,16	11,13	18,24
Subject F	8,12	8,11	X	20,21	28,30	9,11	15,16	11,13	18,24

Backpack 9

	Locus								
Swab	D13	D7	AMEL	D2	D21	D16	D18	CSF	FGA
9Z1UP	8,12,14		X	17,20,**26**	29,30,31	12*	13,16;17*	12	20*,22*
9Z2UP	11*			17	31*	12*	16		
9Z3UP	8,12	8*	X*	17,19,20	30	9*	13,16	**7**,11,12,13	**20,22**
9Z4P+	8,9,10,11,13, 14	8*,10*	X	17,20,24	30,**31**,31.2	12*,13*	**12**,13,14,15*,16	10,11,12	20,21*,22
9THP	8,9*,**11**,12, 13,14	**8,10**	X	17,20;18,22,24*, 26	30,**31**	12,**13**	13,16;14*,20*	10,11,12	20,21*,22
9LSP+	8,14;9,11,**12**	**8**,10	X	16,17,18,20,25*	28,29,30,31	**10**,12,**13**	13,16;12,14,17*,**19**	10,11,12,13	20,22,23
9RSP	8,11,12*		X*	17,20;23,24,26	30*		13,16	10,12	
9NP+	8,14;9,10,**13**	8,10,11*	X,Y*	17,20;18,19	29,30,31, **31.2**,32.2*	12,13	13,16;17*	12	20,21,22
9FMP	8,14;**10**	8*	X	17,20	28*,30,31*	11*,**12,13**	13,16;19*	10,11,12	20,21*,22
9FTP		8,10*	X	17,20;25*	30*	**12**,13	13,16	12	
9RBSWAB 9RB									
Call	8,14	8,10	X	17,20	No call made**	12,13	13,16	12	20,22
Subject A	8,14	8,10	X	17,20	30,31	12,13	13,16	12	20,22

**—Call could not confidently be made between a 30 homozygote or a 30, 31.

Backpack 10

	Locus								
Swab	D13	D7	AMEL	D2	D21	D16	D18	CSF	FGA
10Z1UP	11,12	**9**,10*	X,Y	19,25	29,30	11,12	12,13,16	10,**11**,12,13	19*,**20**,**21**,22,23
10Z2UP	11,12	9*,12*	X,**Y**	18,19	**28**,29*,**30**	10*,**11**	**12**,13*,16,18	12,13*	**20**,22*
10Z3P	11,12	9,**12**	X	18,19,25	29,30	11,**12**	13,16;**15**	12,13,14	22,23
10Z4P	12		X	20,25	29*,30	11,**12**	13*,16	12,13	**22**,23,24*,25
10Z5P	**12**	9,11*	X	**18**,19	29*	9*,10*,**11**	13,16	12,13	20*,**22**,23
10THP	11,12	9,**12**	X	19,25	29,30	11,12*	13,16	12,13;10,11	22,23;20*,24*
10LSP+	11,12,**13**	9,10,12	X	19,25;17*, 18,24	26,29,30, **31.2**	11,**12**	13,14,**15**,16	12,13;11,14*	22,23
10RSP+	11,12	9,**10**,11*12	X	19,25;**16**, 17,18	29,30;**31**	11,12*,**9**	13,16;**10**,15,17,21*	12,13;10	22,23
10NP	11,12	9,12	X	19,25;**23**	29,30;**31**	11,12*	13,16;12	12,13	22,23
10FMP	11,12*	9,**12***	X	18,19,25*	29,30	11,**12**	13,16	12,13;10*,11*	22,23*
10FTP	11	9	X	17,**19**, 20	29	11*,12*	13*	12,13*	
10RBSWAB								**12**	48.2*
10RB			X*,**Y**					10*	
Call	11,12	9,12	X	19,25	29,30	11	13,16	12,13	22,23
Subject H	11,12	9,12	X	19,25	29,30	11	13,16	12,13	22,23

Backpack 11 + control	Locus								
Swab	D13	D7	AMEL	D2	D21	D16	D18	CSF	FGA
11Z1UP	8,11;12*	9*	X,Y*	18,19,20,25	29*,31.2	12*	15,16*	10,12	21*,23,**24**
11Z2UP								**12**	
11Z3UP	8,11	9,12	X	18,25	**30**,31.2	11,12	12,15	12	23,24;**21**
11Z4UP	8*		X	25	31.2				24*,25*
11THP	8,11;12*	9,12;11*	X	18,25	28,31.2	11,12	15,17*,18*	12	23,24
11LSP	8,11	9,12	X,Y*	18,25	31.2	11,12	15	12	23,24
11RSP	8,11;12,14*	9,12;8*	X	18,25;19	31.2	11,12;9*	12*,15,16*	**9**,12	23,24;20*
11NP	8,11;12*	9,11,12	X,Y	18,25;19,20	28,31.2	11,12;9	15;16	10,12	21,23,24
11FMP	8,11	9,**12**	X	18,25	31.2	11,12	15;16*	12	23,24
11FTP	8,10*,11,12*		X	18,25	31.2	11*,12*	15	12	23,**24**
11RBSWAB 11RB									
Call	8,11	9,12	X	18,25	31.2	11,12	15	12	23,24
Handler	8,11	9,12	X	18,25	31.2	11,12	15	12	23,24

Note: Data from this backpack were not incorporated into analyses.

Backpack 12 - control									
	Locus								
Swab	D13	D7	AMEL	D2	D21	D16	D18	CSF	FGA
12Z1UP	11	10*	X,Y	19,**20,24**	28,**31**	9,15	15,16	10,**12**	21,23
12Z2UP									
12Z4P	11*						**13**		
12THP							12*,16*		20*
12LSP								10*	
12RSP									
12NP			Y*	19				11*	
12FMP				**19**					
12FTP									
12RBSWAB									
12RB									

Note: Data from this backpack were not incorporated into analyses.

APPENDIX B. DNA quantitation data

DNA samples were quantified in triplicate using Quantifiler™. The averages and standard deviations were calculated for all samples and are reported in nanograms. Reactions in which the threshold cycle of the internal positive control was above 30 are noted. Areas swabbed were labeled as; backpack#/identifer/P. The identifiers used were: Z# for zippers (# replaced by 1, 2, 3, 4, or 5 depending upon the zipper's origin; if the origin of the zipper was unidentifiable a 'U' was added after the number), TH for top handle, LS and RS for left and right strap, respectively, FM for front middle, FT for front tab, and N for neck region. For example, 3THP indicates the swab is from the top handle of backpack 3, while 3Z1UP indicates that the swab is from the first unidentifiable zipper of backpack three.

Backpack 3	Replicate				
Swab	1	2	3	Average	Standard Deviation
3Z1UP	0.113	0.0874	0.0970	0.0991	0.0129
3Z2UP	0.0689	0.0909	0.0461	0.0686	0.0224
3Z3UP	0.0451	0.0671	0.0310	0.0476	0.0184
3Z4P	0.0943	0.116	0.118	0.109	0.0131
3Z5P	0.0389	0.0160	0.0189	0.0246	0.0125
3THP	0.196	0.271	0.179	0.215	0.0490
3LSP	0.238	0.241	0.192	0.224	0.0275
3RSP	0.641	0.347*	0.267**	0.418	0.197
3NP	0.322	0.302	0.251	0.292	0.0366
3FTP	0.0416	0.0172	0.0248	0.0279	0.0125

*—IPC had a threshold cycle of 37.31.

**—IPC did not reach the threshold cycle by 40 cycles.

Backpack 4

Swab	Replicate 1	2	3	Average	Standard deviation
4Z1UP	0.154	0.152	0.188	0.165	0.0202
4Z2UP	0.427	0.387	0.364	0.393	0.0319
4Z3UP	0.153	0.192	0.103	0.149	0.0446
4Z4UP	0.291	0.202	0.261	0.251	0.0453
4THP	0.803	0.650	0.743	0.732	0.0771
4LSP	0.926	0.963	0.787	0.892	0.0928
4RSP	1.25	1.16	1.29	1.233	0.0666
4NP	1.06	1.62	1.07	1.25	0.320
4FMP	0.895	0.890	0.759	0.848	0.0771
4FTP	0.410	0.369	0.375	0.385	0.0221

Backpack 5

Swab	Replicate 1	2	3	Average	Standard deviation
5Z1UP	0.00638	0.00972	0.0119	0.00933	0.00278
5Z2UP	0.00660	0.00915	0.0129	0.00955	0.00317
5THP	0.326	0.339	0.306	0.324	0.0166
5LSP	0.152	0.187	0.208	0.182	0.0283
5RSP	0.0596	0.0466	0.0402	0.0488	0.00989
5NP	0.0171	0.0194	0.0143	0.0169	0.00255
5FMP	0.0633	0.0285	0.0388	0.0435	0.0179
5FTP	0.0300	0.0173	0.000	0.0158	0.0151

Backpack 6

Swab	Replicate 1	2	3	Average	Standard deviation
6Z1UP	0.00221	0.0104	0.0131	0.00857	0.00567
6Z2UP	0.00270	0.000	0.00419	0.00230	0.00212
6THP	0.0554	0.0476	0.0681	0.0570	0.0103
6LSP	0.0524	0.0279	0.0400	0.0401	0.0123
6RSP	0.0538	0.0611	0.0438	0.0529	0.00869
6NP	0.0651	0.0735	0.0537	0.0641	0.00994
6FMP	0.0484	0.0320	0.0172	0.0325	0.0156
6FTP	0.000	0.0211	0.00320	0.00810	0.0114

Backpack 7

Swab	Replicate 1	2	3	Average	Standard deviation
7Z1UP	0.0846	0.132	0.0955	0.104	0.0248
7THP	0.293	0.280	0.347	0.307	0.0355
7LSP	0.182	0.187	0.198	0.189	0.00819
7RSP*	0.260	0.162	0.217	0.213	0.0491
7NP	0.158	0.238	0.180	0.192	0.0413
7FMP	0.368	0.536	0.618	0.507	0.127
7FTP	0.0907	0.0522	0.0482	0.0637	0.0235

*—IPC did not reach the threshold cycle by 40 cycles for all three replicates.

Backpack 8

Swab	Replicate 1	2	3	Average	Standard deviation
8Z1UP	0.214	0.158	0.124	0.165	0.0454
8Z2UP	0.260	0.265	0.202	0.242	0.0350
8Z3UP	0.150	0.146	0.152	0.149	0.00306
8Z4UP	0.0559	0.125	0.0939	0.0916	0.0346
8Z5P	0.282	0.273	0.334	0.296	0.0329
8THP	0.404	0.595	0.699	0.566	0.150
8LSP	0.775	0.874	0.767	0.805	0.0596
8RSP	0.271	0.221	0.322	0.271	0.0505
8NP	0.573	0.487	0.541	0.534	0.0435
8FTP	0.0245	0.00489	0.0180	0.0158	0.00999

Backpack 9

Swab	Replicate 1	2	3	Average	Standard deviation
9Z1UP	0.0105	0.0252	0.0182	0.0180	0.00735
9Z2UP	0.0171	0.0151	0.000	0.0107	0.00935
9Z3UP	0.0109	0.0101	0.000	0.00700	0.00608
9Z4P	0.0332	0.0312	0.0161	0.0268	0.00935
9THP	0.0492	0.00866	0.0326	0.0302	0.0204
9LSP	0.0560	0.0735	0.0308	0.0534	0.0215
9RSP	0.0429	0.0306	0.0332	0.0356	0.00648
9NP	0.0529	0.0860	0.0348	0.0579	0.0260
9FMP	0.0520	0.0269	0.0287	0.0359	0.0140
9FTP	0.0241	0.0199	0.00759	0.0172	0.00858

Backpack 10					
	Replicate				
Swab	1	2	3	Average	Standard deviation
10ZIUP	0.0457	0.0160	0.0152	0.0256	0.0174
10Z2UP	0.0209	0.00918	0.00752	0.0125	0.00729
10Z3P	0.0147	0.0436	0.0276	0.0286	0.0145
10Z4P	0.0188	0.0229	0.0493	0.0303	0.0166
10Z5P	0.0139	0.0208	0.00486	0.0132	0.00799
10THP	0.0606	0.0725	0.108	0.0804	0.0247
10LSP	0.0817	0.0564	0.0593	0.0658	0.0138
10RSP	0.0341	0.0490	0.0888	0.0573	0.0283
10NP	0.0315	0.063	0.0815	0.0586	0.0253
10FMP	0.0150	0.0326	0.000	0.0159	0.0163
10FTP	0.000	0.00422	0.000	0.00141	0.0024

REFERENCES

Akane A, Matsubara K, Nakamura H, Takahashi S, Kimura K. 1994. Identification of the heme compound copurified with deoxyribonucleic acid (DNA) from bloodstains, a major inhibitor of polymerase chain reaction (PCR) amplification. *J Forensic Sci.* 39:362-72.

Al-Soud WA, Rådström P. 2000. Effects of amplification facilitators on diagnostic PCR in the presence of blood, feces, and meat. *J Clinical Microbiology.* 38:4463-70.

Applied Biosystems. 2006, October. The next generation of STR technology begins with the AmpFlSTR® MiniFiler™ PCR amplification kit, available soon from Applied Biosystems. Forensic News. Retrieved February 11, 2008, from http://marketing.appliedbiosystems.com/images/enews/ForensicNews_Vol7/PDF/00_ForensicNews.pdf

Applied Biosystems. 2006a. Quantifiler™ human DNA quantification kit and Quantifiler™ Y human male DNA quantification kit. User guide.

Applied Biosystems. 2007. AmpFlSTR® MiniFiler™ PCR amplification kit. Product Bulletin.

Applied Biosystems. 2007a. AmpFlSTR® MiniFiler™ PCR amplification kit. User guide.

Atkinson R. 2007a, October 1. 'There was a two-year learning curve...and a lot of people died in those two years.' *Washington Post.* p. A01. Retrieved March 18, 2008, from http://www.washingtonpost.com/wp-dyn/content/article/2007/09/30/AR2007093001675_2.html?sid=ST2007092900754

Atkinson R. 2007b, October 3. 'If you don't go after the network, you're never going to stop these guys. Never.' *Washington Post.* p. A01. Retrieved March 18, 2008, from http://www.washingtonpost.com/wp-dyn/content/article/2007/10/02/AR2007100202366.html?sid=ST2007092900754

Bessetti J. 2007, March. An introduction to PCR inhibitors. *Profiles in DNA.* 10:9-10.

Bolz F, Dudonis K, Schulz DP. 2005. The counterterrorism handbook. CRC Press. Boca Raton, FL.

Bourke MT, Scherzinger CA, Ladd C, Lee HC. 1999. NaOH treatment to neutralize inhibitors of Taq polymerase. *J Forensic Sci.* 44:1046-50.

Braid MD, Daniels LM, Kitts CL. 2003. Removal of PCR inhibitors from soil DNA by chemical flocculation. *J Microbiological Methods.* 52:389-93.

Bright J, Petricevic SF. 2004. Recovery of trace DNA and its application to DNA profiling of shoe insoles. *For Sci Int.* 145:7-12.

Budowle B, Moretti TR, Baumstark AL, Defenbaugh DA, Keys JM. 1999. Population data on the thirteen CODIS core short tandem repeat loci in African Americans, U.S. Caucasians, Hispanics, Bahamians, Jamaicans, and Trinidadians. *J Forensic Science.* 44:1277-86.

Budowle B, Hobson DL, Smerick JB, Smith JAL. 2001. Low copy number-consideration and caution. Presented at Twelfth International Symposium on Human Identification. Biloxi, MS, USA.

Burke R. 2000. Counter-terrorism for emergency responders. CRC Press LLC. Boca Raton, FL.

Butler JM. 2005. Forensic DNA typing 2nd ed. Elsevier Academic Press. Boston, MA.

Butler JM. 2006. Genetics and genomics of core short tandem repeat loci used in human identity testing. *J Forensic Sci.* 51:253-65.

Butler JM, Shen Y, McCord BR. 2003. The development of reduced size STR amplicons as tools for analysis of degraded DNA. *J Forensic Sci.* 48(5):1054-64.

Chertoff M. 2007, October 19. Preventing IED attacks. DHS Leadership Journal (discussion forum). Retrieved February 25, 2008, from http://www.dhs.gov/journal/leadership/2007_10_01_archive.html

Clayton TM, Whitaker JP, Sparkes R, Gill P. 1998. Analysis and interpretation of mixed forensic stains using DNA STR profiling. *For Sci Int.* 91:55-70.

Coble MD, Butler JM. 2005. Characterization of new miniSTR loci to aid analysis of degraded DNA. *J Forensic Sci.* 50(1):43-53.

Collins PJ, Hennessy LK, Leibelt CS, Roby RK, Reeder DJ, Foxall PA. 2004. Developmental validation of a single-tube amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: the AmpFlSTR Identifiler PCR amplification kit. *J Forensic Sci.* 49:1265-77.

Esslinger KJ, Siegel JA, Spillane H, Stallworth S. 2004. Using STR analysis to detect human DNA from exploded pipe bomb devices. *J Forensic Sci.* 49:481-4.

Foran DR. 2006. Relative degradation of nuclear and mitochondrial DNA: an experimental approach. *J Forensic Sci.* 51:766-70.

Gehring M. 2004. The recovery and analysis of mitochondrial DNA from exploded pipe bombs. Master's thesis. Michigan State University.

Gill P. 2001. Application of low copy number DNA profiling. *Croatian Medical J.* 42:229-32

Gill P, Whitaker J, Flaxman C, Brown N, Buckleton J. 2000. An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA. *For Sci Int.* 112:17-40.

Green RL, Roinestad IC, Boland C, Hennessy LK. 2005. Developmental validation of the Quantifiler™ real-time PCR kits for the quantification of human nuclear DNA samples. *J Forensic Sci.* 50:809-31.

Grubwieser P, Muhlmann R, Berger B, Niederstatter H, Pavlic M, Parson W. 2006. A new "mini-STR multiplex" displaying reduced amplicon lengths for the analysis of degraded DNA. *Int J Legal Med.* 120:115-20.

Hill CR, Kline MC, Coble MD, Butler JM. 2008. Characterization of 26 miniSTR loci for improved analysis of degraded DNA samples. *J Forensic Sci.* 53(1):73-80.

Holland MM, Parsons TJ. 1999. Mitochondrial DNA sequence analysis – validation and use for forensic casework. *For Sci Rev.*11: 21-51.

Katz DE. 2007, March. The hidden benefits of real-time PCR: Assessing and addressing qualitative challenges. *Profiles in DNA.* 10:6-8.

Kisilevsky AE, Wickenheiser RA. 1999. DNA PCR profiling of skin cells transferred through handling. Proceedings of the Annual Meeting of the Canadian society of Forensic Science. Edmonton, Alberta, Canada.

Kline MC, Duewer DL, Redman JW, Butler JM. 2005. Results from the NIST 2004 Quantitation Study. *J Forensic Sci.* 50:1-7.

Kreader CA. 1996. Relief of amplification inhibition in PCR with bovine serum albumin or T4 gene 32 protein. *Appl Environ Microbiology.* 62:1102-06.

Kremer S, Foran D. 2008. Identification of improvised explosive device assemblers using miniSTRs. Presented at the American Academy of Forensic Sciences 60th annual scientific meeting. Washington, DC, USA.

Krenke BE, Tereba A, Anderson SJ, Buel E, Culhane S, Finis CJ, Tomsey CS, Zachetti JM, Masibay A, Rabbach DR, Amiott EA, Sprecher CJ. 2002. Validation of 16-locus fluorescent multiplex system. *J Forensic Sci.* 47:773-85.

Lowe A, Murray C, Whitaker J, Tully G, Gill P. 2002. The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces. *For Sci Int.* 129:25-34.

Mueller III RS. 2007, March 28. Speech from the director of the FBI. Retrieved March 21, 2008, from http://www.fbi.gov/pressrel/speeches/mueller032807.htm

National Research Council: Committee on defeating improvised explosive devices. 2007. Countering the threat of improvised explosive devices: basic research opportunities, abbreviated version. National Academies Press. Washington, DC. Retrieved October 23, 2007, from http://www.nap.edu/catalog/11953.html

Noe D. 2007. Eric Rudolph: serial bomber (the Olympics bombed). Retrieved February 11, 2008, from http://www.crimelibrary.com/terrorists_spies/terrorists/eric_rudolph/1.html

Parsons TJ, Huel R, Davoren J, Katzmarzyk C, Milos A, Selmanovic A, Smaljovic L, Coble MD, Rizvic A. 2007. Application of novel "mini amplicon" STR multiplexes to high volume casework on degraded skeletal remains. *For Sci Int Genetics.* 1:175-79.

Rådström P, Knutsson R, Wolffs P, Lovenklev M, Lofstrom C. 2004. Pre-PCR processing: Strategies to generate PCR-compatible samples. *Molecular Biotechnology.* 26:133-46.

Ricci U, Giovannucci Uzielli ML, Klintschar M. 1999. Modified primers for D12S391 and a modified silver staining technique. *Int J Legal Med.* 112:342-44.

Robin E, Wong R. 1988. Mitochondrial DNA molecules and virtual number of mitochondria per cell in mammalian cells. *J Cell Physiol.* 136:507-13.

Sageman M. 2004. Understanding terror networks. U. of Penn. Press. Philadelphia, PA.

Shutler G, Gagnon P, Verret G, Kalyn H, Korkosh S, Johnston E, Halverson J. 1999. Removal of a PCR inhibitor and resolution of DNA STR types in mixed human-canine stains from a five year old case. *J For Sci.* 44:623-26.

Stallworth, F/Lt. Shawn E. Bomb squad commander, Michigan State Police.

STRBase. Retrieved March 6, 2008, from http://www.cstl.nist.gov/biotech/strbase/

Sweet D, Lorente M, Lorente JA, Valenzuela A, Villanueva E. 1997. An improved method to recover saliva from human skin: the double swab technique. *J For Sci.* 42:320-2.

Timeline: Madrid investigation. 2004. Retrieved February 11, 2008, from http://news.bbc.co.uk/2/hi/europe/3597885.stm

Van Oorschot RAH, Jones MK. 1997. DNA fingerprints from fingerprints. *Nature.* 387: 767.

Watson RJ, Blackwell B. 2000. Purification and characterization of a common soil component which inhibits the polymerase chain reaction. *Can J Microbiol.*46:633-42.

Whitaker JP, Clayton TM, Urquhart AJ, Millican ES, Downes TJ, Kimpton CP, Gill P. 1995. Short tandem repeat typing of bodies from a mass disaster: high success rate and characteristic amplification patterns in highly degraded samples. *BioTechniques.* 18(4):670-7.

Wickenheiser R. 2002. Trace DNA: a review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact. *J Forensic Sci.* 47:442-50.

Wiegand P, Kleiber M. 2001. Less is more—length reduction of STR amplicons using redesigned primers. *Int J Legal Med.* 114:285-7.

Williams D. Counterterrorism consultant, Counter Terrorism Consulting Group LLC, Harwood, Maryland. November 6, 2007.

Wilson, C. 2007, November 21. Improvised explosive devices in Iraq and Afghanistan: effects and countermeasures. *CRS Report for Congress*. Retrieved February 9, 2008, from http://assets.opencrs.com/rpts/RS22330_20071121.pdf

Yoshida K, Sekiguchi K, Kasai K, Sato H, Seta S, Sensabaugh GF. 1997. Evaluation of new primers for CSF1PO. *Int J Legal Med.* 110:36-38.