This is to certify that the
thesis entitled

RECOVERY AND QUANTIFICATION OF NUCLEAR AND MITOCHONDRIAL DNA FROM IMPROVISED EXPLOSIVE DEVICES USING COMPARATIVE SOAKING AND SWABBING TECHNIQUES

presented by

KAMILA MARYAM GOMEZ

has been accepted towards fulfillment
of the requirements for the

M.S. degree in CRIMINAL JUSTICE

Major Professor's Signature

5/6/09

Date

RECOVERY AND QUANTIFICATION OF NUCLEAR AND MITOCHONDRIAL DNA FROM IMPROVISED EXPLOSIVE DEVICES USING COMPARATIVE SOAKING AND SWABBING TECHNIQUES

By

Kamila Maryam Gomez

A THESIS

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

MASTERS OF SCIENCE

Criminal Justice

2009

ABSTRACT

RECOVERY AND QUANTIFICATION OF NUCLEAR AND MITOCHONDRIAL DNA FROM IMPROVISED EXPLOSIVE DEVICES USING COMPARATIVE SOAKING AND SWABBING TECHNIQUES

By

Kamila Maryam Gomez

The use of improvised explosive devices (IEDs) in terror-related attacks has become increasingly common worldwide, in large part because they are easily assembled and disguised. Fingerprint and impression mark analysis are generally unsuccessful for identifying manufacturers of IEDs, thus DNA has been examined as a new approach for apprehending perpetrators. Previous researchers have used a double swab technique to recover DNA from deflagrated pipe-bombs, which proved to be time consuming due to the small bomb fragments. The purpose of this study was to compare the traditional double swab technique and a novel soaking technique to ascertain which produced higher quantity and quality DNA. Thirty-six volunteers handled either steel or PVC pipe nipples and end caps for about 30 seconds. The pieces were assembled into pipe bombs and deflagrated. Bomb fragments were either swabbed, or soaked at room temperature for 2 hours or overnight at 56 °C to recover the DNA. Subsequent quantitative real-time PCR and STR analyses showed that DNA was recoverable from both types of pipe using both techniques, although higher yields and better sample quality were obtained using the swabbing technique. Full profiles were recovered from the DNA of swabbed fragments, while soaked fragments generated partial or no profiles. The swabbing technique, therefore, could be optimized for laboratory use in cases pertaining to IEDs.

ACKNOWLEDGEMENTS

A heartfelt thank you is extended to my committee members for taking the time to invest in my work, for their invaluable input, and for graciously allowing me the extra time that I needed to get the job done. To my advisor Dr. David Foran I extend a special thank you, not for only being my advisor and guide, but for ensuring that I was always well taken care of, whether it be financial aid, a summer job, or enquiring about whether or not I had a place to stay at the times when family and friends mean the most. As an international student it meant the world. To First Lt. Shawn Stallworth, my greatest appreciation is extended to you and the team from the Michigan State Police Bomb Squad for your dedication and commitment to the research conducted by the Michigan State University Forensic Biology program, and for ensuring that we were able to conduct our research safely. Finally, to Dr. Christina DeJong a gracious thank you for showing enthusiasm for my work, for volunteering to participate and for your academic assistance.

I would also like to thank my lab-mates Shane Hoffmann and Scott Grammer for all their assistance toward my project. I extend a thank you, as well, to my other peers in the lab who have willingly helped whenever I needed them and have kept me in good spirits. To my C.S.A family, I will always be appreciative to you for making me a home away from home. Last by not least, my sincerest gratitude is extended to my family and loved ones, whose unwavering support kept me strong and focused in my times of need.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

IMAGES IN THIS THESIS APPEAR IN COLOR

Introduction

Prior to the 1980's, terror-related incidents in the United States were mainly perpetrated by domestic groups that were either anti-government, anti-legislation, or extremists such as the Ku Klux Klan (FBI Terrorism Report 2002 – 2005). These trends continued through the 1980s, however at the time an increase in major terrorist events against the U.S. was observed overseas. Between the early 1990's and the beginning of the 21st century, there was a change in the nature of terrorist attacks both domestically and internationally (FBI Terrorist Report 2002 – 2005). Before the early 1990's, terrorist acts were more focused on destruction of property, but from 1993 – 2001 the main targets were people. On February 26, 1993, the first attack on the World Trade Center was executed by an international terrorist group using a vehicle, ammonium nitrate, and fuel oil (Burke 2000). Most of the harm was property damage but the attack did send the message that the United States was not immune to terrorist threats. The second attack, on September 11, 2001, marked the pinnacle of fright and terror not only for the U.S., but the world as a whole. It was the deadliest terrorist attack on U.S. soil, causing 2,972 deaths and over 12,000 injuries (FBI Terrorist Report 2000 – 2005). Worldwide, terrorist assaults in the 21st century have become more frequent and elaborate; but while it is impossible to actually stop the planning and execution of every attack, law enforcement agencies can devise methods to prevent or reduce the scale of the problem by trying to identify perpetrators *post facto*.

Whether attacks are large- or small-scale, one common similarity is the use of non-conventional bombs, or improvised explosive devices (IEDs), to cause injury and destruction. As the name suggests, an IED is a bomb that is often "homemade" and is

either assembled or detonated by means that differ from standard military munitions. The bombs can be made from different types of material such as glass, plastic or metal, and one of the most dangerous aspects of these devices is that they can be disguised or hidden in ordinary containers like backpacks and mail packages. Using timers and electronic signals, perpetrators have also exploited electronic technology which has allowed detonation of IEDs from a distance. This has decreased the likelihood of apprehending the criminal since the individual does not have to be at the scene for detonation to occur.

Trends and Statistics on Improvised Explosive Devices

According to Burke (2007), explosives account for over 70% of the devices used in terrorist attacks. Records up until the year 2000 showed that pipe bombs represented 31% of them (Burke 2000). Pipe bombs are common as they are effective, easily assembled and hidden, and the materials can be bought in any neighborhood hardware store without raising suspicion. Typically, all that is required is a length of pipe, end caps, explosive powder, and a fuse or triggering mechanism. Purchase of most of these materials is not regulated by law and criminals frequently escape detection while obtaining their supplies (National Research Council 1998).

The FBI Bomb Data Center General Information Bulletin 97-1 (1997) showed that from 1987 – 1994 there was a steady increase in the number of bombing incidents in the U.S. This was followed by a decrease from 1995 – 1997. Except for a slight increase the next year (FBI Bomb Data Center General Information Bulletin 98-1), Alcohol, Tobacco, Firearms and Explosives (ATF) data demonstrated a continuation in the downward trend from 2000 – 2003 (ATF Explosive Incidents Reports 2000 – 2003).

Summarizing the reports from the FBI and ATF, bombing was the leading domestic terrorist event from 1980 – 2005 (FBI report 2002 – 2005). Civilians or private property were the main targets for the majority of these years, and the devices were usually hidden in mailboxes. Black or smokeless powders were used in most pipe bomb instances, with metal pipes being the primary container. The reports also revealed that in most cases the events occurred either at dusk or dawn, and the main perpetrators were young adults.

Characteristics of Smokeless Powder

Smokeless powders have a variety of functions, thus they are manufactured in an assortment of shapes and sizes to achieve optimal performance (NRC 1998). Physical appearances range from disks and flattened balls to squares and tubes. The powders are usually blended with stabilizers and glazed with graphite to increase the shelf life and decrease sensitivity to friction, static electricity, and heat. Approximately 10 million pounds of smokeless powder are sold annually in the U.S., of which about 70% is sold to manufacturers of ammunition, with the remainder sold to private citizens (NRC 1998).

Smokeless powder is available in three categories: single-base, double-base, and triple-base. The energetic material found in single-base powder is nitrocellulose. Combining this with nitroglycerin produces the energetic material for double-base powders. Triple-base powders include a mixture of these and nitroguanidine. The powder becomes more potent as the base number increases, making triple-base powders the most dangerous and the least available to the general public. Single-based smokeless powders, therefore, are most commonly used in terrorist activity, because they are the least regulated and most available (NRC 1998). Nitrocellulose also has non-explosive uses,

and is found in lacquers, varnishes, and some printing products. Cellulose itself is a polymer made up of units of the sugar glucose, and nitrocellulose is produced by replacing up to three hydroxyl groups with a nitrogen atom, into each sugar monomer. The concentration of nitrogen in cellulose trinitrate is about 14.15% (Yinon and Zitrin 1993). The nitrogen content in single-base smokeless powders ranges between 12.6% and 13.3% (NRC 1998). The percentage determines the powder's sensitivity (ease with which it can be ignited), and as it increases so does the sensitivity, potency, and the heat released during the explosion.

The amount of energy liberated as heat when the powder burns is termed the heat of combustion. When a hydrocarbon (in this case, sugar) combusts, carbon dioxide, water, and heat are produced as it reacts with oxygen. Because the hydroxyl groups in the sugar have been replaced with nitrogen, burning is not as efficient and therefore the amount of heat decreases as the percentage of nitrogen atoms increases (Table 1). The reverse is seen however, when an explosion takes place, as the percentage of nitrogen dictates the sensitivity and potency of the reaction. An explosion occurs as a result of the rapid generation and release of pressure and heat; rapidity being the key element. The more rapid the reaction, the faster the pressure builds, and thus the more heat released.

Table 1: Percent nitrogen (N) contained in nitrocellulose versus the amount of heat released

	N content (%)		
	12.60	**13.15**	**14.0**
Heat of combustion (kcal/kg)	2414	2345	2237
Heat of explosion (kcal/kg)	934.5	1016	1140

Adapted from: (Yinon and Zitrin 1993). Kcal represents kilocalories; kg represents kilograms.

The energy of the gas is dispersed via a shock wave that travels from the blast center to the surrounding space. Shock waves occur for all intensities of explosions, and create an overpressure that may be strong enough to inflict damage; the greater the overpressure the more damage that can ensue (Burke 2000). An overpressure between 0.5 and 1.0 pounds per square inch (psi) has enough force to shatter windows or knock down an individual; 5 psi is capable of rupturing the eardrum, and 15 psi causes lung damage. When the overpressure reaches 35 psi or more, the threshold for fatalities has been reached. A fifty percent rate of fatalities has been seen for overpressures at 50 psi and a 95% rate at 65 psi.

Low explosives such as smokeless powder change their physical state (solid to gas) at a slow rate which is called deflagration. The material burns through thermal conductivity, where the hot layer heats and ignites the cooler layer adjacent to it. The pressure of an explosion as a result of deflagration, however, can reach up to 50,000 psi (Burke 2000). High explosives such as trinitrotoluene (TNT), change states almost immediately, and are said to detonate. The burning is very rapid, propagates via shock waves, and is able to inflict high amounts of damage.

Nuclear and Mitochondrial DNA: the Two Human Genomes

DNA has proven to be a powerful tool for identifying both perpetrators and victims as a result of terrorist activity. DNA is found in two places in the cell: the nucleus and the mitochondria. The human nuclear genome has approximately 3 billion base pairs (bp) (National Human Genome Research Institute), and is inherited from both parents. Nuclear DNA provides high powers of genetic discrimination among individuals and can

be sourced from any cell that contains a nucleus. The most robust genetic results for identification are usually obtained when nuclear DNA is of high quality and quantity. A limitation to the usefulness of nuclear DNA arises when DNA is degraded or is present in very low amounts.

Mitochondrial DNA (mtDNA) is found in the mitochondrion, a small energy-producing organelle within the cell. MtDNA is circular, smaller in size than the nuclear genome with only about 16,569 bp, maternally inherited, and may number in the thousands of copies per cell (reviewed by Holland and Parsons, 1999). Because of the matrilineal inheritance, the genome offers a lower power of discrimination among individuals as all maternal relatives carry the same mitochondrial genomic sequence. However, if a mtDNA reference sample is not available for a suspect, any maternal relative can be used to obtain the profile. The same can be done when identifying victims of mass disasters or war. Because of its high copy number and extra layer of protection within the mitochondrion (Foran 2006), mtDNA can provide robust results when nuclear DNA is degraded.

DNA Profiles: Powers of Discrimination and Frequencies

Current human forensic identification is based on the examination of short tandem repeats (STRs): regions of nuclear DNA that are made up of variable lengths of a repeated DNA sequence usually 2 to 6 bp in length (Butler 2005). The number of repeats at a particular locus varies among individuals. A combination of sizes, or alleles, from different STR loci is used to produce an STR profile. Thirteen such loci have been chosen by the FBI as the standard, and commercial kits such as the PowerPlex® 16 System by

Promega and Identifiler® by Applied Biosystems (ABI) are currently used by forensic laboratories in the U.S. to carry out these analyses.

Instances occur, however, when a DNA sample cannot be assayed due to degradation. One solution to this problem has been the development of miniSTRs. These are smaller in size than the traditional 13 STRs; a feat accomplished by including less of the DNA sequences that flank the repeat region. The smaller size of the target DNA region (amplicon) increases the chance that amplification is successful, even if degradation exists. However, while miniSTRs may be advantageous for analyzing degraded samples, a major disadvantage is that fewer loci can be analyzed at one time which reduces the power of discrimination.

MtDNA variation is mainly observed within the two hyper-variable regions—HV1 and HV2, and is uncovered by sequencing the DNA base by base. Polymorphisms within a profile are compared to a database to determine the profile frequency by dividing the number of times the profile appears by the total number of profiles in the database. The power of discrimination is not as strong as that of nuclear STRs for two reasons: matrilineal relatives share the same mtDNA profile and the number of profiles in the database is small.

Touch Samples and Trace DNA

Objects that have been touched can provide a small amount of retrievable DNA (trace DNA) from cells that have been left behind. Epithelial cells have proven to be a convenient source of DNA as approximately 400,000 of them are shed daily by humans (Raven and Johnson 1986). Herber and Herold (1998) showed that DNA could be

extracted and typed from human dandruff with a single particle producing an average of 4.7 ng of DNA. Van Oorschot and Jones (1997) generated STR profiles from swabs taken from the palm of the hands and objects that were touched, and retrieved 2 – 150 ng of DNA, with the lower amounts being recovered from hands that were dry or previously washed. Also examined were objects that were touched by multiple individuals, which showed that the DNA of the last person to touch an object was usually present, and that of the other handlers was present in varying amounts. It was interesting to note that the strongest profile did not always belong to the last individual that handled the object, demonstrating how diverse epithelial shedding can be.

Alessandrini et al. (2003) examined DNA retrieval and genetic profiling from fingerprints. They focused on washed and unwashed fingers pressed onto various substrates (wood, glass, and metal) for 30 seconds. Quantification results showed that the amount of DNA recovered varied both among donors and within multiple trials of the same donor; indicating that a donor deemed to be a good epithelial shedder in one experiment did not necessarily maintain that status in another. More DNA was recovered from unwashed hands, but unlike Van Oorschot and Jones (1997) the average quantity ranged from less than 100 pg to slightly greater than 300 pg.

Balogh et al. (2003) focused on the relationship between the length of time a substrate was touched and the ability to recover, sequence, and type DNA. Handling time had no effect on the quality of STR profiles obtained, as a substrate that was touched for 2 seconds yielded results as good as a substrate touched for 60 seconds. Sequencing of mtDNA was possible for all the samples analyzed.

Secondary transfer—the passing of epithelial cells belonging to another person to an object—has been a concern in trace DNA analysis since it is not known to what extent extraneous DNA may be recovered. A study by Ladd et al. (1999) indicated that secondary transfer may be negligible, as a full secondary profile was not detectable in most of the analyses. Lowe et al. (2002) showed that secondary transfer can occur under optimum laboratory conditions and a controlled environment, but suggested that the results may not be reproducible when circumstances are not as meticulous. Full secondary profiles were only obtainable in a few cases, but for the most part, mixed profiles were generated. Proff et al. (2006) demonstrated that secondary transfer was possible when using a non-human vector (latent fingerprint brush), that was used at different crime scenes. While transfer did occur, it was only seen when a large surface area was dusted with a brush that was used at several crime scenes. The results from these studies indicate that although secondary transfer is a possibility, its effects may be negligible when dealing with touch samples.

DNA Isolation from Touched Objects

Biological materials for DNA testing are often collected by rubbing a swab across the surface of evidence. The double swab technique, developed by Sweet et al. (1997), is a standard procedure used in the field of forensics to retrieve cells. The technique entails using a wet swab to both hydrate and pick up cells, then a dry swab to soak up any remaining material. The rehydration effect aids in loosening dried materials deposited onto the surface, so that they are easier to pick up when the dry swab is applied. Pang and Cheung (2007) conducted an experiment to ascertain which swab in the technique

produced a higher quantity of DNA, since both swabs were capable of retrieving the biological material. It was shown that both swabs were equally effective in cell recovery. STR analysis proved that profiles were obtainable from both wet and dry swabs, though there were instances where a profile was obtained from the dry swab while none was generated from the corresponding wet swab.

DNA Amplification and Quantification

Quantifying DNA has become a critical step in forensic analysis as STR analysis requires specific amounts of starting material for optimal performance. Quantification techniques have become more technologically advanced over the years, allowing for quick, efficient testing with more accurate results. A critical step in DNA quantification is the use of the polymerase chain reaction (PCR) to amplify a specific target sequence. The amplification process entails numerous cycles of DNA replication, using a polymerase to generate the new strands of DNA. As the amplicon is replicated, it is used as the template in the following cycle, thus doubling the amount of DNA. The quantity of DNA continues to grow exponentially until resources are depleted. A reaction that initially started with a few copies of DNA produces billions of copies upon completion.

Quantitative (real-time) PCR (qPCR) has made the PCR technique more convenient as it simultaneously amplifies and quantifies the target DNA in real time. DNA levels are monitored after each cycle, and the efficiency of amplification is calculated using a computer program. The premise of the method is the same as regular PCR, except that a dye-labeled oligonucleotide—a short piece of DNA complimentary to the target sequence, or probe—is used for detection of double-stranded DNA. If a target

sequence is successfully replicated, there is increased fluorescence from the reporter dye on the probe, thus greater fluorescence signifies more DNA amplification (ABI Protocol 2005). The ability to quantify more than one sample in the same reaction (multiplexing) through the use of different probes is another advantage of the qPCR technique. Commercial kits for quantifying nuclear DNA include the Quantifiler™ Human DNA Quantification Kit, produced by ABI. The Quantifiler assay is sensitive enough to be used for degraded and low copy number (LCN) DNA, and contains an internal positive control (IPC) template and oligonucleotide that amplifies along with the sample DNA. The control aids in identifying problems that may hinder sample analysis, such as inhibitors.

Independent research laboratories have also developed quantification assays. Timken et al. (2005) quantified nuclear and mtDNA in a duplex reaction. The nuclear target DNA was TH01, one of the 13 standard STR loci, with a sequence ranging approximately 170 – 180 bp. The locus was chosen because the amplicon size was mid-range among the target sequences of the 13 loci, and would have given a more accurate quantification value for larger amplicons. The mitochondrial target was a 69 base pair region of the NADH dehydrogenase subunit 1 (ND1) gene. The quantification method worked well with both high quality and degraded samples, the latter of which was compared to Quantifiler™. Results showed that the Quantifiler assay, which targets a 62 base pair region, detected significantly more DNA than the Timken et al. (2005) assay. Subsequent STR analysis on the degraded samples revealed that Quantifiler overestimated the quantity of DNA for larger amplicons, as a large portion of the longer templates failed to amplify.

Previous Studies of DNA Isolation from Pipe Bombs

New approaches are being used to pinpoint suspects by recovering and analyzing DNA that may have been left on pieces of evidence. Studies focusing on recovering DNA from deflagrated pipe bombs and their carrying containers have been carried out at Michigan State University. Esslinger et al. (2004) explored obtaining DNA profiles from exploded pipe bombs after having volunteers handle the pipes. One full profile was recovered, while 4 of 20 bombs gave partial results that were consistent with the donor's known profile. Alleles from an additional 8 bombs were obtained, but were not called because they were below the fluorescence threshold of the study. Obtaining a successful profile correlated with the concentration of DNA recovered, the level of pipe fragmentation, and the number of bomb pieces collected. The type of pipe material, PVC or steel, was not a critical factor in obtaining a profile. Gehring (2004) focused on sequencing mtDNA from deflagrated pipe bombs, and was able to correctly assign 18 of 38 bombs to a single donor and 7 to a subset of donors. Twelve of the bombs were not assignable and 1 was incorrectly assigned. Unlike the Esslinger et al. (2004) study, the Gehring (2004) research was conducted blind. Although better success was achieved by Gehring, trends similar to Esslinger (2004) were observed where lower amounts of DNA were recovered as the amount of pipe fragmentation increased. Kremer (2008) combined miniSTR analysis of nuclear DNA with mtDNA sequencing to identify bomb handlers, which proved to be even more informative. Results showed that more than half of the bombs could be assigned to a single donor if both genomes were used. In a subsequent study, Hoffmann (2008) focused on recovering DNA (and ultimately miniSTRs) from IED carrying containers and correctly assigned all eight backpacks to their donors, again

under blind conditions. The key to the success was independently testing different areas of a backpack and building a consensus DNA profile.

Aim of the Current Study

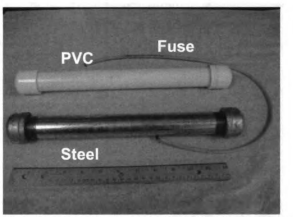
While swabbing has been successfully used to recover DNA from pipe bomb fragments, small bomb pieces have proven to be very tedious and time-consuming to process. It begs the question, therefore, of whether there is an easier and more efficient way to recover DNA from bomb pieces. Thus, a novel soaking technique was developed in an attempt to improve the speed with which DNA can be retrieved and the amount that can be recovered. The goal was to compare the double swab technique and the soaking technique, examining the quantity and quality of the recovered DNA. Two hypotheses were tested: (1.) the more pieces of pipe collected, the more DNA would be recovered; and (2.) the soaking technique would recover more DNA than the swabbing technique because the pieces of pipe would be submerged in a buffer and more pieces would be used for analysis. Pipe bombs were handled by volunteers, deflagrated, and collected and analyzed. The pipe fragments for the soaking technique were immersed into a cell-lysing buffer that covered their entire surface area. The recovered DNA from each technique was quantified and STR analysis was performed.

Materials and Methods

Pipe Bomb Preparation

Foot-long pipe nipples, one inch in diameter, along with matching end caps were purchased at a local hardware store; half of the pipes were PVC and half galvanized steel. Two end caps were allocated to each pipe and a ¼ inch hole was drilled into the center of one of them. The pipes and caps were sterilized by soaking in a 10% bleach solution for one hour, followed by rinsing with deionized water and UV irradiation on flip sides for 5 minutes each (approximately 7.5 J/cm^2). The materials were wiped with ELIMINase® as per the manufacturer's instructions, and rinsed a second time with deionized water. PVC caps without the drilled holes were glued onto the PVC pipes using PVC cement (Figure 1). Each pipe was stored in a separate brown paper bag until deflagration.

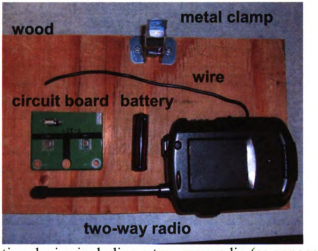
Figure 1: Steel and PVC pipe bombs



Steel and PVC pipe bomb nipples with attached end caps. Also shown is a length of fuse, used to ignite the bombs.

Pipe bombs, their corresponding detonation devices (Figure 2), and carrying containers (either a backpack or cardboard box), the latter two of which were part of a separate study, were assigned a number. The detonation device was made up of a cellular phone or two-way radio, a circuit board, a battery, a clamp for holding the pipe bomb, a piece of wire, Velcro, and a piece of wood, that were cleaned of extraneous DNA. The carrying containers were autoclaved for an hour, followed by exposure to UV radiation for 5 minutes on flip sides. Ten pipes were deflagrated as is, ten were assigned to backpacks, and eighteen were assigned to both a carrying container and detonation device (Table 2). Volunteers were asked to draw a number and a letter prior to handling the components of a specific pipe, container, and device and the numbers were recorded out of view of the analysts to conduct bomb assignation blindly. Approval for the use of human subjects was granted by the Michigan State University Committee on Research Involving Human Subjects.

Figure 2: An assembled detonation device, with a two-way radio



Components of a detonation device including a two-way radio (as opposed to a cellular phone), metal clamp, battery, wire, circuit board, and piece of wood. Each component was attached to the piece of wood using Velcro, so that they could be easily removed, handled and replaced by the volunteer. The pipe bomb was fitted into the clamp just prior to deflagration.

Bombs, detonation devices, and cardboard boxes were handled for approximately 30 seconds, while backpacks were used by volunteers for one week. The pipe bomb designated as the positive control was handled for about a minute once a day for three days, while the negative control underwent no handling. Following handling, the bombs were stored in separate brown paper bags until deflagration. A control buccal swab was collected from each volunteer and protective gear (gloves, masks, sleeves and lab coats) were worn by the analysts while dealing with the various components.

Table 2: Pipe bombs with corresponding carrying container and/ or detonation device

Pipe #	Pipe material	Container	Detonation device
3	Steel	Backpack	-
4	Steel	Backpack	-
5	Steel	Backpack	-
6	Steel	Backpack	-
7	Steel	Backpack	-
8	PVC	Backpack	-
9	PVC	Backpack	-
10	PVC	Backpack	-
11 (pos)	PVC	Backpack	-
12 (neg.)	PVC	Backpack	-
13 (neg.)	Steel	Cardboard box	Yes
14	Steel	Backpack	Yes
15	Steel	Cardboard box	Yes
16	Steel	Backpack	Yes
17	Steel	Cardboard box	Yes
18	Steel	Backpack	Yes
19	Steel	Cardboard box	Yes
20	Steel	Backpack	Yes
21	Steel	Cardboard box	Yes
22	PVC	Backpack	Yes
23	PVC	Cardboard box	Yes
24	PVC	Backpack	Yes
25	PVC	Cardboard box	Yes
26	PVC	Backpack	Yes
27	PVC	Cardboard box	Yes
28	PVC	Backpack	Yes
29	PVC	Cardboard box	Yes
30	PVC	Backpack	Yes
31	Steel	-	-

Table 2 continued			
32	Steel	-	-
33	Steel	-	-
34	Steel	-	-
35	Steel	-	-
36	PVC	-	-
37	PVC	-	-
38	PVC	-	-
39	PVC	-	-
40	PVC	-	-

The number of each pipe bomb, the material it was made from, along with its corresponding carrying container and detonation device is shown. The symbol (-) denotes that a particular component was not designated. Pipe bomb 11 was the positive control and pipe bombs 12 and 13 were negative controls.

Pipe Bomb Deflagration

Twenty-eight bombs were initially deflagrated at a local fire fighter training facility, with the aid of the Michigan State Police bomb squad. An additional ten bombs were deflagrated in the same manner at a later time. The process took place in a windowless, brick room with a metal door as the only access. Inside was a ventilated metal box into which the bombs were placed (Figure 3).

Figure 3: Metal box used to contain deflagrated pipe pieces

The metal box was supplied by the Michigan State Police bomb squad. Its purpose was to reduce the scatter of the pipe fragments upon deflagration, so that they could be easily collected. Ventilation holes were cut into the sides and lid so that the pressure from the blast could be released.

The bombs were filled with 1.5 oz of Green Dot Smokeless Shotshell Powder. Drilled PVC end caps were glued to the pipe and the steel end caps were screwed on. Bombs were deflagrated using an approximate 45 second fuse, which was inserted into the hole in the end cap, clipped on to its detonation device using the clamp, and placed into its corresponding container (Figure 4). Bombs that were not assigned a carrying container or detonation device were placed on the bottom of the metal box. Once deflagrated, the pieces of pipe, the container, and the components of the detonation device were collected and placed into their respective paper bags. The base of the metal box was either swept or vacuumed between deflagrations to remove remaining fragments, and the floor of the room was swept to prevent cross contamination. Fragments found after the collection and clean-up of a bomb were discarded. Gloves, masks, and sleeves were worn throughout the process by the bomb squad personnel and the analysts.

Figure 4: Pipe bomb with detonation device and carrying container

Fuses were inserted into the pipes through the end cap holes and the bombs were clipped onto the detonation devices using the metal clamps (A). The bombs and detonation devices were placed into the carrying containers and put into the metal box to be deflagrated (B).

Processing Pipe Fragments

Buffer, plastic pouches, microcentrifuge tubes, and swabs were UV irradiated for 10 minutes prior to processing the pipe bomb fragments. The soaking technique was used on PVC pipe bombs 8 – 12 and 22 – 30, and small steel fragments from bombs 31 – 35. Fragments were soaked in a plastic pouch, constructed by heat-sealing the top and bottom of a of Seal-A-Meal® bag (Figure 5). Twenty mL of digestion buffer along with 25 µL of proteinase K (20 mg/mL) were added and the bags were sealed. Pouches were agitated on a shaker for 2 hours and incubated. Soaked steel bomb fragments 3 – 21 were incubated overnight at 56ºC while steel bomb fragments 31 – 35 were not.

Figure 5: Plastic pouch containing PVC fragments for soaking

The PVC fragments of pipe bomb #10 being prepared for soaking. The plastic pouch was constructed by heat-sealing the top and bottom of the bag (arrows). The sides were pre-sealed by the manufacturer.

Large steel bomb fragments and PVC fragments from pipe bombs 36 – 40 were swabbed using the double swab technique (Sweet et al. 1997). One hundred and fifty µL of digestion buffer was used to moisten the first swab, which was rubbed across the galvanized covered surfaces of the steel fragments and the outer surfaces of the PVC fragments, in a circular motion. A dry swab then followed and both were placed into a 1.5 mL microcentrifuge tube along with 500 µL of digestion buffer and 4 µL of proteinase K. The tubes were vortexed for 10 seconds and incubated overnight at 56ºC. Reagent blanks were also initiated. Following DNA isolation, the mass of the bomb fragments was recorded.

Optimization of Vivascience Ultrafiltration Devices

Vivascience Vivaspin ultrafiltration devices were optimized for DNA use by testing filters that retained 5,000, 10,000, 30,000, and 100,000 molecular weight (MW)

material. Microcentrifuge tubes, 50 mL polypropylene centrifuge tubes, plastic caps, and buffer were UV irradiated for 10 minutes. The ultrafiltration devices were UV irradiated for 20 minutes, with the filters being detached and flipped over after 10 minutes. Volunteers handled 3 plastic caps for about 1 minute, and placed the caps into a 50 mL polypropylene centrifuge tube. To the tube was added 20 mL of buffer along with 25 μL of proteinase K. The solution was incubated overnight at 56°C. Buffer solutions were transferred to each of the devices and centrifuged at 4000 x g for 20 minutes, or until the liquid was concentrated to 500 μL.

Organic extractions were performed on the concentrated buffer solutions as well as 500 μL of flow-through from each device. Five hundred μL of phenol was added to the solution, vortexed, and centrifuged at 14,000 rpm for 5 minutes. The top aqueous layer was placed into a new 1.5 mL tube, to which an equal volume of chloroform was added. The mixture was vortexed and centrifuged at 14,000 rpm for 5 minutes. The aqueous layer was transferred to a Microcon YM-30 spin column. One hundred microliters of 10mM Tris/ 1mM EDTA (TE) was added and the column was centrifuged at 14,000 x g for 10 minutes. Three washes were subsequently performed by adding 300 μL of TE to the column and centrifuging at 14,000 x g for 10 minutes. The DNA was eluted in 20 μL of TE after inverting the column into a new 1.5 mL tube, and centrifuging at 1000 x g for 4 minutes.

MtDNA PCR analysis was conducted on all the extracts. The reaction mix included the addition of 1 μL of: mitochondrial primers F15 and R285 (20 μM); 10X PCR reaction buffer (USB); magnesium chloride (25 mM, USB); dNTPs (20 μM); bovine serum albumin (BSA) (0.2 mg/ μL); 1 unit HotStart-It® Taq DNA polymerase

(5u/ μL, USB); 2 μL of DNA sample; and 1.8 μL deionized water for a total of 10 μL. Cycling parameters included denaturing at 94°C for 30 seconds, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute for 38 cycles, followed by a final extension at 72°C for 5 minutes. PCR products were separated via electrophoresis on a 2% agarose gel and subsequently prepped for mtDNA sequencing.

Recovered DNA was transferred to a Montage column, and rinsed three times with 300 μL of deionized water, at 1000 x g for 15 minutes. The DNA was resuspended in 30 μL of deionized water and reactions of 4 μL of Quick Start mix (Beckman Coulter), 1 μL of primer, and 5 μL of DNA (50 – 100 fM) were prepared. Sequencing parameters included denaturing at 96°C for 20 seconds, annealing at 50°C for 20 seconds, and extension at 60°C for 4 minutes for 30 cycles. DNA was prepared for electrophoresis by adding 2.5 μL of stop solution, which included 5 μL of 3 M NaOAC, 5 μL of 100 M EDTA, and 2.5 μL of glycogen (Beckman Coulter). The DNA was precipitated through addition of 30 μL of 95% ethanol and centrifuging at 14,000 x rpm for 15 minutes, followed by two rinses in 180 μL of 70% ethanol, at 14,000 x rpm for 5 minutes. The ethanol was removed, the DNA pellet was vacuum-dried for about 20 minutes, and resuspended in 40 μL of Sample Loading Solution (Beckman Coulter). One drop of mineral oil was added to each well. Sequencing was performed on a Beckman Coulter CEQ™ 8000 Genetic Analysis System. The program used was LFR-1 and was carried out at 50°C. Samples were denatured at 90°C for 120 seconds, and injected for 15 seconds at 20 kV. Bomb and reference sequences were analyzed using BioEdit sequence alignment software (Ibis Biosciences).

Pipe Bomb DNA Extraction

Centrifuge tubes and Microcon columns were UV irradiated for 10 minutes and the Vivaspin 10,000 MW ultrafiltration devices were UV irradiated for 20 minutes with the filters being detached and inverted after 10 minutes. The buffer from the soaked pipe fragments was transferred to a 50 mL polypropylene tube and centrifuged at 4000 x g for 15 minutes, to pellet any debris. The liquid was transferred to the Vivaspin device and centrifuged at 4000 x g for 20 minutes or until the liquid was concentrated to 500 µL. The retentate was pipetted into a 1.5 mL microcentrifuge tube. Swabs were transferred to a spin basket and centrifuged at 14,000 revolutions per minute (rpm) for 5 minutes and the liquids combined into one 1.5 mL microcentrifuge tube. Organic extraction, as outlined above, was performed on each buffer solution, as well as 500 µL of flow-through from four randomly selected pipe bombs. The procedure was followed by mtDNA PCR analysis on the pipe bomb DNA and flow-through extracts, using the abovementioned protocol.

Quantification of Pipe Bomb DNA

FAM and HEX dyes supplied by Bio-Rad, and VIC dye purchased from Applied Biosystems, were calibrated as per the iQ™5 manufacturer's instructions. DNA from the pipe bombs was first quantified using a TaqMan assay (Timken et al. 2005). Reactions were prepared in either duplicate or triplicate. Singleplex reactions included 7.5 µL of iQ Supermix; 0.64 µL of primers and probe (Timken et al. (2005) final concentrations maintained); 0.64 µL of BSA (0.2 µg/ µL); 2 µL of undiluted DNA or 2 – 3 µL of diluted DNA, and deionized water to 15 µL. Reactions were analyzed on a Bio-Rad iQ™5

Multicolor Real-Time Detection System. This included a polymerase activation step of 95°C for 10 minutes, a denaturing step at 95°C for 15 seconds, and a dual annealing and extension step at 60°C for 1 minute (Timken et al. (2005)). The latter two steps were carried out for 45 cycles. Ten-fold serial dilutions of single-source male DNA ranging from 20 ng/ μL to 0.02 ng/ μL were prepared as the standards for the nuclear assay, and 10 ng/ μL to 0.01 ng/ μL of Promega genomic standard DNA (K562) were made for the mtDNA assay. Initial mtDNA reactions were carried out utilizing a probe that lacked a minor groove binder (MGB), which was critical for successful amplification. MtDNA quantification was measured by copy number and was calculated using the estimation of about 400 copies of mtDNA per 3.3 pg of K562 DNA. Data were analyzed using the Bio-Rad iQ™5 software.

Pipe bomb DNA was also quantified using a Quantifiler™ Human DNA Quantification Kit, using the same dilutions of unknown DNA described above. Volumes were reduced from 25 μL to 15 μL and consisted of 7.5 μL of reaction mix, 6.3 μL of primer mix, and 1.2 μL of DNA. The Quantifiler™ standard was diluted per manual instructions and ranged from 50 ng/ μL to 0.023 ng/ μL. The same cycling parameters as the Timken et al. (2005) qPCR assay were used, except for a reduction from 45 to 40 cycles. Inhibition categories were created and were: N – none, P – partial, and F – full. DNA samples giving a Quantifiler result of zero or showing signs of inhibition were reanalyzed by preparing fresh dilutions.

STR Analysis

STR analysis was carried out on the first 28 DNA samples (except for bombs 3 and 6) using a MiniFiler™ PCR Amplification Kit. Ten-microliter reactions contained 4 μL of MiniFiler™ Master Mix, 2 μL of MiniFiler™ primer mix, and 4 μL of DNA. Swabbed bomb DNAs were used undiluted, while 1:20 dilutions were prepared for the soaked DNA samples. A positive control contained 3 μL of 007 control DNA (Applied Biosystems) and 1μL of TE. Thermal cycling parameters included an initial incubation at 95°C for 11 minutes followed by 32 cycles of denaturation at 94°C for 20 seconds, annealing at 59°C for 2 minutes, and extension at 72°C for 1 minute, followed by a final extension at 60°C for 45 minutes. Reference (buccal) samples were analyzed using a PowerPlex® 16 System kit. Buccal DNA was diluted 1:100 and 1 μL of the diluted DNA along with 3 μL of TE were added to the reaction.

Capillary electrophoresis was performed on an ABI PRISM® 310 Genetic Analyzer. Reactions included 24.5 μL of formamide, 0.5 μL of GeneScan™ 500 LIZ ® Size Standard (Applied Biosystems) and 1.5 μL of DNA or allelic ladder. The mixtures were incubated at 95°C for 3 minutes and immediately cooled on ice. One drop of mineral oil was added to each tube and DNAs were separated using performance optimized polymer 4 and 1X buffer containing EDTA (Applied Biosystems). Electrophoresis included a run temperature of 60°C, an injection time of 5 seconds at 15 kV, and a run time of 28 minutes at 15 kV.

GeneMapper® ID software v3.2.1 was used to analyze the data. The Minifiler™ analysis method was MiniFiler_GS500_HID_v1, the panel was MiniFiler_GS500_v1, and the matrix was DS-33 Matrix 7-12-07. The PowerPlex analysis method was PP16SH,

the panel was PowerPlex_16ID3.1.0, the size standard was ILS 600 Advanced, and the matrix was Matrix-UMS Test. Peaks over 50 relative fluorescent units (RFUs) were recorded and volunteer profiles were initially called blindly before comparisons were made to the reference samples. Results were categorized as: A – Alleles from volunteers only, B – Volunteer (major profile) + additional alleles, C – Volunteer (non-major profile) + additional alleles, D – Some, but not all alleles from volunteer, E – No alleles from volunteer, F – No alleles obtained. Additional symbols after the lettered category indicated: † = incorrect alleles; • = missing alleles; * = complete allele dropout at 1 locus; ** = complete allele dropout at 2 loci; *** = complete allele dropout at 3 or more loci.

Statistical Analysis

Nuclear and mtDNA quantities were compared to the type of pipe material utilized, mass of the pipe fragments, and level of bomb fragmentation. Nuclear DNA results using the Timken et al. (2005) assay were also compared to those using Quantifiler, and further comparisons were made between the two DNA isolation techniques. DNA quantities within 10 pg of one other using the two nuclear DNA quantification techniques were considered 'similar', while those greater than 10 pg were deemed as 'higher' or 'lower'. Quantification averages were calculated inclusive or exclusive of negative results (no DNA) generated from either low DNA quantities or inhibition. Results were included if corresponding positive quantities were low, and excluded if quantities were high, but showed signs of inhibition. Nuclear or mtDNA quantities were converted from picograms (pg) to femtograms (fg) or mtDNA copies per

gram of pipe, to compare the two DNA isolation techniques based on the quantity of pipe recovered. Deflagrated bombs were assigned to fragmentation categories which were: 1 – low, 2 – medium, 3 – high, and 4 – complete.

The Pearson correlation coefficient (r) was calculated to ascertain correlative relationships between: the Timken et al. (2005) and Quantifiler assays, the nuclear and mtDNA yields, and the mass of the fragments and the amount of DNA recovered. The formula was $r = \Sigma(x - X)(y - Y)/ \sqrt{[\Sigma(x - X)^2][\Sigma(y - Y)^2]}$, where x and y represented DNA quantities, and X and Y denoted the mean of the quantities. Computation of 'r' generated a scale that ranged from -1 to +1, where -1 indicated that the correlation had a perfect negative relationship, -0.5 was a moderate negative relationship, 0.0 was no relationship, +0.5 was a moderate positive relationship, and +1 was a perfect positive relationship.

DNA quantities from the nuclear DNA, mtDNA and Quantifiler assays were compared using t-tests. DNA concentrations for the swab and soak techniques were averaged separately, and the standard deviation for each technique was computed and used to calculate the degrees of freedom. A significance level of 0.05 for a two-tailed t-test was used together with the degrees of freedom to ascertain the critical values of the t-test calculation.

Results

Optimization of Vivascience Ultrafiltration Devices

Vivaspin devices with larger molecular weight cutoffs concentrated the buffer solution in a faster time. The Vivaspin 100,000 MW device filtered the solution in about 5 minutes, followed by the 30,000 MW device, which took about 10 minutes, the 10,000 MW device took about 20 minutes, and the 5,000 MW took over an hour. MtDNA PCR showed that DNA was recovered from the concentrated buffer solution from all the devices, but it was also retrieved from the flow-through of the 30,000 and 100,000 MW devices. Subsequent mtDNA sequencing generated the haplotypes of the volunteers. Therefore the Vivaspin 10,000 MW device was used for the remainder of this study.

Post Bomb Deflagration and DNA Isolation Observations

PVC pipe bombs underwent a higher level of fragmentation upon deflagration, than did steel pipe bombs. While the entire PVC pipe broke into pieces, most of the fragmentation of the steel pipes was at end caps and less with the nipple. Therefore, the steel nipples were swabbed while the caps and other fragments were soaked.

Blackening of the swabs occurred with both types of pipe, while soaking produced varying amounts of buffer discoloration after incubation. The buffer from soaked PVC bomb fragments became grayish-brown, and from soaked steel fragments incubated overnight, a red rust color. Closer inspection of the steel bomb fragments revealed that a white crystal-like solid covered their surfaces. While oxidation occurred

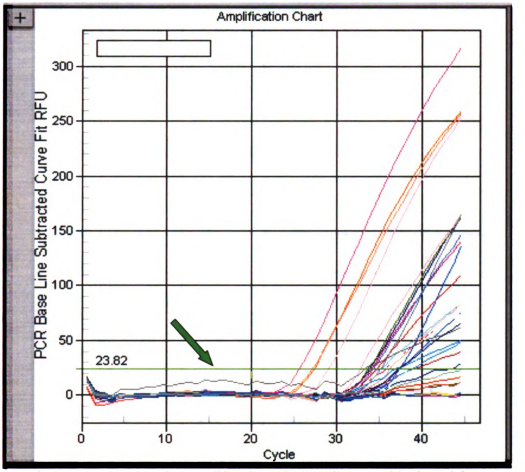
on all the soaked steel fragments, it was more pronounced in the fragments that were soaked overnight.

Centrifugation of the overnight soak solutions resulted in much larger pellets from steel fragments than from PVC fragments, and included the white substance as well as debris. After centrifugation the buffer from the steel fragments soaked overnight lost most of its color and became almost colorless. The PVC soak buffers, however, had a brownish hue, which was retained after the concentration step. The organic extraction was not effective in removing all of the coloration, leaving the eluted DNA from soaked PVC fragments brown in color. The eluted DNA from both the swabbed and soaked steel fragments was colorless.

DNA Electropherograms from Swabbed and Soaked Bomb Fragments.

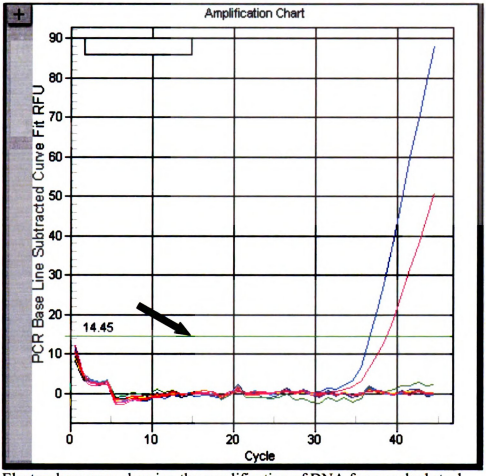
DNA from swabbed steel fragments amplified more frequently (Figure 6) than did DNA from the soaked fragments (Figure 7), and was achieved without DNA dilution. Positive DNA quantities were more often attained from the former than the latter, although neither isolation technique showed signs of inhibition according to the Quantifiler assay.

Figure 6: Amplification of DNA from swabbed steel fragments



Electropherogram showing the amplification of DNA from swabbed steel fragments. Relative fluorescence units (RFUs) are displayed on the y-axis using a linear scale. Cycle number is denoted on the x-axis. The horizontal line (arrow) is the threshold and each line represents one DNA sample. Nineteen of the 24 samples crossed the threshold between cycles 32 and 35 and maintained the sigmoidal curve generated from successful amplification.

Figure 7: Amplification of DNA from soaked steel fragments

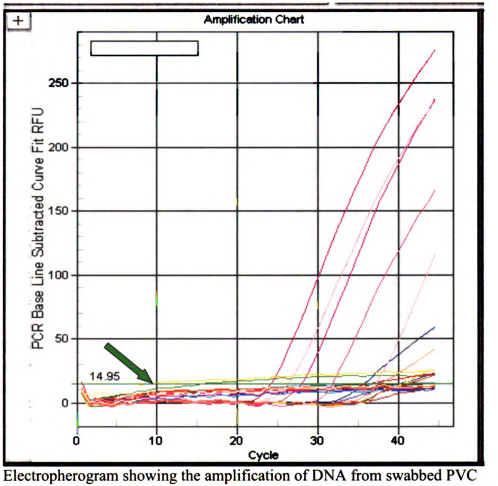


Electropherogram showing the amplification of DNA from soaked steel fragments. RFUs are displayed on the y-axis using a linear scale, while cycle number is denoted on the x-axis. The horizontal line (arrow) is the threshold and each line represents one DNA sample. Only 2 of the 6 samples crossed the threshold and maintained the sigmoidal curve generated from successful amplification.

Standard and positive control DNAs amplified and produced expected results during analysis of PVC DNA samples. DNA amplification was also successful with the swabbed PVC fragments (Figure 8), and quantification values were obtained without diluting the DNA. Soaked PVC fragments, however, had lower amplification success (Figure 9) as amplification curves failed to cross the threshold for at least one of the replicate reactions (Appendix A1). As a result, repeated trials had to be performed to

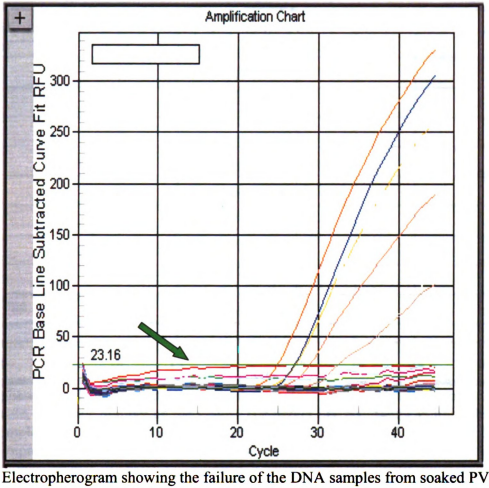
obtain positive outcomes. Results from the Quantifiler assay showed that 11 of the 12 DNA samples from the soaked PVC fragments were either partially or fully inhibited (Figures 10 and 11, respectfully).

Figure 8: Amplification of DNA from swabbed PVC fragments



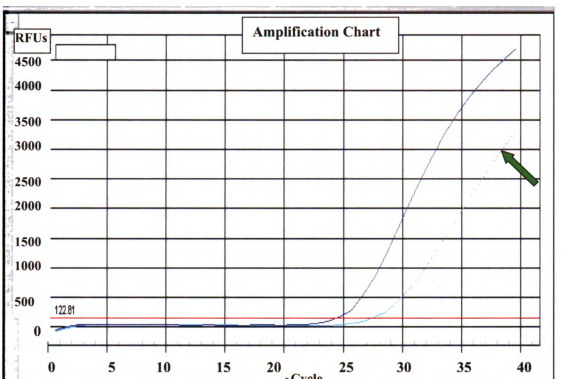
Electropherogram showing the amplification of DNA from swabbed PVC fragments. RFUs are displayed on the y-axis using a linear scale, while cycle number is denoted on the x-axis. The horizontal line (arrow) is the threshold and each line represents one DNA sample. Eight of the 12 samples crossed the threshold between cycles 32 and 35, and maintained the sigmoidal curve generated from successful amplification.

Figure 9: Amplification of DNA from soaked PVC fragments before dilution



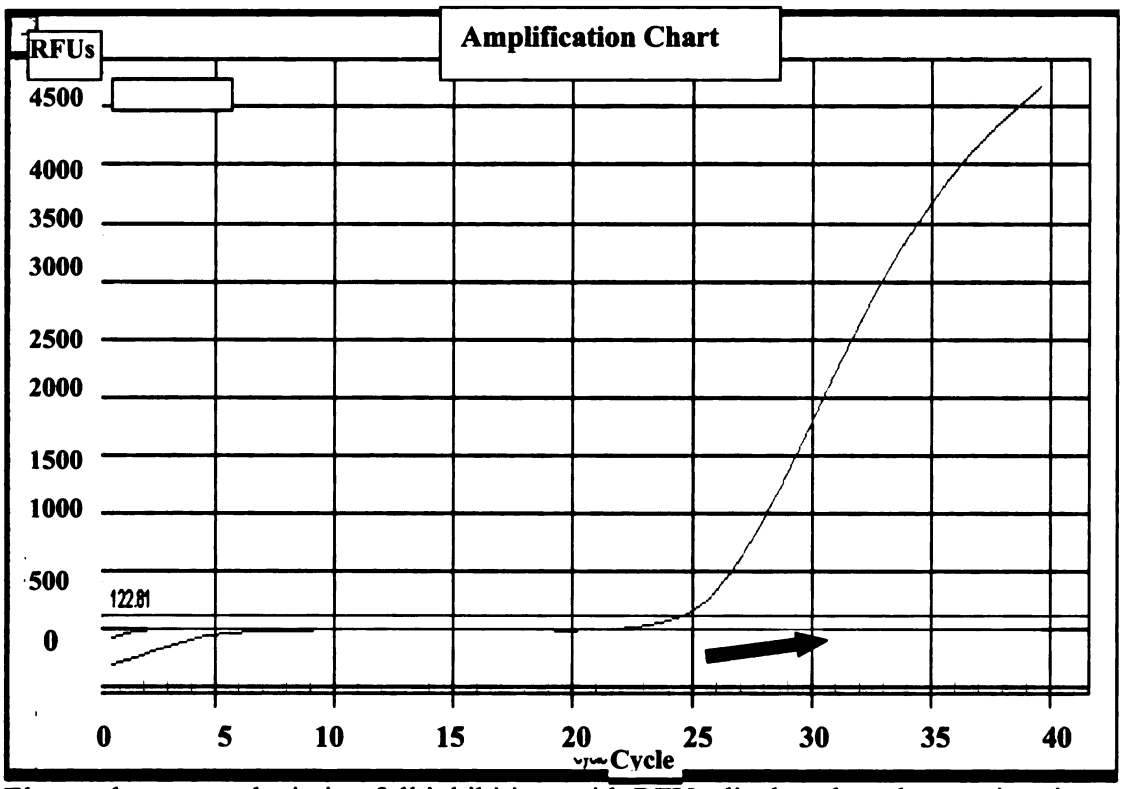
Electropherogram showing the failure of the DNA samples from soaked PVC fragments to amplify. RFUs ARE displayed on the y-axis using a linear scale, and cycle number on the x-axis. The horizontal line (arrow) is the threshold. Amplification of the standard DNA samples and the positive control is seen, with each line representing one DNA sample. None of the samples crossed the threshold.

Figure 10: Amplification of Quantifiler IPC showing partial inhibition



Electropherogram depicting partial inhibition, with RFUs displayed on the y-axis using a linear scale, and cycle number on the x-axis. The darker line represents an uninhibited DNA sample as the IPC line crosses the threshold at cycle number 25. The lighter line (arrow) represents a partially inhibited DNA sample as the IPC line crosses at a later cycle number.

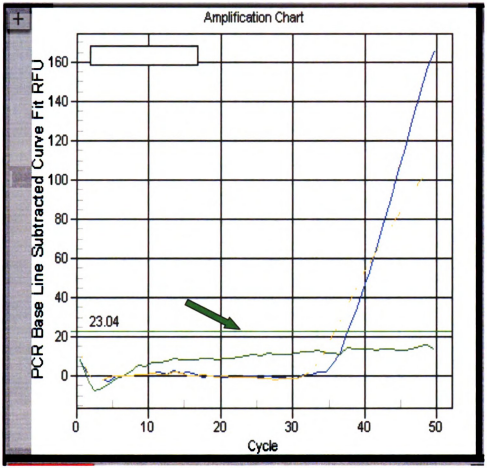
Figure 11: Amplification of Quantifiler IPC showing full inhibition



Electropherogram depicting full inhibition, with RFUs displayed on the y-axis using a linear scale, and cycle number on the x-axis. The darker line represents an uninhibited DNA sample as the IPC line crosses the threshold at cycle number 25. The horizontal IPC line parallel to the threshold (arrow) represents a fully inhibited DNA sample as the line does not cross the threshold.

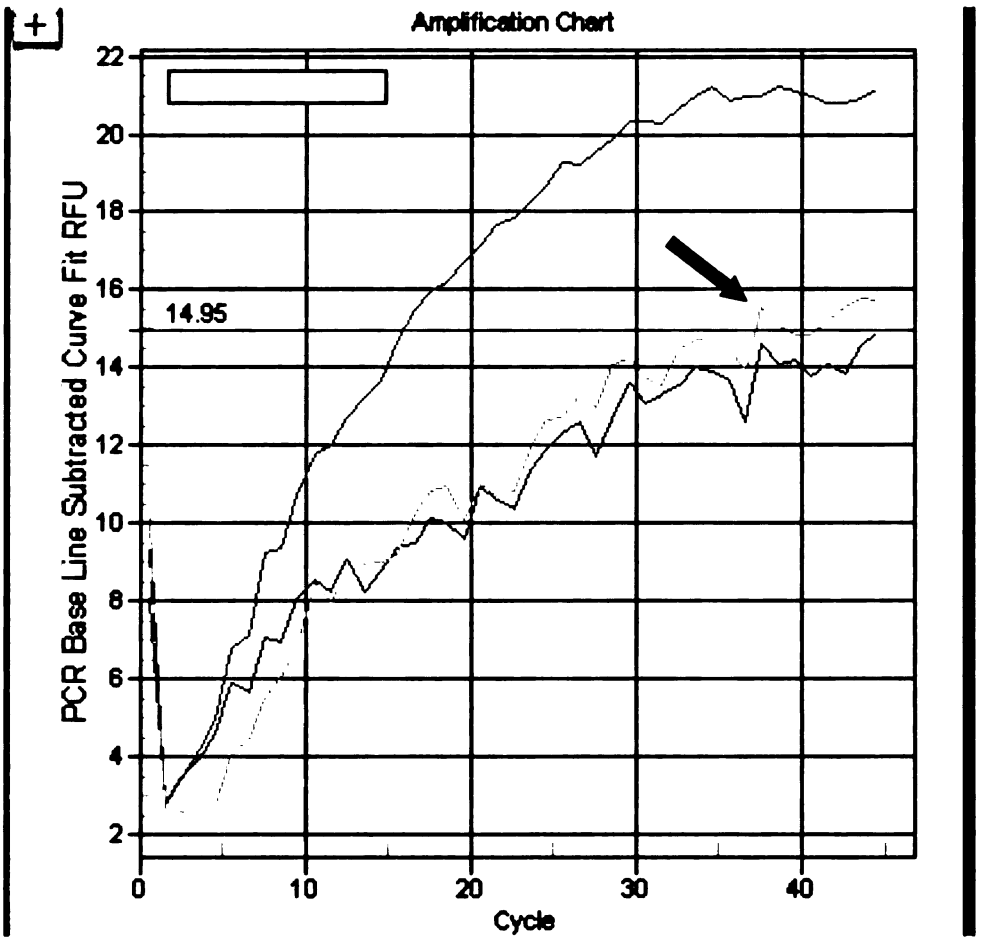
DNAs from soaked PVC fragments amplified after 1:10 or 1:20 DNA dilution (Figure 12). There were also instances of false positive quantification results, in which amplification curves crossed the threshold prematurely or amplification was abnormal as the curves did not maintain the sigmoidal shape (Figure 13). Table 3 displays a summary of the results from the qPCR nuclear, mtDNA and STR assays.

Figure 12: Amplification of DNA from soaked PVC fragments after DNA dilution



Electropherogram showing the amplification of DNA from soaked PVC fragments after dilution. RFUs are displayed on the y-axis using a linear scale, and cycle number is denoted on the x-axis. The horizontal line (arrow) is the threshold and each line represents one DNA sample. Two of the triplicate samples crossed the threshold, and maintained the sigmoidal curve generated from successful amplification.

Figure 13: Electropherogram depicting erroneous amplification curves



Depiction of false positive amplification curves, where the line crossed the threshold at a very early cycle or was uncharacteristically shaped. One of the lines crossed the threshold at about cycle 18, whereas the other (arrow) crossed at about cycle 36 despite being abnormally shaped. RFUs are displayed on the y-axis using a linear scale and cycle number is denoted on the x-axis. Each line represented one DNA sample.

Table 3: Results from qPCR nuclear, mitochondrial, and STR assays

Bomb #	DNA Isolation Tech.	Pipe Material	Recovered Mass (g)	Level of fragmentation	Average amt. of DNA (pg)	Femtogram of DNA per gram of pipe	Average amt. of DNA based on Quantifiler (pg)	Average amt. mtDNA (copies)	STR category	Level of inhib.
3	swab	steel	630.72		0.00	0.00	-	-	-	-
3	soak	steel	242.96	3	0.00	0.00	-	-	-	-
4	swab	steel	561.92		46.95	83.55	7.19	1240	B †	N
4	soak	steel	393.01	4	0.00	0.00	-	-	-	-
5	swab	steel	676.18		(1.91)	(2.83)	2.44	230	C †•	N
5	soak	steel	211.50	2	0.00	0.00	-	-	-	-
6	swab	steel	493.57		0.00	0.00	-	-	-	-
6	soak	steel	406.43	3	0.00	0.00	-	-	-	-
7	swab	steel	650.69		23.48	36.08	15.70	1540	B †•	N
7	soak	steel	236.99	1	0.00	0.00	-	-	-	-
8	soak	PVC	74.21	4	(2.58)	(34.80)	62.4, inhib	5624	B †•*	P
9	soak	PVC	93.6	4	(11.91)	(127.22)	0, inhib	2699	D†•	P
10	soak	PVC	91.12	4	(7.19)	(78.85)	14.20	2461	D†•**	P
11 (pos)	soak	PVC	69.96	4	(4.21)	(60.14)	Inhib	490	D†***	F
12 (neg)	soak	PVC	69.48	4	0.00	0.00	0, inhib	110		F
13 (neg)	swab	steel	620.96		(0.98)	(1.57)	2.79	829		P
13 (neg)	soak	steel	266.72	1	0.00	0.00	-	-	-	-
14	swab	steel	768.32		45.95	59.81	36.90	1543	C •	N
14	soak	steel	151.34	2	0.00	0.00	-	-	-	-
15	swab	steel	625.03		8.50	13.60	19.20	1317	C †•	N
15	soak	steel	274.97	2	0.00	0.00	-	-	-	-
16	swab	steel	577.15		7.77	13.46	19.60	1433	B •	N
16	soak	steel	267.07	3	0.00	0.00	-	-	-	-
17	swab	steel	498.02		72.00	144.57	34.9	1657	B *	N
17	soak	steel	475.25	3	0.00	0.00	-	-	-	-
18	swab	steel	598.03		2.64	4.41	10.10	1320	B *	N
18	soak	steel	289.65	1	0.00	0.00	-	-	-	-
19	swab	steel	400.37		(6.75)	(16.86)	0, inhib	3180	B †	P
19	soak	steel	487.89	4	0.00	0.00	-	-	-	-
20	swab	steel	680.79		29.08	42.72	29.20	9297	C *	N
20	soak	steel	206.80	3	0.00	0.00	-	-	-	-
21	swab	steel	572.22	4	(2.50)	(4.37)	10.1	782	B †	N
21	soak	steel	306.05		0.00	0.00	-	-	-	-
22	soak	PVC	101.83	4	32.20	316.21	34.2	1022	D ***	P
23	soak	PVC	70.03	4	(3.61)	(51.48)	0, inhib	469	E •***	F
24	soak	PVC	76.15	4	(5.53)	(72.55)	0, inhib	966	D •*	P

Th
disp
Tw
as
the
the
sho
A –
fro
* =
eac

Table 3 continued

25	soak	PVC	94.04	4	20.35	216.40	41.1	1956	D †•***	P
26	soak	PVC	83.65	4	5.73	68.50	0.00	1128	D †•*	P
27	soak	PVC	86.81	4	67.33	775.60	69.4	4145	F	P
28	soak	PVC	113.1	4	(0.38)	(3.36)	0.00	4533	E† ***	N
29	soak	PVC	53.36	4	(4.15)	(77.81)	0.00	434	D†•	P
30	soak	PVC	121.68	4	0.00	0.00	0, inhib	864	D†• **	P
31	swab	steel	598.9		0.00	0.00	0.00	8	-	N
31	soak	steel	218.25	2	(7.69)	(35.22)	0.00	943	-	N
32	swab	steel	700.84		0.00	0.00	0.00	1799	-	N
32	soak	steel	186.84	1	0.00	0.00	18.45	8	-	N
33	swab	steel	694.18		12.12	17.46	20.65	1394	-	N
33	soak	steel	193.5	1	(1.27)	(6.56)	0.00	19	-	N
34	swab	steel	635.37		16.25	25.58	0.00	751	-	N
34	soak	steel	252.31	2	(1.35)	(5.34)	0.00	21	-	N
35	swab	steel	650.11		7.66	11.78	85.30	3204	-	N
35	soak	steel	237.57	1	0.85	3.58	0.00	16	-	N
36	swab	PVC	73.82 **46.71**	4	47.10	1008.35	6.55	2085	-	N
37	swab	PVC	83.10 **54.87**	4	53.79	980.32	161.50	5398	-	N
38	swab	PVC	84.57 **57.63**	4	(45.28)	(785.70)	0.00	2097	-	N
39	swab	PVC	74.56 **39.69**	4	41.29	1040.31	103.5	3050	-	N
40	swab	PVC	58.34 **52.57**	4	38.27	727.98	0.00	1718	-	N

The 1st column denotes pipe bomb numbers; bomb 11 was the positive control (pos), and bombs 12 and 13 were the negative controls (neg). The 2nd and 3rd columns display the DNA isolation technique used and the type of pipe bomb material respectively. The 4th column shows the recovered mass of each pipe bomb post deflagration. Two masses are displayed for pipe bombs 36 – 40: the mass of all the pieces collected, and the mass of the swabbed pieces only (bold). Bomb fragmentation levels are as noted: 1 – low, 2 – medium, 3 – high, and 4 – complete. The 6th column displays the average amount of DNA recovered from each pipe bomb in picograms (pg) using the Timken et al. (2005) assay. Values in parentheses signify the average quantity of DNA inclusive of zero values (the Ct value was not reached). The 7th column displays the amount of DNA in femtograms (fg) per gram of pipe. Column 8 gives the quantity of nuclear DNA in picograms (pg) based on the Quantifiler assay and column 9 shows the quantity of mtDNA in copy numbers. The symbol (-) indicates that no analysis was conducted. The 10th column shows STR results based on categories: A – Alleles from volunteers only, B – Volunteer (major profile) + additional alleles, C – Volunteer (non-major profile) + additional alleles, D – Some, but not all alleles from volunteer, E – No alleles from volunteer, F – No alleles obtained. The additional symbols after the lettered category indicate: † = incorrect alleles; • = missing alleles; * = complete allele dropout at 1 locus; ** = complete allele dropout at 2 loci; *** = complete allele dropout at 3 or more loci. Column 11 shows the level of inhibition for each sample, based on the results of the Quantifiler IPC. Inhibition categories were: N – none, P – partial and F – full.

Comparison of Timken et al. (2005) and Quantifiler Assays: Nuclear DNA

The Timken et al. (2005) and Quantifiler assays had a correlation value of 0.48, indicating that there was a moderate positive relationship. Seven of the DNA samples from the Timken et al. (2005) assay generated values higher (greater than 10 pg) than Quantifiler, with quantities for samples 4, 36, 38 and 40 giving more than 30 pg greater than the latter assay. The Quantifiler assay produced values that were higher than the Timken et al. (2005) assay for eight of the DNA samples, three of which (35 swab, 37 and 39) were more than 60 pg greater than those of Timken et al. (2005). The remaining 23 DNAs were similar in concentration (less than 10 pg difference) between the assays. No amplification was observed for 19 of the samples using the Quantifiler assay, 4 of which (12, 30, 31 swab and 32 swab) produced the same results using the Timken et al. (2005) assay. There were five instances where the Quantifiler assay produced a positive value for one of its replicates but no amplification in another (8, 10, 27, 32 soak and 33 swab).

DNA Quantity Relative to Pipe Material, Pipe Mass Recovered, and Isolation Technique

DNA yields based on the type of pipe material and its recovered mass are displayed in Table 3. DNA amounts in descending order are shown in Appendix A2 and A3 respectively. Fifteen of the 18 steel bombs (83.3%) generated DNA, while no DNA was recovered from bombs 3, 6 and 32 (16.7%). Assessing the steel bombs according to isolation technique, 14 of the 18 swabbed bombs (77.8%) and 4 of the 18 soaked bombs (22.2%) yielded DNA. Further breakdown of the soaked steel fragments show that 0 of the 13 pipe bombs that were soaked overnight produced DNA, while 4 of the 5 bombs

incubated at room temperature for 2 hours generated positive results. Quantification values were obtained for 16 of the 17 PVC bombs (94.1%). DNA was recovered from all 5 swabbed PVC bombs (100%) and 11 of the 12 soaked bombs (91.7%).

Higher, but not statistically different quantities of nuclear DNA per gram of pipe were retrieved from swabbed steel fragments versus the soaked fragments (bombs 33 – 35). The mass of bomb recovered and DNA yields showed a modest positive correlation of 0.29 using the Timken et al. (2005) assay, and 0.36 using Quantifiler. However, bombs with larger masses did not necessarily generate the highest DNA quantities. As an example, bomb 32 had a mass of 700.84 g but no DNA was recovered based on either quantification assay, while bomb 16 had a lower mass of 577.15 g but generated 7.77 pg/ μL of DNA using Timken et al. (2005) and 19.6 pg/ μL using Quantifiler. DNA quantities from soaked steel fragments were similar to each other, all being less than 10 pg/ μL.

Swabbed PVC fragments (36 – 40) yielded higher, but not statistically different quantities of nuclear DNA per gram of pipe than soaked PVC fragments. Recovered bomb masses for the swabbed fragments were among the lowest analyzed, ranging from 39.69 – 57.63 g. Masses of soaked PVC fragments, ranged from 53.36 – 121.68 g. More DNA was generated from the higher fragment masses among soaked PVC samples, although their DNA quantities were still less than that recovered from swabbed samples. The mass of PVC bomb recovered and DNA yields showed a moderate negative relationship of -0.47 using the Timken et al. (2005) assay and -0.31 using Quantifiler.

Recovered Nuclear DNA Quantities and Levels of Pipe Fragmentation

Fragmentation levels from 6 steel bombs fell into the low category, 5 into medium, 3 into high, and 3 into complete fragmentation. Table 3 shows the level of pipe fragmentation versus the quantity of DNA retrieved for each steel pipe. The average quantity of DNA for each fragmentation category is recorded in Appendix A4. Using DNA quantities from the Timken et al. (2005) assay, the greatest amount was recovered from bombs in the high fragmentation category, followed by bombs in the complete category. Bombs in the medium fragmentation category produced the third highest DNA yields, while those in the low category generated the lowest amount of DNA.

The Quantifiler assay showed that bombs in the high fragmentation category produced the most DNA, followed by those in the low fragmentation category (Appendix A4). Bombs in the medium category yielded the third highest quantity of DNA, while those that underwent complete fragmentation generated the lowest amount of DNA.

MtDNA Yields and Pipe Material, Pipe Mass and Isolation Technique

Steel fragments 3 – 7 and 13 – 21, incubated overnight, were not analyzed for mtDNA quantity as no DNA was obtained for either the preliminary mtDNA PCR analysis or the Timken et al. (2005) nuclear quantification assay. Apart from those bombs, pipe bomb material did not appear to influence mtDNA retrieval as DNA was generated for all other pipes (Table 3). Average mtDNA quantities, in descending order of mass, and separated by pipe material and isolation technique, are shown in Appendix A5. The correlation between the mass of pipe recovered and mtDNA yield was -0.04 for PVC pipes and 0.12 for steel pipes. Swabbed PVC mtDNA yields ranged from 1718 –

5394 copies, while soaked quantities ranged from 110 – 5624 copies. Swabbed steel fragments also tended to generate higher quantities of DNA (8 – 9297 copies) than did the soaked fragments (8 – 943 copies; Appendix A6). Pipe bomb 32 yielded a positive mtDNA result despite not producing any nuclear DNA in either the Timken et al (2005) or Quantifiler assays.

Disparities among mtDNA triplicate yields were small (Appendix A1), but instances occurred where two quantities were similar to each other while the third was much higher or lower. Examples include pipe bomb 7 which generated 2170, 2036 and 413 copies, and pipe bomb 9 that produced 1600, 2291 and 4206 copies. A few of the pipes produced a positive result for only two of the three replicates, while generating no DNA for the third. These included bomb 24, which yielded 927 and 1004 copies, and bomb 38 that produced 2109 and 2085 copies, with no DNA being obtained for the third triplicate in both instances. This trend was not more prominent with either PVC or steel pipes, nor for a particular isolation technique.

MtDNA Quantities and Levels of Pipe Fragmentation

Appendix A7 shows the average quantity of mtDNA recovered per gram of pipe for each pipe fragmentation category. Pipes in the high fragmentation category generated the most DNA, followed by complete pipe fragmentation, low pipe fragmentation, and medium fragmentation. The order of the mtDNA categories were the same as the Timken et al. (2005) nuclear assay, except that the last two categories were reversed. Only the high fragmentation category was the same as Quantifiler, which had the low

fragmentation category with the second highest yields, followed by medium fragmentation, and lastly complete fragmentation.

Recovered Nuclear DNA and MtDNA Quantities

Table 4 displays the rank of nuclear and mtDNA quantities, with nuclear DNA in descending order, while Table 5 shows the rank of the DNAs in descending order of mtDNA quantity. The correlation value between the Timken et al. (2005) assay and the mtDNA assay was 0.33, while the value between the Quantifiler and mtDNA assays was 0.52, showing a moderate positive relationship in both instances. Overall rankings are seen in Appendix A8, with samples producing a quantification value of zero not ranked.

Table 4: Comparison of nuclear DNA quantities in descending order to mtDNA quantities

Rank	Pipe #	Isolation Technique	Pipe Material	Average amt. of nuclear DNA (pg)	Average amt. of mtDNA (copy #)
1	17	swab	steel	72.00	1657
2	27	soak	PVC	67.33	4145
3	37	swab	PVC	53.79	5398
4	36	swab	PVC	47.10	2085
5	4	swab	steel	46.95	1240
6	14	swab	steel	45.95	1543
7	38	swab	PVC	45.28	2097
8	39	swab	PVC	41.29	3050
9	40	swab	PVC	38.27	1718
10	22	soak	PVC	32.20	1022
11	20	swab	steel	29.08	9297
12	7	swab	steel	23.48	1540
13	25	soak	PVC	20.35	1956
14	34	swab	steel	16.25	751
15	33	swab	steel	12.12	1394
16	9	soak	PVC	11.91	2699
17	15	swab	steel	8.50	1317
18	16	swab	steel	7.77	1433
19	31	soak	steel	7.69	943

Table 4 continued					
20	35	swab	steel	7.66	3204
21	10	soak	PVC	7.19	2461
22	19	swab	steel	6.75	3180
23	26	soak	PVC	5.73	1128
24	24	soak	PVC	5.53	966
25	11	soak	PVC	4.21	490
26	29	soak	PVC	4.15	434
27	23	soak	PVC	3.61	469
28	18	swab	steel	2.64	1320
29	8	soak	PVC	2.58	5624
30	21	swab	steel	2.50	782
31	5	swab	steel	1.91	230
32	34	soak	steel	1.35	21
33	33	soak	steel	1.27	19
34	13	swab	steel	0.98	829
35	35	soak	steel	0.85	16
36	28	soak	PVC	0.38	4533
37	12	soak	PVC	0.00	110
38	30	soak	PVC	0.00	864
39	31	swab	steel	0.00	8
40	32	swab	steel	0.00	1799
41	32	soak	steel	0.00	8

Column 1 shows the rank of the nuclear DNA quantities in descending order. Pipe bomb number is reported in column 2, while columns 3 and 4 display the isolation technique and the type of pipe material, respectively. Average amount of nuclear DNA in picograms (pg) is shown in column 5, while column 6 displays the average quantity of mtDNA in copy number.

Pipes that generated high quantities of nuclear DNA did not necessarily produce high mtDNA quantities. Pipe bomb 22 ranked 10th using the Timken et al. (2005) nuclear assay and 9th using Quantifiler, but 25th on the mtDNA scale. Interestingly, pipe bomb 8 provided one of the lowest quantities of nuclear DNA using Timken et al. (2005) assay (29th), ranked 5th in nuclear DNA using the Quantifiler assay and yielded the 2nd highest quantity of mtDNA (Appendix A8).

Ranking DNA quantities based on mtDNA yield, pipe bomb 20 produced the most mtDNA but was 11th in the amount of nuclear DNA using the Timken et al. (2005) assay (Table 4), and 10th using the Quantifiler assay. Pipe bomb 19 ranked 7th on the mtDNA scale, but 22nd and 26th on the Timken et al. (2005), and Quantifiler assays respectively. The largest disparity was seen with bomb 28, which ranked 4th on the mtDNA scale, but 36th using the Timken et al. (2005) nuclear assay and produced no results using Quantifiler despite there being no sign of inhibition.

Table 5: Comparison of mtDNA quantities in descending order to nuclear DNA quantities

Rank	Pipe #	Isolation Technique	Pipe Material	Average amt. of mtDNA (copy #)	Average amt. of nuclear DNA (pg)
1	20	swab	steel	9297	29.08
2	8	soak	PVC	5624	2.58
3	37	swab	PVC	5398	53.79
4	28	soak	PVC	4533	0.38
5	27	soak	PVC	4145	67.33
6	35	swab	steel	3204	7.66
7	19	swab	steel	3180	6.75
8	39	swab	PVC	3050	41.29
9	9	soak	PVC	2699	11.91
10	10	soak	PVC	2461	7.19
11	38	swab	PVC	2097	45.28
12	36	swab	PVC	2085	47.10
13	25	soak	PVC	1956	20.35
14	32	swab	steel	1799	0.00
15	40	swab	PVC	1718	38.27
16	17	swab	steel	1657	72.00
17	14	swab	steel	1543	45.95
18	7	swab	steel	1540	23.48
19	16	swab	steel	1433	7.77
20	33	swab	steel	1394	12.12
21	18	swab	steel	1320	2.64
22	15	swab	steel	1317	8.50

Table 5 continued					
23	4	swab	steel	1240	46.95
24	26	soak	PVC	1128	5.73
25	22	soak	PVC	1022	32.20
26	24	soak	PVC	966	5.53
27	31	soak	steel	943	7.69
28	30	soak	PVC	864	0.00
29	13	swab	steel	829	0.98
30	21	swab	steel	782	2.50
31	34	swab	steel	751	16.25
32	11	soak	PVC	490	4.21
33	23	soak	PVC	469	3.61
34	29	soak	PVC	434	4.15
35	5	swab	steel	230	1.91
36	12	soak	PVC	110	0.00
37	34	soak	steel	21	1.35
38	33	soak	steel	19	1.27
39	35	soak	steel	16	0.85
40	31	swab	steel	8	0.00
41	32	soak	steel	8	0.00

Column 1 shows the rank of the mtDNA quantity in decreasing order. Pipe bomb number is reported in column 2, while columns 3 and 4 display the isolation technique and the type of pipe material, respectively. Average amount of mtDNA in copy number is shown in column 5. Column 6 displays the average quantity of nuclear DNA in picograms (pg).

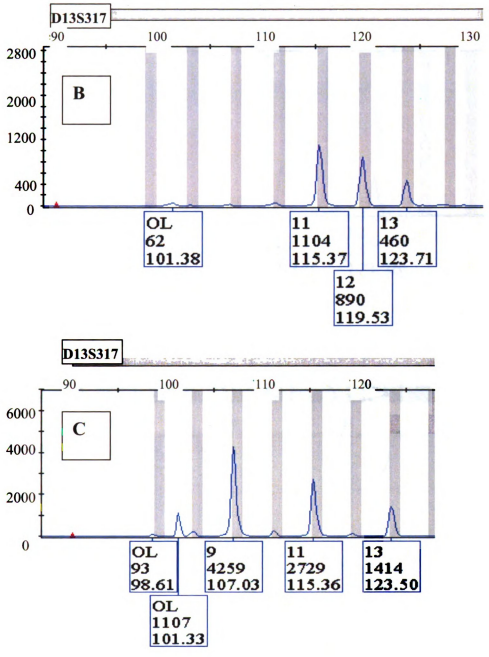
STR Results for Swabbed and Soaked DNA Samples

Total input DNA for STR analysis based on the Timken et al. (2005) assay were: 20 samples had less than 100 pg of DNA, 4 had 100 – 200 pg, and 2 had 200 – 300 pg of DNA; based on Quantifiler: 19 samples had less than 100 pg of DNA, 5 had 100 – 200 pg, and 2 had 200 – 300 pg of DNA. STR results based on the number of alleles observed are shown in Table 3 and complete results are in Appendix B. Figures 14 and 15 show examples of electropherograms for STR categories B and C, and D and F respectively. Seven of the 11 swabbed steel pipes fell into category B, wherein although there were additional alleles, the volunteer constituted the major profile. Four of those 7 swabbed pipes had incorrect alleles at one locus, 2 had at least one missing allele, and 2 had

complete allele drop-out at the FGA locus. The remaining 4 of the 11 swabbed pipes fell into category 'C' where the volunteer alleles were recovered along with additional alleles, but were not the major profile. Two of the 4 pipes produced incorrect alleles at one locus, 3 of the pipes had at least one missing allele, and 1 pipe had total allele drop-out at the FGA locus.

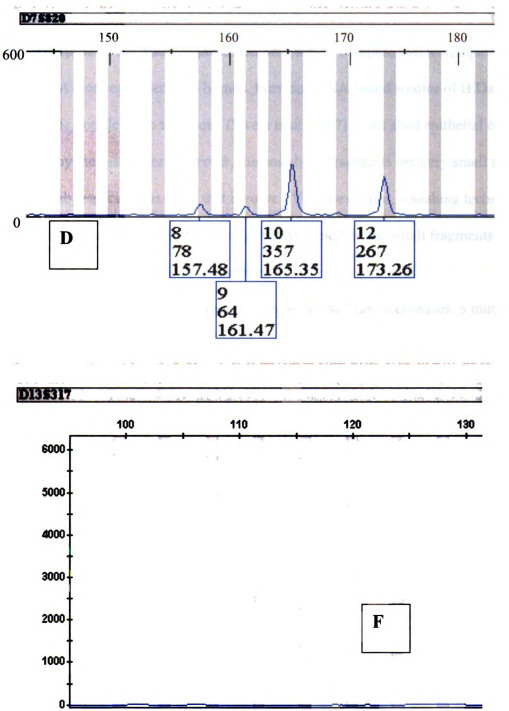
DNA from the soaked PVC pipe fragments fell into categories D – F, with only one sample falling into category 'B' (8 soak). Eight pipes were categorized as 'D' (some of the volunteer's alleles obtained), 2 pipes as 'E' (no volunteer alleles obtained), and 1 as 'F' (no alleles obtained). The positive control (11) was categorized as 'D', with at least one locus with incorrect alleles and complete allelic drop-out at three or more loci. STR results compared to the level of inhibition showed that samples that generated the most allelic drop-out were soaked PVC fragments, which tended to be inhibited. One of the negative controls (12) showed sporadic contamination as only one allele was obtained, while the other (13) produced alleles at every locus, 7 of which were consistent with one of the analysts. Alleles were recovered at three loci for sample 30, although no quantification results were obtained for the nuclear DNA assays.

Figure 14: Electropherograms of STR categories B and C



Electropherograms showing STR categories B and C (volunteer (major profile) + other alleles and volunteer (non-major profile) + other alleles) respectively. The number of RFUs is displayed on the y-axis, while the size of the alleles is denoted on the x-axis. The numbers within the boxes reflects the number of STR repeats, the number of RFUs, and the length of the repeat (in bp), respectively. Vertical columns refer to grouped 'bins' into which the alleles fall. Category B depicts the volunteer alleles (11, 12) as the major peaks among additional alleles, and category C shows the volunteer alleles (13, 13) as the minor peak among additional alleles.

Figure 15: Electropherograms of STR categories D and F



Electropherograms showing STR categories D and F respectively. The number of RFUs is displayed on the y-axis, while the size of the alleles is denoted on the x-axis. The numbers within the blue boxes reflects the number of STR repeats, the number of RFUs, and the length of the repeat (in bp), respectively. Vertical columns refer to grouped 'bins' into which the alleles fall. Category D depicts an instance where only some of the volunteer alleles (11, 12) are present, and category F shows no alleles were generated.

Discussion

The purpose of the research presented here was to compare two techniques used to recover DNA from exploded pipe bombs. Previous DNA-based studies of IEDs incorporated the double swab technique (Sweet et al. 1997) to lift shed epithelial cells left on the device by the assembler. However, the post-blast fragments are very small and difficult to swab, especially in cases that involve PVC pipes. Thus, a soaking technique was developed as a means of retrieving cells that remained on the small fragments, which typically would not be processed by swabbing.

Various advantages were offered by each of the isolation techniques, a mutual one being that they were both relatively easy. A major benefit of the swabbing technique was that fewer reagents—including buffer, proteinase K, and other laboratory supplies—were used. A minimal amount of buffer was applied to the swab before it was rubbed over the pipe surface and less than 1 mL was used to digest the retrieved cells. The small amount of moisture also limited the generation of rust from the steel bomb fragments. Another advantage was that the swab was applied directly to the surface of the fragments, and the pressure likely aided in loosening and lifting any cells adhering to the surface. A third benefit was that fewer steps were performed between swabbing the fragments and DNA extraction, which decreased the chances of DNA loss.

One disadvantage of the swabbing technique was that it was tedious and time-consuming. Each fragment needed to be carefully swabbed to ensure that the entire surface was covered. Fragments from a steel bomb took about 20 minutes to swab,

allowing about 3 bombs to be processed in an hour. Swabbed PVC bombs took even longer as there were many small fragments to be processed.

The soaking technique allowed more bombs to be set up for analysis in a shorter period of time. The time taken to prepare the fragments and pouches for several bombs was short, as was adding the buffer and sealing the pouches for incubation. A second advantage was that more fragments per bomb were analyzed since the very small pieces, not normally swabbed, could be processed in the pouch.

However, the soaking technique required large quantities of reagents. Further, when the fragments were being shaken or incubated in the plastic pouches, the buffer solution settled to the bottom of the pouch, leaving some of the top fragments uncovered. Unless the pouch was frequently flipped over, some of the cells may not effectively be lysed. Also, it was not possible to remove all of the buffer from the pouch and pipe pieces, and generally only about 15 of the 20 mL was recovered. DNA was undoubtedly left in the residual liquid or adhered to the bomb fragments. Greater DNA retrieval may have been possible if a different device was used to soak the fragments. Instead of the flexible pouch, a sturdier container that can be centrifuged may be advantageous. Not only would the liquid be drawn to the bottom, but it would reduce the number of times that the buffer was transferred during its cleanup. This, in turn, could reduce the amount of liquid, and ultimately DNA, that is lost. Centrifugation would pellet the debris that accumulated in the buffer as it was washed off the fragments. While centrifugation can be a benefit, the downside is that DNA may be trapped and pelleted as well.

Owing to the large volume of buffer used in the soaking technique, the solution had to be concentrated before extraction. The ultrafiltration devices used were intended

for protein concentration, thus needed to be tested on DNA. Different size filters that retained proteins up to a specific molecular weight were tested before choosing the one used in the experiment. The Vivaspin 10,000 MW device was selected because it retained DNA while allowing for relatively fast filtering. MtDNA PCR analysis confirmed that no DNA was entering the flow-through, as no PCR product was obtained for flow-through samples. However, another problem was encountered during the concentration step, when debris that did not pellet during centrifugation became concentrated along with the DNA. As a result, the debris was eluted with the DNA in the final step of the organic extraction. Subsequent DNA analysis of these samples proved to be challenging due to inhibition and amplification failure.

A final drawback of the soaking technique occurred during the processing of the steel fragments. The soak allowed for rust formation, seen in greater amounts for the fragments that were left overnight. It became apparent that overnight incubation had an adverse effect on the steel fragments and the final buffer quality, as a white substance, not seen on fragments incubated at room temperature for two hours, formed on the pieces. The substance was crystal or salt-like. It could have been generated from one or more of the reagents in the buffer, such as the TRIS, EDTA, or SDS as it reacted with the metal, presumably through oxidation. It is not known to what extent the substance affected the DNA, although no PCR product was obtained for any of those DNA samples and there were no signs of inhibition. The DNA could have been destroyed, or trapped and pelleted with the debris. The latter is quite possible since the formerly rust-filled solution became almost colorless after centrifugation. The rust in buffer solutions from steel fragments incubated at room temperature was removed during Microcon

centrifugation, as the final extract yielded clean DNA that, according to the Quantifiler assay, showed no signs of inhibition.

Correlation between the two nuclear quantification assays was particularly important as it gave an indication of the extent to which the two assays were in sync. A moderate positive relationship of +0.48, signified that there were differences in the quantification results from each assay. The deviances between the assays might have arisen due to pipetting error from stochastic sampling effects (the random chance that the target DNA is sampled and amplified stemming from the small quantities of DNA recovered), or from the number of times each DNA sample had to be assayed to obtain positive results. This influenced quantification results as the chance of obtaining a positive outcome increased as the number of trials increased. Samples assayed using Timken et al. (2005) were run repeatedly until positive results were obtained, whereas those assayed using Quantifiler were run once in duplicate, decreasing the number of positive results obtained. Repeat runs sometimes required diluting the DNA 1:10 or 1:20, which exacerbated stochastic effects. No positive quantification values were obtained once the dilution was greater than 1:20.

A moderate positive correlation between the two nuclear assays begged the question as to which was more accurate at quantifying the DNA. Subsequent correlations to the mtDNA assay and the mass of the recovered pipe fragments indicated that Quantifiler was more accurate, in spite of there being no amplification for almost half of the DNAs assayed. This signified that the zero values were real and could not be discounted. While the stochastic effects and small DNA quantities influenced quantification results, the accuracy of Quantifiler could also be attributed to components

found in its reagents. The buffer from the Quantifiler kit was optimized for real-time quantification (Quantifiler Kit User Manual), and it is possible that proprietary reagents within the buffer and primer mix are more effective for quantification than are the reagents found in the Bio-Rad buffer used for the Timken et al. (2005) assay, resulting in more accurate Quantifiler results.

The only counterintuitive result seen between DNA quantity and recovered pipe amounts was for swabbed PVC bombs, where DNA yields increased with decreasing amounts of recovered pipe. This is contrast to the soaked samples, generated from greater amounts of retrieved pipe overall, which demonstrated a positive relationship between DNA quantity and pipe obtained, although DNA yields were still less than those recovered from the swabbed fragments. Interestingly, high quantities of DNA were consistently generated by the 5 swabbed bombs based on the Timken et al. (2005) assay, while only three of those same bombs amplified using Quantifiler. Two of the bombs analyzed using the latter assay (bombs 37 and 39), one of which (39) had the smallest swabbed mass, had DNA quantities in excess of 100 pg/ μL, values that were higher than any other recovered DNA quantity for either assay. Bombs 37 and 39 also had two of the highest levels of DNA recovered based on the Timken et al. (2005) assay as well, thus the results seem real, and it appears these bombs, in spite of their high fragmentation, actually contained large amounts of DNA, with bomb 39 causing the negative correlation between DNA and bomb mass yields.

STR analysis aided in ascertaining the quality of DNA recovered from the pipe fragments. MiniFiler required a total of 500 – 750 pg of input DNA for optimum performance; hence, none of the samples were optimal. Good results, however, were

obtained from the swabbed pipe fragments, indicating that a lack of PCR inhibition was important to successful STR analysis. For example, volunteer alleles were retrieved from swabbed pipe bomb 5, which was analyzed using 3.61 pg of DNA based on Timken et al. (2005) and 7.64 pg based on Quantifiler. Conversely, no alleles were recovered for soaked pipe bomb 27 which was analyzed using 269.32 pg and 277.60 pg in the same assays respectively. Pipe bomb 5 showed no sign of inhibition based on the Quantifiler assay, while pipe bomb 27 was partially inhibited. Thus, adding 4 μL of DNA for STR analysis versus 1.2 μL for quantification likely contributed to the failure of obtaining alleles for bomb 27. The cleanness of the DNA sample, therefore, made a difference in the generation of STR results. Also of note was bomb 8 which was the only soaked bomb to be classified in category B (volunteer alleles along with additional alleles were obtained, with the volunteer alleles being the major profile). This DNA showed partial inhibition, where 10.32 pg of DNA was added based on the Timken et al. (2005) assay, while 249.60 pg was added based on Quantifiler. In this instance, even though the DNA sample contained inhibitors, the quantity of DNA added (based on Quantifiler values) aided in producing volunteer alleles. These results help confirm that Quantifiler was the better assay for nuclear DNA quantification.

Complete allele drop-out generally occurred at the largest locus (FGA) for swabbed fragments, while it was more variable from the soaked fragments. Larger loci tend to drop-out first when DNA is degraded (Whitaker et al. 1995), which would explain the lack of amplification success of the FGA locus from the swabbed fragments. Although this would have also occurred for soaked fragments, there was the additional issue of diluting those DNAs to reduce PCR inhibition, and hence an increase in

stochastic effects. It is therefore understandable why a higher level of allelic drop-out was experienced with the DNA samples from the soaked fragments.

Stutter, an artifact of STR analysis, occurs as a result of slippage by the polymerase, making the target DNA one repeat unit shorter than the true allele. Most of the additional alleles at a locus were one repeat unit shorter than the true allele, and were observed at various loci for each sample. It was difficult to ascertain whether those alleles were real or were the result of stutter. Usually stutter products are about 5 – 10% of the actual allele's peak height. However, under LCN conditions stutter products are enhanced and their peak heights can be greater than the 10% (Whitaker et al. 2001). Examining the peak height intensities, therefore, did little to identify real alleles because in some instances the intensity of an additional peak and that of an actual peak were almost the same. Low intensity peaks were especially challenging to categorize as they could have been called in various ways: they may have been stutter, a real allele from a volunteer, or an allele from a minor contributor. All the scenarios demonstrate the difficulties that arise when analyzing LCN samples. These could potentially be eliminated by either testing different portions of the bomb to build a consensus profile from repeated peaks, or comparing the unknown alleles to known profiles to ascertain whether a minor profile existed.

Allele drop-in was also common in the STR results, occurring at about half the loci for swabbed samples, and at all the loci for soaked samples. Drop-in is sporadic, and is usually not reproducible when samples are retested. It was difficult to establish, however, whether an allele was drop-in, a result of contamination, or the result of stutter, as the DNAs were only tested once. Previous studies in the laboratory demonstrated the

benefits of repeated testing in generating a consensus profile (Hoffmann 2008), in which only alleles that appeared more than once were called, and the chances of identifying all correct alleles increased. A few of the alleles that were established to be drop-in after making comparisons to the reference samples had higher peak intensities than those from the volunteer, and were falsely deemed as the major allele. Although it was possible that some of the drop-in was a result of contamination from the analysts, identifying an allele as drop-in would have been easier had repeat testing been done.

The purity of the DNA recovered from each type of pipe was determined using the Quantifiler IPC. DNA from swabbed steel and PVC fragments showed no signs of inhibition and amplification curves were generated without difficulty. Interestingly, samples from soaked steel fragments also showed no signs of inhibition, despite there being rust and the white substance prior to extraction. It was likely that the higher temperature of 56 °C for the overnight soaks sped up the rate of chemical reactions taking place between the buffer and the steel, causing the formation of rust and the white salt. Pelletting of the white substance and subsequent use of the Microcon columns, removed the debris and rust from the final elute. In contrast, the reduced soak time of 2 hours and incubation at room temperature, which retarded the rate of chemical reactions, allowed for successful DNA retrieval and amplification of soaked steel samples. Low quantities or absence of DNA from the soaked steel fragments could be attributed to degradation of the nuclear DNA by Mg^{2+}-dependent exonucleases, even though EDTA was added to the buffer to chelate the Mg^{2+} ions and inactivate such enzymes. A high concentration of Fe^{2+} stemming from the steel pipes may have overwhelmed the EDTA, rendering it ineffective. Two hour incubations, along with increasing the concentration of EDTA,

could reduce the chance of saturating the buffer with divalent cations, and allow the EDTA to effectively chelate the Mg^{2+}ions. Future soaks of steel fragments should be done using various time intervals, to determine the shortest time possible for optimum DNA recovery.

MtDNA was less challenging to amplify as results were always obtained after one quantification attempt. Amplification success was likely due to dilution of the inhibited DNA, which reduced the concentration of the inhibitors and increased polymerase activity, coupled with factors such as higher copy number and better survivability of mtDNA (Foran 2006). Three of the bombs generated mtDNA quantities for only two of three triplicate samples, with the DNA from one of those showing partial inhibition while the others were uninhibited. Inhibitors from that single bomb may be responsible for the amplification failure of its third triplicate, whereas the failure of the uninhibited DNA from the other two bombs could be due to stochastic effects, or inhibitors introduced into the sample from the PCR tube or pipette tip.

Amplicon size also likely influenced the successful amplification of mtDNA. The mtDNA target of 69 bp was smaller than that of the Timken et al. (2005) nuclear target of 170 – 180 bp. Timken et al. (2005) examined the amplification success of degraded DNA as it related to amplicon size. High quality DNA was degraded to various extents and analyzed using Quantifiler, which amplifies a 62 bp region, and the Timken et al. (2005) assay. Subsequently, larger STR loci failed to amplify, indicating that while the two assays were effective in quantifying amplicons of their respective sizes, they both overestimated the quantities for longer amplicons when the DNA was degraded. Quantifiler had a higher overestimation as its amplicon was shorter and more likely to be

intact and in greater amounts when the DNA was degraded. Thus, due to high copy number, high quality and the small size of the mtDNA amplicon, amplification was more successful.

Esslinger et al. (2004) and Gehring (2004) both found that as the level of pipe fragmentation decreased, the amount of recovered DNA increased. This trend was not seen in the current study as the low fragmentation category collectively produced the least nuclear DNA based on Timken et al. (2005) quantification results, and the second lowest quantity based on mtDNA. This fragmentation category, however, had the second highest DNA yield based on the Quantifiler assay, although most of this could be attributed to bomb 35 which generated a very high value of 131.21 fg per gram of pipe. The same bomb only generated 15.36 fg per gram of pipe for the Timken et al. (2005) assay. While the Quanitfiler value seemed to be an outlier, mtDNA quantities for bomb 35 was one of the highest obtained, thus more closely agreeing with the Quantifiler nuclear quantity. It is likely, therefore, that the Quantifiler value was not an outlier, but another confirmation that the assay was more reliable.

Low bomb fragmentation meant that a large portion of pipe nipple survived, along with several small end cap pieces, providing a large surface area for processing. The nipple and end caps were coated with zinc, which, if removed, would result in the loss of cells. Zinc removal, however, was minimal when fragmentation was low, becoming more frequent as the level of fragmentation increased. It is not certain, therefore, why pipes with the most surface area did not generate the highest DNA quantities. It is possible that large fragments retained the heat from the blast for a longer time and as a result, there was further DNA degradation. In spite of this, cells containing quality DNA were still

recovered as all of the DNA from low fragmentation bombs fell into STR category B—the volunteer alleles constituted the major profile. This indicated that, although yields were low, the DNA was of good quality, and potentially represented a caliber of DNA typical of large pipe fragments. Another explanation for low quantities of DNA from category 1 fragments is linked to the amount of cells shed by a volunteer. There were instances in the high and complete fragmentation categories, including bombs 37 and 39 detailed above, where certain pipes generated DNA quantities that were much higher than the others, perhaps owing to a 'good shedder'. This included steel bomb 17 in the high fragmentation category which had a quantification value of 144.57 fg per gram of pipe, while the other two high fragmentation steel bombs only generated 42.72, and 13.46 fg per gram, and steel bomb 4 which had the highest value in the complete fragmentation category with 83.55 fg per gram of pipe, versus 16.86 and 4.37 fg per gram for the other two complete fragmentation steel bombs. Low DNA yields for the large category 1 bomb fragments may have also resulted from few cells being deposited or cells being left behind during swabbing. In this regard, there were times when the nipple became dry during the application of the wet swab, which could result in cells re-adhering to the surface of the pipe and not being effectively lifted by the dry swab.

Overall, two hypotheses were proposed for this study: 1) more recovered pipe would generate more DNA, and 2) the soaking technique would recover more DNA than the swabbing technique because it allowed all sized fragments to be utilized. The first hypothesis was not necessarily supported, particularly when analyzing PVC pipes, as the amount of DNA recovered depended less on the amount of pipe recovered than it did on the DNA isolation technique used. Swabbed PVC fragments produced more DNA than

soaked PVC fragments, despite having the least amount of recovered pipe available. It could be that there is a limit to the size of a fragment from which DNA can be recovered, regardless of the technique. Smaller pipe pieces may be generated from hotter pipes, which cause more cells to be either degraded by heat or removed by the impact of the blast. If this is the case, even though more pieces are incorporated into the analysis during the soaking technique, many would not contribute DNA.

The hypothesis that the soaking technique would recover more DNA than the swabbing technique was not supported either, as more DNA was recovered from swabbed fragments. It was thought that the soaking technique would have been more successful for two main reasons: 1) in the case of PVC pipes, more of the recovered pipe could be utilized, and 2) the fragments were fully immersed in the buffer. However, in each instance there were limitations that hindered the amount of DNA that could be retrieved. Improvements in the soaking technique, such as using a sturdier container or a more effective cleanup method, may assist in producing greater quantities of DNA.

Although the swabbing technique was found to be more effective at DNA recovery, its application exposed minor adjustments that could be incorporated to make it more efficient. Swabs could be re-wetted when analyzing large surface areas to ensure that cells are hydrated, or multiple wet swabs may be used on the same surface before the dry swab is applied. The effectiveness (or ineffectiveness) of the soaking technique could also be tested by swabbing the soaked fragments to determine if DNA remains. Further optimization of the two isolation techniques could prove beneficial in the analysis of IEDs.

Conclusion

The goal of this study was to compare the double swab and soaking techniques, to ascertain which method was better at efficiently and effectively providing a higher quantity and quality of recovered DNA. While the double swab technique was more tedious, it was more effective at DNA retrieval as extracts from swabbed fragments were cleaner, and generated greater quantities of DNA. Amplification success was more frequent and better STR results were also obtained from swabbed bombs. The soaking technique was quicker than the double swab technique, but was less effective at DNA retrieval as the isolated DNA was more challenging to analyze. This resulted from an inability to remove debris that co-extracted with the DNA, which caused PCR inhibition, and because the soaked samples had to be diluted as a consequence. Thus, stochastic effects were exacerbated.

MtDNA amplification was more successful than nuclear DNA amplification for both isolation technique, which was attributed to high mtDNA copy number, the small size of the amplicon, and the possibility that the mitochondrion protected the DNA during deflagration more than did the nucleus. Correlative relationships between nuclear DNA, mtDNA, and recovered pipe masses indicated that Quantifiler was a more accurate nuclear DNA quantification assay, in addition to its benefit of determining PCR inhibition levels. Finally, successful recovery of both types of DNA was independent of pipe material, the level of pipe fragmentation, or the amount of recovered pipe used for analysis, with the DNA isolation technique being the primary factor influencing DNA recovery.

Appendix A

Nuclear and MtDNA Data

Table A1: Nuclear DNA and mtDNA quantities for each sample

Pipe #	Isolation Technique	Pipe Material	Nuclear DNA Values	MtDNA values
3	swab	steel	0, 0	-
3	soak	steel	0, 0	-
4	swab	steel	23.85, 54.50, 43.95, 65.50	604, 1297, 1818
4	soak	steel	0, 0	-
5	swab	steel	5.10, 2.06, 0.49, 0	75, 291, 324
5	soak	steel	0, 0	-
6	swab	steel	0, 0	-
6	soak	steel	0, 0	-
7	swab	steel	14.00, 29.40, 20.55, 29.95	2170, 2036, 413
7	soak	steel	0, 0	-
8	soak	PVC	1.20, 9.13, 0, 0	810, 7479, 8582
9	soak	PVC	47.63, 0, 0, 0	1600, 2291, 4206
10	soak	PVC	5.07, 23.67, 0, 0	1224, 2655, 3503
11	soak	PVC	16.83, 0, 0, 0	117, 396, 958
12	soak	PVC	0, 0, 0, 0	28, 55, 246
13	swab	steel	1.95, 0	859, 630, 999
13	soak	steel	0, 0	-
14	swab	steel	48.65, 43.25	1394, 1491, 1745
14	soak	steel	0, 0	-
15	swab	steel	10.10, 6.90	1309, 1321, 1321
15	soak	steel	0, 0	-
16	swab	steel	13.05, 2.49	1208, 1333, 1758
16	soak	steel	0, 0	-
17	swab	steel	72.00, 0	1673, 1770, 1527
17	soak	steel	0, 0	-
18	swab	steel	0.12, 5.15	1624, 967, 1370
18	soak	steel	0, 0	-
19	swab	steel	13.50, 0*	3382, 3309, 2848
19	soak	steel	0, 0	-
20	swab	steel	37.65, 20.50	10982, 9176, 7733
20	soak	steel	0, 0	-
21	swab	steel	5.00, 0	962, 642, 742
21	soak	steel	0, 0	-
22	soak	PVC	42.60, 21.80, 0, 0*	777, 1016, 1273
23	soak	PVC	1.85, 1.87, 10.7, 0	465, 608, 333
24	soak	PVC	22.10, 0, 0, 0	927, 1004, 0

Table A1 continued				
25	soak	PVC	18.90, 21.80, 0, 0, 0*	1903, 1855, 2109
26	soak	PVC	5.42, 8.61, 3.17, 0, 0, 0*	867, 1345, 1171
27	soak	PVC	67.33,0 ,0*	4533, 3248, 4655
28	soak	PVC	1.14, 0, 0	3406, 4315, 5849
29	soak	PVC	13.67, 1.32, 1.62, 0	522, 284, 496
30	soak	PVC	0, 0, 0, 0	573, 1078, 941
31	swab	steel	0, 0, 0, 0, 0	6, 10, 0
31	soak	steel	5.65, 8.75, 16.35, 0	1143, 1236, 450
32	swab	steel	0, 0, 0, 0	947, 2788, 1661
32	soak	steel	0, 0, 0, 0	7, 8, 0
33	swab	steel	0.64, 18.05, 18.5, 11.3	885, 1806, 1491
33	soak	steel	2.68, 1.60, 0.80, 0	17, 12, 27
34	swab	steel	28.30, 10.60, 9.85	690, 713, 851
34	soak	steel	1.30, 4.09, 0, 0	24, 25, 14
35	swab	steel	0.28, 9.65, 13.05	1842, 2739, 5030
35	soak	steel	1.67, 0.03, 0	9, 22, 0
36	swab	PVC	16.80, 52.50, 72.00	1903, 1745, 2606
37	swab	PVC	31.45, 35.45, 170.0, 19.20, 12.85	4606, 5467, 6121
38	swab	PVC	7.90, 105.00, 113.00, 0, 0	2109, 2085, 0
39	swab	PVC	8.30, 8.70, 59.50, 11.75, 61.00, 98.50	2873, 3030, 3248
40	swab	PVC	40.95, 47.20, 62.50, 2.41	960, 2097, 2097

Column 1 shows the pipe bomb number, while columns 2 and 3 depict the isolation technique and pipe material respectively. Column 4 displays the nuclear DNA quantities and column 5 the mtDNA quantities. The symbol * indicates that the zero values were recognized and included in the calculation of the DNA average.

Table A2: Average amount of DNA in descending order

Pipe #	Isolation Technique	Pipe Material	Avg. amt (pg)
17	swab	steel	72.00
27	soak	PVC	67.33
37	swab	PVC	53.79
36	swab	PVC	47.10
4	swab	steel	46.95
14	swab	steel	45.95
38	swab	PVC	45.28
39	swab	PVC	41.29
40	swab	PVC	38.27
22	soak	PVC	32.20
20	swab	steel	29.08
7	swab	steel	23.48
25	soak	PVC	20.35
34	swab	steel	16.25
33	swab	steel	12.12

Table A2 continued			
9	soak	PVC	11.91
15	swab	steel	8.50
16	swab	steel	7.77
31	soak	steel	7.69
35	swab	steel	7.66
10	soak	PVC	7.19
19	swab	steel	6.75
26	soak	PVC	5.73
24	soak	PVC	5.53
11	soak	PVC	4.21
29	soak	PVC	4.15
23	soak	PVC	3.61
18	swab	steel	2.64
8	soak	PVC	2.58
21	swab	steel	2.50
5	swab	steel	1.91
34	soak	steel	1.35
33	soak	steel	1.27
13	swab	steel	0.98
35	soak	steel	0.85
28	soak	PVC	0.38
3	swab	steel	0.00
3	soak	steel	0.00
4	soak	steel	0.00
5	soak	steel	0.00
6	swab	steel	0.00
6	soak	steel	0.00
7	soak	steel	0.00
12	soak	PVC	0.00
13	soak	steel	0.00
14	soak	steel	0.00
15	soak	steel	0.00
16	soak	steel	0.00
17	soak	steel	0.00
18	soak	steel	0.00
19	soak	steel	0.00
20	soak	steel	0.00
21	soak	steel	0.00
30	soak	PVC	0.00
31	swab	steel	0.00
32	swab	steel	0.00
32	soak	steel	0.00

Column 1 shows the pipe bomb number, while columns 2 and 3 depict the isolation technique and pipe material respectively. Column 4 depicts the average quantity of nuclear DNA in picograms, and in descending order. Sample # 11 was the positive control, while samples # 12 & 13 were negative controls.

Table A3: Quantity of DNA recovered in descending order by mass

Pipe #	Pipe Material	Isolation Technique	Mass (g)	Avg. amt (pg)	*fg per gram of pipe*	Avg. of Quantifiler (pg)	*fg per gram of pipe*
14	steel	swab	768.32	45.95	***59.81***	36.90	***48.03***
32	steel	swab	700.84	0.00	***0.00***	0.00	***0.00***
33	steel	swab	694.18	12.12	***17.46***	20.65	***29.75***
20	steel	swab	680.79	29.08	***42.72***	29.20	***42.89***
5	steel	swab	676.18	1.91	***2.83***	2.44	***3.61***
7	steel	swab	650.69	23.48	***36.08***	15.70	***24.13***
35	steel	swab	650.11	7.66	***11.78***	85.30	***131.21***
34	steel	swab	635.37	16.25	***25.58***	0.00	***0.00***
15	steel	swab	625.03	8.50	***13.60***	19.20	***30.72***
13	steel	swab	620.96	0.98	***3.14***	2.79	***4.49***
31	steel	swab	598.90	0.00	***0.00***	0.00	***0.00***
18	steel	swab	598.03	2.64	***4.41***	10.10	***16.89***
16	steel	swab	577.15	7.77	***13.46***	19.60	***33.96***
21	steel	swab	572.22	2.50	***4.37***	10.10	***17.65***
4	steel	swab	561.92	46.95	***83.55***	7.19	***12.80***
17	steel	swab	498.02	72.00	***144.57***	34.90	***70.08***
19	steel	swab	400.37	6.75	***16.86***	0.00	***0.00***
34	steel	soak	252.31	1.35	***5.34***	0.00	***0.00***
35	steel	soak	237.57	0.85	***3.58***	0.00	***0.00***
31	steel	soak	218.25	7.69	***35.22***	0.00	***0.00***
33	steel	soak	193.50	1.69	***1.27***	0.00	***0.00***
32	steel	soak	186.84	0.00	***0.00***	18.45	***98.75***
30	PVC	soak	121.68	0.00	***0.00***	0.00	***0.00***
28	PVC	soak	113.10	0.38	***3.36***	0.00	***0.00***
22	PVC	soak	101.83	32.20	***316.21***	34.20	***335.85***
25	PVC	soak	94.04	20.35	***216.40***	41.10	***437.05***
9	PVC	soak	93.60	11.91	***127.22***	0.00	***0.00***
10	PVC	soak	91.12	7.19	***78.85***	14.20	***155.84***
27	PVC	soak	86.81	67.33	***775.60***	69.40	***799.45***
26	PVC	soak	83.65	5.73	***68.50***	0.00	***0.00***
24	PVC	soak	76.15	5.53	***72.55***	0.00	***0.00***
8	PVC	soak	74.21	2.58	***34.80***	62.40	***840.86***
23	PVC	soak	70.03	3.61	***51.48***	0.00	***0.00***
11	PVC	soak	69.96	4.21	***60.14***	0.00	***0.00***
12	PVC	soak	69.48	0.00	***0.00***	0.00	***0.00***
38	PVC	swab	57.63	45.28	***785.70***	0.00	***0.00***
37	PVC	swab	54.87	53.79	***980.32***	161.50	***2943.32***
29	PVC	soak	53.36	4.15	***77.81***	0.00	***0.00***
40	PVC	swab	52.57	38.27	***727.98***	0.00	***0.00***
36	PVC	swab	46.71	47.10	***1008.35***	6.55	***140.23***
39	PVC	swab	39.69	41.29	***1040.31***	103.50	***2607.71***

Column 1 shows the pipe bomb number, while columns 2 and 3 depict the pipe material and isolation technique respectively. Column 4 depicts the mass of the recovered fragments for each bomb in descending order. Column 5 shows the average quantity of nuclear DNA based on the Timken et al. (2005) assay, while column 6 depicts the amount of femtograms per gram of pipe for column 5. Column 7 refers to the average quantity of nuclear DNA based on the Quantifiler assay, while column 8 depicts the amount of femtograms per gram of pipe for column 7. Sample # 11 was the positive control, while samples # 12 & 13 were negative controls.

Table A4: Average amount of DNA recovered from each fragmentation category

A	B	C	D	E	F	G
Pipe #	**Pipe Material**	**Frag. Category**	**Total using Avg. amt (fg)**	**Avg. of column 'D' (fg)**	**Total using Avg. of Quantifiler (fg)**	**Avg. of column 'F' (fg)**
7	steel	1	36.08	12.95	24.13	34.41
13	steel	1	3.14		4.49	
18	steel	1	4.41		16.89	
32	steel	1	0.00		0.00	
33	steel	1	18.73		29.75	
35	steel	1	15.36		131.21	
5	steel	2	2.83	28.47	3.61	16.47
14	steel	2	59.81		48.03	
15	steel	2	13.60		30.72	
31	steel	2	35.22		0.00	
34	steel	2	30.92		0.00	
16	steel	3	13.46	66.92	33.96	48.98
17	steel	3	144.57		70.08	
20	steel	3	42.72		42.89	
4	steel	4	83.55	34.93	12.80	10.15
19	steel	4	16.86		0.00	
21	steel	4	4.37		17.65	

Column 1 shows the pipe bomb number, while columns 2 depicts the pipe material. Column 3 shows categories for the level of fragmentation: 1 – low, 2 – medium, 3 – high, and 4 – complete. Column 4 shows the total average quantity of DNA based on the Timken et al. (2005) assay, where total DNA refers to the sum of the swabbed and soaked results for each sample. Column 5 depicts the average for each fragmentation category in column 4. Column 6 represents the total average quantity of DNA based on the Quantifiler assay, while column 7 shows the average for each fragmentation category in column 6.

Table A5: Average mtDNA quantity according to pipe material and isolation technique

Pipe #	Isolation Technique	Pipe Material	Average mtDNA (copy #)
31	soak	steel	943
32	soak	steel	8
33	soak	steel	19
34	soak	steel	21
35	soak	steel	16
4	swab	steel	1240
5	swab	steel	230
7	swab	steel	1540
13(neg)	swab	steel	829
14	swab	steel	1543
15	swab	steel	1317
16	swab	steel	1433
17	swab	steel	1657
18	swab	steel	1320
19	swab	steel	3180
20	swab	steel	9297
21	swab	steel	782
31	swab	steel	8
32	swab	steel	1799
33	swab	steel	1394
34	swab	steel	751
35	swab	steel	3204
8	soak	PVC	5624
9	soak	PVC	2699
10	soak	PVC	2461
11(pos)	soak	PVC	490
12(neg)	soak	PVC	110
22	soak	PVC	1022
23	soak	PVC	469
24	soak	PVC	966
25	soak	PVC	1956
26	soak	PVC	1128
27	soak	PVC	4145
28	soak	PVC	4533
29	soak	PVC	434
30	soak	PVC	864
36	swab	PVC	2085
37	swab	PVC	5398
38	swab	PVC	2097
39	swab	PVC	3050
40	swab	PVC	1718

Column 1 shows the pipe bomb number, while columns 2 and 3 depict the isolation technique and pipe material respectively. Column 4 depicts the average quantity of mtDNA in copy number. Sample # 11 was the positive control, while samples # 12 & 13 were negative controls.

Table A6: Mitochondrial DNA copy number in descending order of mass

Pipe #	Pipe Material	Isolation Technique	Mass (g)	Avg. mtDNA (copy #)	Copies per gram of material
14	steel	swab	768.32	1543	2.01
32	steel	swab	700.84	1799	2.57
33	steel	swab	694.18	1394	2.01
20	steel	swab	680.79	9297	13.66
5	steel	swab	676.18	230	0.34
7	steel	swab	650.69	1540	2.37
35	steel	swab	650.11	3204	4.93
34	steel	swab	635.37	751	1.18
15	steel	swab	625.03	1317	2.11
13	steel	swab	620.96	829	1.34
31	steel	swab	598.9	8	0.01
18	steel	swab	598.03	1320	2.21
16	steel	swab	577.15	1433	2.48
21	steel	swab	572.22	782	1.37
4	steel	swab	561.92	1240	2.21
17	steel	swab	498.02	1657	3.33
19	steel	swab	400.37	3180	7.94
34	steel	soak	252.31	21	0.08
35	steel	soak	237.57	16	0.07
31	steel	soak	218.25	943	4.32
33	steel	soak	193.5	19	0.10
32	steel	soak	186.84	8	0.04
30	PVC	soak	121.68	864	7.10
28	PVC	soak	113.1	4533	40.08
22	PVC	soak	101.83	1022	10.04
25	PVC	soak	94.04	1956	20.80
9	PVC	soak	93.6	2699	28.84
10	PVC	soak	91.12	2461	27.00
27	PVC	soak	86.81	4145	47.75
26	PVC	soak	83.65	1128	13.48
24	PVC	soak	76.15	966	12.68
8	PVC	soak	74.21	5624	75.78
23	PVC	soak	70.03	469	6.69
11	PVC	soak	69.96	490	7.01
12	PVC	soak	69.48	110	1.58
38	PVC	swab	57.63	2097	36.39
37	PVC	swab	54.87	5398	98.38
29	PVC	soak	53.36	434	8.13
40	PVC	swab	52.57	1718	32.68
36	PVC	swab	46.71	2085	44.63
39	PVC	swab	39.69	3050	76.85

Column 1 shows the pipe bomb number, while columns 2 and 3 depict the pipe material and isolation technique respectively. Column 4 depicts the mass of the fragments for each pipe in descending order. Column 5 shows the average quantity of mtDNA in copy number.

Table A7: Average number of copies recovered from each fragmentation category

Pipe #	Pipe Material	Fragmentation Category	Average mtDNA per gram (copy #)	Avg. per category
4	steel	4	2.21	3.84
19	steel	4	7.94	
21	steel	4	1.37	
16	steel	3	2.48	6.49
17	steel	3	3.33	
20	steel	3	13.66	
5	steel	2	0.34	2.01
14	steel	2	2.01	
15	steel	2	2.11	
31	steel	2	4.33	
34	steel	2	1.27	
7	steel	1	2.37	2.60
13	steel	1	1.34	
18	steel	1	2.21	
32	steel	1	2.61	
33	steel	1	2.10	
35	steel	1	4.99	

Column 1 shows the pipe bomb number, while column 2 depicts the pipe material. Column 3 shows categories for the level of fragmentation: 1 – low, 2 – medium, 3 – high, and 4 – complete. Column 4 represents the average quantity of mtDNA in copy number. Column 5 depicts the average for each fragmentation category in column 4.

Table A8: MtDNA ranking v.s. corresponding nuclear DNA and Quantifiler ranking

Pipe #	MtDNA rank	Nuclear DNA rank (Avg amt.)	Quantifiler rank
20	1	11	*10*
8	2	29	**5**
37	3	**3**	**1**
28	4	36	-
27	5	**2**	**4**
35 (swab)	6	20	**3**
19	7	22	*26*
39	8	**8**	**2**
9	9	*16*	-
10	10	21	*16*
38	11	**7**	-
36	12	*4*	*20*
25	13	**13**	*6*
32 (swab)	14	-	-

Table A8 continued			
40	15	*9*	-
17	16	**1**	*8*
14	17	**6**	*7*
7	18	*12*	**15**
16	19	**18**	*12*
33 (swab)	20	**15**	*11*
18	21	*28*	**17**
15	22	**17**	13
4	23	**5**	**19**
26	24	**23**	-
22	25	10	9
24	26	**24**	-
31 (soak)	27	*19*	-
30	28	-	-
13	29	**34**	*21*
21	30	**30**	18
34 (swab)	31	14	-
11	32	*25*	-
23	33	*27*	-
29	34	*26*	-
5	35	**31**	22
12	36	-	-
34 (soak)	37	**32**	-
33 (soak)	38	**33**	-
35 (soak)	39	**35**	-
31 (swab)	40	-	-
32 (soak)	41	-	14

Column 1 shows the pipe bomb number along with the DNA isolation technique used, while column 2 depicts the rank of the pipe according to mtDNA quantity. Column 3 refers to the nuclear DNA rank of the samples based on the Timken et al. (2005) assay. Bold values depict rankings that are within 5 places of each other and italic values refer to values that are within 10 places of each other. Column 4 refers to the nuclear DNA rank of the samples based on the Quantifiler assay. Symbol (-) indicates that the value was zero and was not ranked.

Table A9: Statistical differences of isolation techniques using t-tests

	Avg. amt (swab)	Avg. amt (soak)	Quantifiler (swab)	Quantifiler (soak)	MtDNA (swab)
Avg. amt (swab)	-	-	NO	-	-
Avg. amt (soak)	NO	-	-	NO	-
Quantifiler (swab)	NO	NO	-	NO	-
Quantifiler (soak)	-	-	NO	-	-
MtDNA (soak)	-	-	-	-	NO

Using the 1st column: row 2 refers to the average quantity of nuclear DNA recovered from swabbed fragments using the Timken et al. (2005) qPCR assay; row 3 refers to the average quantity of nuclear DNA recovered from soaked fragments using the Timken et al. (2005) qPCR assay; Quantifiler denotes the average quantities of nuclear DNA recovered from the swabbing and soaking techniques using the Quantifiler assay and mtDNA denotes the average quantity of mtDNA recovered using the Timken et al. (2005) qPCR assay. (-) denotes that no comparison was made between the two averages.

Appendix B

STR Data

Complete STR results for samples 4 – 30

Alleles highlighted in **bold** indicate a major peak and the symbol (-) denotes that a peak over the threshold value (50 RFUs) was not obtained. "OL" indicates that the allele was off ladder. The locus D2S1338 was not included in the PowerPlex 16 kit, and therefore, could not be compared to the known sample. Sample 11 was the positive control, while samples 12 and 13 were the negative controls. All soak samples were analyzed using 1:20 dilutions

Key:

[A]	Volunteer alone
[B]	Volunteer (major profile) + other alleles
[C]	Volunteer (non-major profile) + other alleles
[D]	At least 1 volunteer allele
[E]	No volunteer
[F]	No alleles
	No comparison made to a known DNA sample

	Sample #							
Locus	**4 swab**		**5 swab**		**7 swab**		**13 swab (neg)**	
D13S317	8, 11	*[A]*	11, 12, 13	*[C]*	**8**, 9, **11**, **12**	*[B]*	**11**, 16	
D7S820	9, 10	*[A]*	10, 11	*[C]*	**8**, 9 10, 11 **12**	*[B]*	10, 11	
Amel	**X**, Y	*[B]*	X	*[A]*	**X**, Y	*[B]*	X, Y	
D2S1338	19, **20**, 22, **25**		23, 25, 28		**17**, **20**, 24		17, **19**, **20**, 21, 24	
D21S11	27, 30	*[A]*	29	*[D]*	28, 30, 31	*[C]*	**28**, **31**, 32	
D16S539	12, 13	*[A]*	10	*[A]*	9, 10, 11	*[C]*	**9**, 11, 15	
D18S51	13, 16	*[A]*	16, 19	*[C]*	15, 17	*[C]*	**15**, **16**, 18	
CSF1PO	11, 12, 13	*[C]*	11, 12	*[A]*	10, 11, 12	*[C]*	**10**, 11, **12**	
FGA	23.2	*[E]*	24, 25	*[E]*	24	*[E]*	16.2, 19, 22.2, 25, 27	

	Sample #			
Locus	**14 swab**	**15 swab**	**16 swab**	**17 swab**
D13S317	**9**, **11**, 13 *[B]*	11, 12, 13 *[B]*	8, **11**, **13**, 16 *[B]*	**10**, **11**, 12, 13, 14, 16 *[B]*
D7S820	8, 10, **12** *[B]*	10, **11**, 12 *[C]*	**7**, **8**, 11 *[B]*	**8**, 10, **11** *[B]*
Amel	**X**, Y	X, Y *[A]*	X *[A]*	**X**, Y *[B]*
D2S1338	**20**, **22**, **24**, 25, 26, 27	21, 22, 23	17, **19**, **20**, 21	**17**, **19**, 20, 21
D21S11	32, 32.2 *[A]*	28 *[A]*	**29**, 31.2, 32.2 *[C]*	28, **29**, **30**, 31 *[B]*
D16S539	**9**, 10, **12**, 13 *[B]*	12, 12.2, 13 *[C]*	11, 12 *[A]*	9, 11 *[A]*
D18S51	**14**, 15, 16, 17, **18** *[C]*	13, **18** *[D]*	12 *[A]*	**12**, 15, 16, **17***[B]*
CSF1PO	**9**, **10**, 11 *[C]*	10, 11, 12 *[C]*	10, **11**, **13** *[B]*	10 *[A]*
FGA	23 *[D]*	27 *[E]*	25, 26, 28.2 *[D]*	- *[F]*

	Sample #			
Locus	**18 swab**	**19 swab**	**20 swab**	**21 swab**
D13S317	**11**, 13, 14 *[C]*	9, 11, 12 *[C]*	**11**, **12**, 13 *[B]*	12 *[A]*
D7S820	**8**, **10**, 12 *[B]*	**10**, 11 **12** *[B]*	9, 11 *[A]*	**8**, 9 **10** *[B]*
Amel	**X**, Y *[B]*	X, Y *[A]*	X, Y *[A]*	**X**, Y *[B]*
D2S1338	19, **22**, 23, **24**, 25	**18**, 19, **24**	20, 21	19, 25
D21S11	29 *[A]*	29, 31 *[A]*	30, 30.2, 32.2 *[C]*	30, 30.2 *[A]*
D16S539	**10**, 12, 13 *[C]*	8, 12 *[A]*	9, 12 *[C]*	9, **11** *[A]*
D18S51	12, **15**, **16** *[B]*	13, 15, 16.2, 17, 18 *[C]*	11, 12, 13, 15, 17, 18 *[C]*	10, 12, 14, 15, **16** *[C]*
CSF1PO	10, 11 *[A]*	**10**, 11, **12** *[B]*	10, 12 *[C]*	11, 12 *[A]*
FGA	- *[F]*	24, 26 *[E]*	- *[F]*	26 *[E]*

	Sample #			
Locus	**8 soak**	**9 soak**	**10 soak**	**11 soak (pos)**
D13S317	8, 12 *[A]*	8, 12 *[D]*	11 *[D]*	8 *[D]*
D7S820	- *[F]*	10 *[D]*	11 *[E]*	- *[F]*
Amel	X *[A]*	X, *[A]*	X, Y *[C]*	- *[F]*
D2S1338	20 *[D]*	17, 20 *[A]*	- *[F]*	15, 18
D21S11	28, 30 *[A]*	31, 32.2 *[D]*	29, 30 *[A]*	25 *[E]*
D16S539	9, 11 *[A]*	10, 12.2 *[E]*	11, 15 *[C]*	- *[F]*
D18S51	15, 16 *[A]*	13, 16 *[A]*	15, 16 *[D]*	15 *[A]*
CSF1PO	11, 13 *[A]*	11, 12 *[C]*	10 *[E]*	- *[F]*
FGA	22, 27 *[E]*	23 *[E]*	- *[F]*	- *[F]*

	Sample #				
Locus	**12 soak (neg)**	**22 soak**	**23 soak**	**24 soak**	**25 soak**
D13S317	-	11 *[E]*	11 *[A]*	OL *[F]*	- *[F]*
D7S820	-	- *[F]*	- *[F]*	- *[F]*	- *[F]*
Amel	-	X *[A]*	X *[A]*	X, Y *[A]*	X, Y *[A]*
D2S1338	19	18	OL *[F]*	17, 19, 20	17, 19, 20, 26
D21S11	-	- *[F]*	OL *[F]*	OL *[F]*	OL *[F]*
D16S539	-	6, 12 *[C]*	11 *[D]*	8, 11 *[D]*	- *[F]*
D18S51	-	19 *[D]*	- *[F]*	15, 16 *[D]*	12, 13 *[D]*
CSF1PO	-	- *[F]*	- *[F]*	10, 12 *[C]*	10 *[D]*
FGA	-	- *[F]*	- *[F]*	22, 23 *[D]*	23 *[E]*

	Sample #				
Locus	**26 soak**	**27 soak**	**28 soak**	**29 soak**	**30 soak**
D13S317	- *[F]*	- *[F]*	8 *[E]*	9, 14 *[D]*	11, 14 *[E]*
D7S820	8, 13 *[A]*	- *[F]*	- *[F]*	8, 9, 10, 12 *[D]*	11 *[D]*
Amel	X, Y *[A]*	- *[F]*	X *[A]*	X, Y *[B]*	X *[A]*
D2S1338	20, 23	- *[F]*	24	17, 18, 19, 21, 25, 23, 27	17
D21S11	OL *[F]*	- *[F]*	27 *[E]*	27, 30.2 *[E]*	- *[F]*
D16S539	9, 11, 12 *[C]*	- *[F]*	11 *[A]*	10, 11, 12.2 *[C]*	11 *[A]*
D18S51	13, 15 *[A]*	- *[F]*	15 *[E]*	12, 14, 16, 19 *[C]*	- *[F]*
CSF1PO	10, 11 *[C]*	- *[F]*	- *[F]*	9, 10, 11, 12 *[C]*	10 *[E]*
FGA	20 *[E]*	- *[F]*	- *[F]*	22, 25, 26, 27, 27.2 *[E]*	- *[F]*

References

Alessandrini F, Cecati M, Pesaresi M, Turchi C, Carle F and Tagliabracci A . (2003). Fingerprints as evidence for a genetic profile: Morphological Study on fingerprints and analysis of exogenous and individual factors affecting DNA typing. J Forensic Sci. 48(3).

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

Applied Biosystems. (2005). TaqMan ® Gene Expression Assays: Protocol.

Australian Federal Police. Publications and Reports. (2002-2006). Australian Bomb Data Center (ABDC) annual reports. Website http://www.afp.gov.au/about/publications/annual_reports/abdc.html. Accessed March 14, 2008.

Bachman R and Paternoster R. (2004). Statistics for criminology and criminal justice, 2, 383-384. New York, NY: McGraw-Hill.

Balogh MK, Burger J, Bender K, Schneider PM and Alt KW. (2003). STR genotyping and mtDNA sequencing of latent fingerprint on paper. For Sci International 137: 188-195.

Bright J and Perticevic SF. (2004). Recovery of trace DNA and its application to DNA profiling of shoe insoles. For Sci International 145(1):7-12.

Budowle B, DiZinno JA, Wilson MR. (1999). Interpretation guidelines for mitochondrial DNA sequencing. Websitehttp://www.promega.com/geneticidproc/ussymp10proc/content/37Budowle.pdf. Accessed May 1, 2008.

Bureau of Alcohol, Tobacco, Firearms and Explosives (ATF). Arson and Explosives. U.S. Bomb Data Center, Statistics. Explosive Incidents Reports for 2000-2003. Website http://www.atf.gov/aexis2/statistics.htm. Accessed March 19, 2008.

Burke R. (2007). Counter-terrorism for emergency responders. CRC Press LLC, Boca Raton.

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

Butler, JM (2005). Forensic DNA typing. Biology, technology, and genetics of STR markers, 2. Burlington, Massachusetts: Elsevier

Esslinger KJ, Siegel JA, Spillane H and Stallworth S. (2004). Using STR analysis to detect human DNA from exploded pipe bomb devices. J Forensic Sci. 49(3): 481-484.

Federal Bureau of Investigations (FBI). FBI Bomb Data Center, General Bulletin 97-1. Website http://www.milnet.com/domestic/1997bombrep.pdf. Accessed March 24, 2008.

Federal Bureau of Investigations (FBI). FBI Bomb Data Center, General Bulletin 98-1. Website http://library.sau.edu/bestinfo/Majors/Criminal/Bomb.pdf. Accessed March 24, 2008.

Federal Bureau of Investigations (FBI). Reports and Publications. Terrorism 2000-2001. Website http://www.fbi.gov/publications/terror/terror2000_2001.pdf. Accessed March 24, 2008

Federal Bureau of Investigations (FBI). Reports and Publications. Terrorism 2002-2005. Website http://www.fbi.gov/publications/terror/terrorism2002_2005.pdf.Accessed March 24, 2008.

Foran, DR (2006). Relative degradation of nuclear and mitochondrial DNA: an experimental approach. J Forensic Sci. 51(4):766-770.

Gehring M. (2004). The recovery and analysis of mitochondrial DNA from exploded pipe bombs. Masters Thesis. Michigan State University.

Herber B and Herold K. (1998). DNA typing of human dandruff. J Forensic Sci. 43(3):648-656.

Hoffmann SGP. (2008). Investigative studies into the recovery of DNA from improvised explosive device containers. Masters Thesis. Michigan State University.

Holland MM and Parsons TJ. (1999). Mitochondrial DNA sequence analysis – Validation and use for forensic casework. For. Sci Review 11:21.

Kremer S, Foran D. (2008). Identification of improvised explosive device assemblers using miniSTRs. Masters Thesis. Michigan State University.

Ladd C, Adamowicz MS, Bourke MT, Scherczinger CA and Lee HC. (1999). A systematic analysis of secondary DNA transfer. J Forensic Sci. 44(6):1270-1272.

Locard E. (1930). The analysis of dust traces – Part I. Amer J Pol Sci. 1:276-298.

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

National Human Genome Research Institute. Human Genome Project. Website http://www.genome.gov. Accessed November 17, 2008.

National Research Council (NRC). (1998). Black and smokeless powders: technology for finding bombs and the bomb makers. National Academy Press, Washington D.C.

Pang BCM and Cheung BKK. (2007). Double swab technique for collecting touched evidence. Legal medicine 9:181-184.

Phipps M and Petricevic SF. (2007). The tendency of individuals to transfer DNA to handled items. For Sci International 168:162-168.

Proff C, Schmitt C, Schneider PM, Foerster G and Rothschild MA. (2006). Experiments on the DNA contamination risk via latent fingerprint brushes. International Congress Series 1288:601-603.

Raven PH and Johnson GB. (1986). Biology. Times Mirror/Mosby College Publishing, St Louis, 906.

Sweet D, Lorente M, Lorente JA, Valenzuela A and Villanueva E. (1997). An improved method to recover saliva from human skin: The double swab technique. J Forensic Sci. 42(2):320-322.

Timken MD, Swango KL, Orrego C and Buoncristiani MR. (2005). A duplex real-time qPCR assay for the quantification of human nuclear and mitochondrial DNA in forensic samples: implications for quantifying DNA in degraded samples. J Forensic Sci. 50(5):1044-1060.

Van Oorschot RAH and Jones MK. (1997). DNA fingerprints from fingerprints. Nature 387:767.

Whitaker JP, Clayton TM, Urquhart AJ, Millican ES, Downes TJ, Kimpton CP and 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: 670 – 677.

Whitaker JP, Cotton EA and Gill P. (2001). A comparison of characteristics of profiles produced with the AMFISTR® SGM Plus multiplex system for both standard and low copy number (LCN) STR DNA analysis. For Sci International 123 (2-3): 215 – 223.

Wickenheiser RA. (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(3):442-450.

Yinon J and Zitrin S. (1993). Modern methods and applications in analysis of explosives. John Wiley & Sons Ltd, Chichester, West Sussex, England.