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USING STR ANALYSIS TO DETECT HUMAN DNA
ON EXPLODED PIPE BOMB DEVICES

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USING STR ANALYSIS TO DETECT HUMAN DNA ON EXPLODED PIPE BOMB DEVICES

By

Kelly Jean Esslinger

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ABSTRACT

USING STR ANALYSIS TO DETECT HUMAN DNA
FROM EXPLODED PIPE BOMB DEVICES

By

Kelly Jean Esslinger

This study investigated whether it was possible to recover a bomb assembler's DNA from an exploded pipe bomb device. Each subject handled components (pipe, caps and fuse) of one metal and one PVC pipe bomb with a 10 second handling time per component, thus transferring sloughed skin cells onto the pipe bomb pieces. Using disposable gloves, the Michigan State Police bomb squad assembled and deflagrated each pipe bomb in separate holes in the ground; each hole was covered with a large rock to contain the fragments. The fragments from each bomb were collected separately and swabbed to recover any remaining skin cells. An AmpF*l*STR® Profiler Plus™ kit as well as an ABI 310 Genetic Analyzer® with Genescan® 2.0.2 and Genotyper® 2.1 software were utilized to generate DNA profiles from these swabbed bomb fragments. The results indicated that DNA recovery and subsequent generation of a genetic profile from the person who handled the bomb was successful in several instances. Overall, 3 of the 20 bombs (15%) rendered useful DNA profile matches to known DNA profiles. These findings are promising. However, problems such as allele dropout, heterozygote imbalance, elevated stutter, and contamination were observed with some samples due to low amounts of DNA and the extreme sensitivity of the method. Suggested improvements in the method could potentially double the success rate and eliminate some problems, which is exciting to consider and should be explored with future research.

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I would like to thank several people for helping to make this project a success. The Michigan State Police Crime Laboratory of Northville, Michigan must be recognized for the use of their equipment and other supplies used for this study. Also, each member of the DNA/Biology unit and the Bomb Squad/Firearms unit are deserving of special thanks for their commitment and support of this project. Heather Spillane of the DNA/Biology unit and Detective Sergeant Shawn Stallworth of the Bomb Squad/ Firearms unit are owed specific acknowledgment for their vision, guidance, patience, and constructive criticism. I would also like to thank Dr. Jay Siegel for his commitment to the education and well being of each of his students, as well as his continued support and advice for this project.

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LIST OF ABBREVIATIONS

A	– letter designation "A" was assigned to samples that were not exploded
ASCLD	– American Society of Crime Laboratory Directors
B	– letter designation "B" was assigned to samples that were exploded
CE	– capillary electrophoresis
DNA	– deoxyribonucleic acid
ILC	– internal laboratory control
NV	– nothing visible
NR	– no results
PCR	– polymerase chain reaction
QA	– quality assurance
QB	– QuantiBlot®
QC	– quality control
RFU	– reflective fluorescent unit
STR	– short tandem repeat

INTRODUCTION

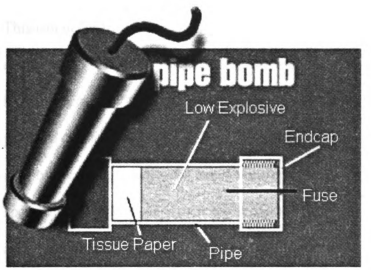
Terrorism is a very real problem today all over the world, and recent events such as September 11, 2001, the 1993 World Trade Center bombing, and the 1995 Oklahoma City bombing have taught us that it can strike anywhere at anytime. Explosives are used more than 70% of the time in terrorist attacks, and although large-scale attacks usually involve more sophisticated incendiary devices, pipe bombs account for 31% of all improvised explosive devices used (7).

When there are reports on the news about bombs in schools, and there are "how-to" pages for building bombs on the Internet, it suggests that anyone can build improvised explosive devices like pipe bombs. For example, in the 1999 Columbine High School shooting several pipe bombs were discovered although they did not explode in this instance. Additionally, pipe bombs or other similarly improvised explosives were utilized in the Olympic Park bombing in Georgia in 1996, the bombing of IRS buildings by Dean Hicks in 1991, and the 17-year reign of terror by the Unibomber. (7,22,29,40). Unlike shootings, those who set off bombs are often gone when the device explodes, thus making it more difficult to connect the person with the crime (5). But what if sufficient human DNA could be recovered from the fragments of a bomb to determine who had been handling it prior to the explosion? In this study, low explosions were performed on metal and PVC pipe bombs to determine what effect if any an explosion has on the successful recovery of human DNA.

Pipe Bombs

As seen in Figure 1, a pipe bomb is a fairly simple device; it is literally a length of pipe that is capped on both ends and filled with an explosive (22). They come in many shapes, sizes, and can be composed of metal, plastic, or some related material. The explosive inside can be filled with low explosives (like smokeless powder) or a higher explosive. They can be rigged to go off instantly or with some type of timed delay (5, 16). Depending upon the agenda of the bomb manufacturer, pipe bombs can also be packed with nails and/or screws to heighten the damage caused due to shrapnel effect (5, 16).

FIGURE 1: Schematic of a Pipe Bomb Device



Statistically speaking, the most commonly encountered explosive device in the United States is the pipe bomb (40). According to Detective Sergeant Shawn Stallworth of the Michigan State Police Bomb Squad and Firearms Unit, at least 60% of their caseload in explosives deal with pipe bombs. What makes the pipe bomb such a popular method of destruction is really two factors. First, the materials can be purchased at any

hardware store, and the powder and time fuse at a sporting or hobby store. Secondly, they are easy to assemble for even the least mechanically inclined (5, 16, 40).

Smokeless Powder

Smokeless powders or propellants are mixtures of chemicals designed to burn very rapidly under controlled conditions, and are manufactured for the use in firearms (27). Specifically, they are used in the reloading of cartridge cases and shot shells, and are thus readily available at hunting or sporting goods stores. Aside from their legitimate use in firearms, smokeless powders are often employed in the production of pipe bombs (16).

Initiation of the powder occurs when it is heated above its ignition temperature. This can occur by exposing the powder to a flame, an electrical spark, or other form of nearby heat (17). When smokeless powder burns, a large amount of gas is produced at a high temperature. Naturally, if the powder is confined to a container the gas creates pressure on that container and it will eventually cause the container to explode or in the case of firearms, to expel the bullet at a high velocity (25). However, if there is too much space or enough gas can escape the container, the pressure can be kept at a low level and an explosion can be avoided (17, 27).

This quality of smokeless powder, a low explosive, makes it different from high explosives, such as dynamite because it is almost impossible to vent the effects of a detonation. Smokeless powder also differs from simple black powder in that black powder burns at essentially the same rate whether it is confined or not. In contrast, smokeless powder burns very differently unconfined as opposed to when confined in a

container. When in the open, smokeless powder burns inefficiently, whereas when confined (i.e. under pressure) smokeless powder burns much more rapidly (5, 27).

There are many different types of smokeless powder available with varying burn rates. For non-military purposes, powders are available in two types of composition, single-base and double base. Single-base smokeless powder's main source of energy is from nitrocellulose, whereas double-base powders derive its energy from a combination of both nitrocellulose and nitroglycerin (1). There are three common shapes of smokeless powder (30).

1. Extruded or tubular powder is cut into rods whose length equals or exceeds the diameter. It is typically used in rifle cartridges and is usually a single-base powder.
2. Spherical or Ball Powder is all double-base, and can look like tiny round balls or grains that are flattened. In general, it is more difficult to ignite than extruded powder.
3. Flake powder is usually double-base, fast burning, and flat in shape.

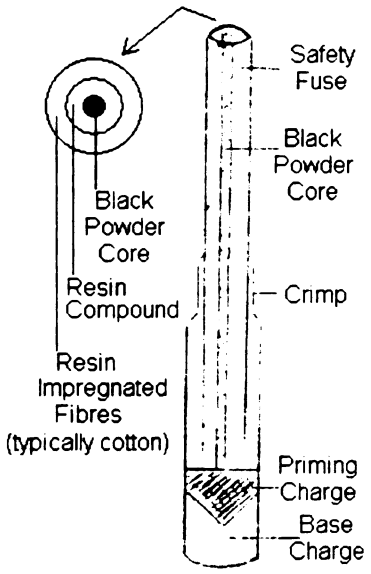
As mentioned previously, smokeless powder is a low explosive whereas dynamite is a high explosive. The major difference between the two types lies in the speed of the explosion. Low explosives like smokeless powder deflagrate; high explosives detonate (7). Deflagration is a rapid burn that occurs slower than the speed of sound where the solid material changes to a gas relatively slowly. A detonation causes the solid material to instantaneously change to a gas form and with it the production of high heat and a

pressure shock wave (7). So these two terms (detonation and deflagration) refer to different types of explosions with deflagration being the correct term to use for this study.

Safety Fuses

A safety fuse is one manner in which the bomber can make his or her escape before the explosion occurs. The outer portion of the fuse has a waxy semblance of resin coated cotton fibers and it can be orange, white, black, striped, or green (military) in color. As shown in Figure 2, the inside is filled with black powder that is produced to burn at a predictable rate, approximately 20 – 40 seconds per foot depending on the manufacturer (17, 23).

FIGURE 2: Diagram of a Safety Fuse

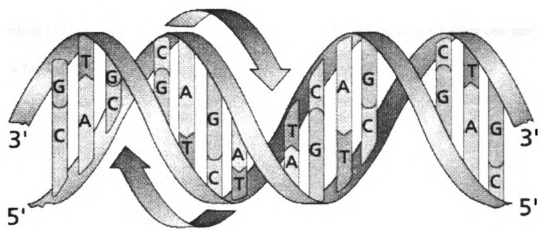


DNA and STR's

Deoxyribonucleic acid (DNA) is a very long macromolecule that is often referred to as the fundamental building block of all living things. DNA is found in the nucleus of a cell, and it determines the cell's form and function. It also passes genetic information from one generation to the next by making copies of itself (8, 33).

As shown in Figure 3, DNA is composed of two nucleotide strands coiled around one another in a ladder-like arrangement called a double helix (36). In this analogy, the alternating sidepieces are phosphate and a deoxyribose sugar. The ladder rungs, which carry the genetic information, consist of the purine and pyrimidine bases, adenine, guanine, cytosine, and thymine (21).

FIGURE 3: The Structure of DNA

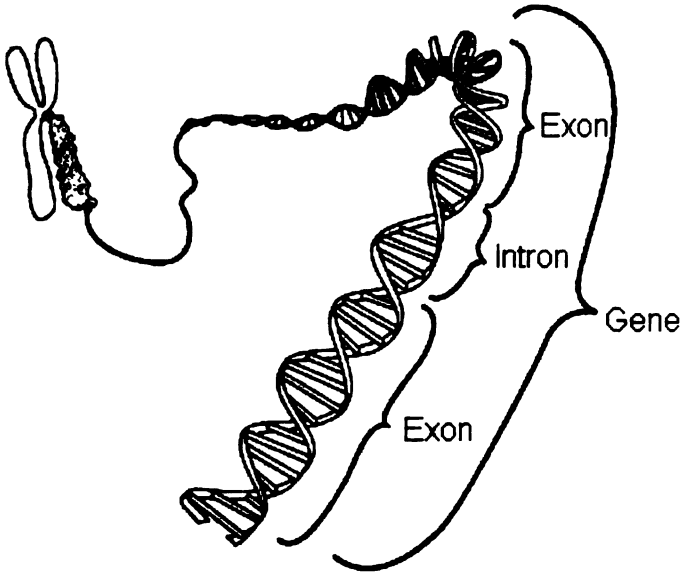


The nuclear DNA in our cells is divided into packages called chromosomes. Each human, with the exception of individuals with genetic diseases such as Down's Syndrome, have a total of 46 chromosomes, or 23 homologous pairs of chromosomes. A

homologous pair means that a copy of every gene is in the same location (locus) on every chromosome of each pair. Each parent contributes one chromosome in each of the 23 pairs to their offspring. All of the possible variations (polymorphisms) for a locus are called alleles (8).

Chromosomes are composed of coding and non-coding regions. The coding regions are called genes, and are directly involved with the body's production of proteins. As shown in Figure 4, genes also are divided into two regions; they have coding regions known as exons and non-coding regions called introns (30). According to Butler, "genes only make up about 5% of human genomic DNA, so the remaining portion of our chromosomal material is composed of non-coding regions" (8). Any non-coding region of DNA is commonly referred to as "junk DNA" because they are not directly involved with the production of proteins. The non-coding regions of chromosomes as well as the non-coding regions within genes are the areas of interest to forensic scientists in identity testing because of the high variability (polymorphisms) in these areas from one person to the next (8).

Figure 4: Schematic of a Gene



Within these non-coding polymorphic regions there are sequences that repeat in 4 base units over and over again. This type of repeat unit is called a short tandem repeat or STR. What is significant about these repeating units is that the number of repeats at a given location (locus) varies greatly between individuals (8).

To review, all the possible variations at a particular locus are called alleles. Alleles are named based upon the number of repeat units at a particular locus. Using the D3S1358 locus as an example, an individual could have 12 repeats on one chromosome at that locus and 14 repeats on the other chromosome at the same locus. This individual would be called heterozygous at this particular genetic locus, as their alleles are different. If each parent were to contribute the same number of repeats at a particular locus, they are referred to as homozygous at that locus (8).

Many individuals can have certain alleles in common at a particular locus; in fact, similarities between individuals are expected. However, by viewing several locations (13 loci in forensics) no two individuals in a population are expected to have the exact same

combination of alleles (number of repeats) at every locus. Very often, the statistical calculation of how often we would expect to see a particular genetic profile using all 13 loci is once in several billion or more people.

Polymerase Chain Reaction (PCR)

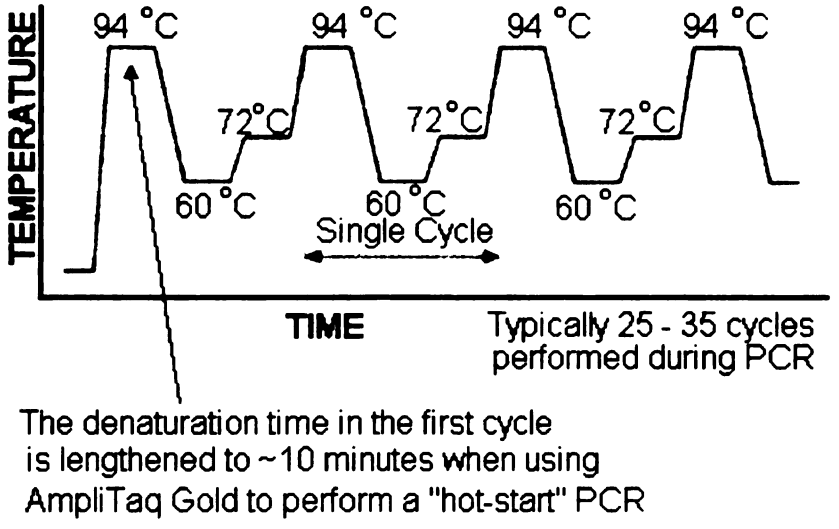
PCR is an enzymatic process whereby multiple copies of certain DNA sequences are produced (8). According to Butler, "PCR has revolutionized molecular biology with the ability to make millions of copies of a specific sequence of DNA in a matter of only a few hours" (8). DNA typing in forensic science has also taken advantage of PCR technology. For forensic analysis in the United States alleles at 13 STR loci are copied using commercially prepared kits such as Profiler Plus™ and Cofiler™. PCR is desirable for forensic samples because often the sample size is small and the DNA is degraded. This generally allows scientists to conserve some of the evidence for repeat testing if necessary, and still have enough evidentiary material to produce a genetic profile.

The components in commercially available kits include primers, reaction mix, and DNA polymerase. Primers are short pieces of DNA that complimentary attach to the DNA strand just outside of the region to be copied. They target the correct sequence of DNA to be copied and help drive the reaction forward. The reaction mix contains buffers to maintain pH and a large amount of each of the four nucleotide bases that are the building blocks of making new DNA strands. The DNA polymerase adds the appropriate nucleotide base to the newly forming DNA strand as it is extended. Taq polymerase is used because it is stable even at the high temperatures required for DNA denaturation (2,8).

The PCR process begins with an incubation step and is referred to as a hot start. This step allows sufficient time for the AmpliTaq Gold™ polymerase to be activated. AmpliTaq Gold™ is a specially designed heat activated DNA polymerase. It prevents the formation of non-specific products at a lower temperature (8).

Then, the cycling of the actual PCR process begins. As displayed in Figure 5, each cycle consists of three precisely timed reactions, for which different temperatures are needed (8).

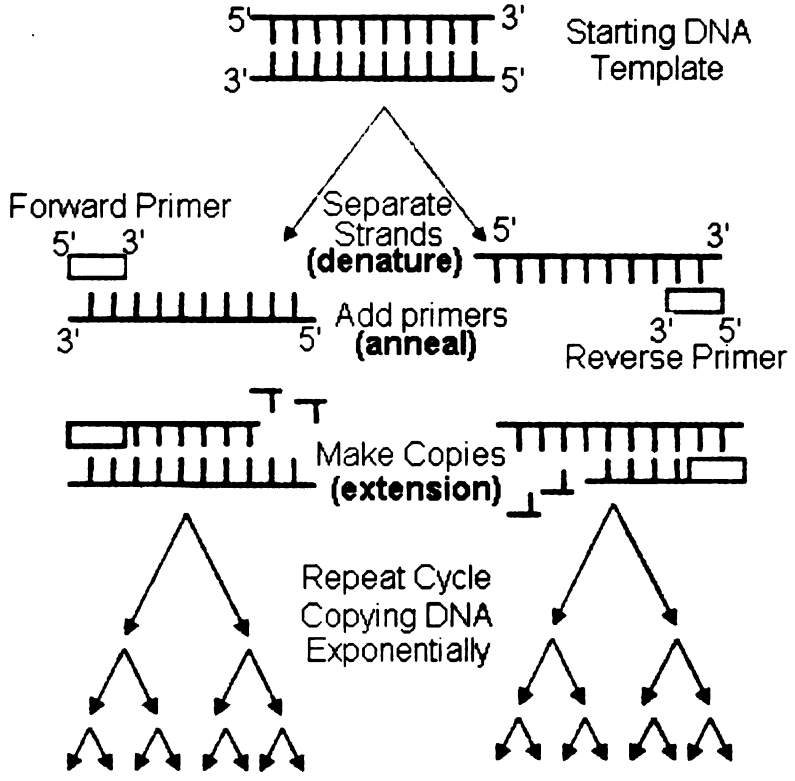
FIGURE 5: Thermocycling Temperatures for PCR



First, the DNA segment to be amplified is heated to separate the DNA into single strands (denatured). These single strands of DNA then serve as the template for synthesis of the new DNA (14, 33). In the second step of the cycle, the strands are cooled and the primers attach (anneal) to the strands. In the final step of the cycle, the four nucleotide bases adenine, guanine, cytosine, and thymine by the actions of DNA polymerase (Taq) are added to each strand in a complimentary fashion. A pairs with T, and only C pairs

with G. It is this final cycle step (extension) that synthesizes new DNA, and exponentially increases the amount of DNA present. Figure 6 demonstrates how the PCR amplification process works (8).

FIGURE 6: PCR Amplification Process



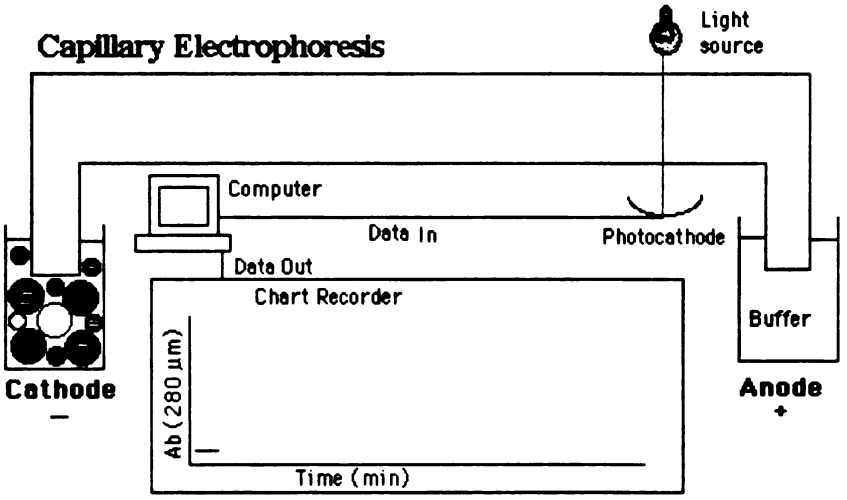
Capillary Electrophoresis

Once the PCR process generates millions of copies of STR alleles from each locus, it becomes necessary to separate these fragments from one another to identify what alleles are present (8). Electrophoresis is a method utilized to separate charged molecules using an electrical current. DNA is a negatively charged molecule. Thus, all of the negatively charged DNA fragments begin at the negatively charged cathode and migrate

to the positively charged anode as depicted in Figure 7 (9). However, the fragments do not all migrate at the same rate. One type of electrophoresis is called capillary electrophoresis. A capillary is a very thin tube (50-100μm diameter), which is filled with a polymer solution to act as a sieve. Smaller DNA fragments pass through at a faster rate than larger fragments, thus a size based separation is accomplished (8, 9).

During the PCR process, fluorescently tagged primers are attached to the DNA fragments. As these fragments progress through the capillary, they pass a detector window with a laser that excites the fluorescent tags. A detector measures the emitted fluorescence, and the computer software uses this data to generate a genetic profile for each sample (8).

FIGURE 7: Schematic of Capillary Electrophoresis



Summary

Previous research has shown that DNA can be recovered from a variety of objects handled by the human hand, but it is unknown if DNA can withstand the effects of a low

explosion. The purpose of this research is to determine if it is possible to recover human DNA from exploded pipe bomb devices from the person who handled or manufactured the bomb in order to generate a DNA profile that would be useful to the law enforcement community.

REVIEW OF THE LITERATURE

In their article, "Fingerprints from Fingerprints", Roland A.H. Van Oorschot and Maxwell K. Jones were the first scientists to demonstrate that one's genetic profile could be generated from swabs taken from a persons hand, or objects a person touched (38). Since this 1997 study, others have used this study as a springboard for other important related research questions.

In 2000 Renterghem, Leonard, and De Greef experimented with the potential use of latent fingerprints as a source of DNA for the purposes of forensic casework. The protocol they developed in this study is now utilized in their laboratory for casework samples (28).

Scientists from the Royal Canadian Mounted Police Forensic Laboratory have produced a list of 37 objects that have been successfully DNA typed when that object was handled by the human hand. In many cases a fingerprint was unable to be visualized, yet a DNA profile was successfully generated (13, 41).

These studies, among others demonstrate that regardless of what objects one may touch, Locard's Exchange Principle applies. It states that a cross-transfer of evidence takes place whenever a person comes in contact with another person, object, or scene (20). In other words, when we touch something, we will take something with us (whether it is dust, fibers, etc.), and we will leave something of ourselves behind, which in this example will be in the form of sloughed skin cells, hairs, latent prints, and so forth.

Once it was demonstrated that a DNA profile can be generated from sloughed skin cells on an object, a new question can be asked. What other variables can be tested

with these objects to simulate real-world events to determine the success of DNA profiling? One such variable to test involves exposing DNA on an object to some type of explosion.

When a gun is fired, a miniature version of an explosion occurs. The first part of the ignition chain is the primer (a primary explosive). When struck, the primer detonates and in turn ignites the propellant (powder), which burns at a controlled rate. This burning propellant produces high-pressure gases that accelerate the bullet down the barrel. These high-pressure gases can reach about 50,000 pounds per square inch and a temperature of 3500 °F (25).

One study by Yoelit Migron, et al examined fired cartridge cases from AK-47's, M16's, and Parabellum's under laboratory conditions for the presence of fingerprints. They had some success with partial fingerprints from brass M16 cartridge cases, but the other cartridge cases were not as successful (19). Another study conducted by four scientists in Magdeburg, Germany demonstrated that a mitochondrial DNA type from the gun user could be detected on fired cartridge cases (35).

In a 1997 article from the JFS entitled, "Suicidal Terrorist Bombings in Israel – Identification of Human Remains", the authors remark on the success of DNA identity testing from tissue samples that have been exposed to very high temperatures (15).

Finally, in a Bachelor of Science thesis by Haylee De-Arne Bechaz from the University of Cambodia, a study was conducted at the Australian Federal Police Forensic Laboratory Service, that involved the recovery of nuclear DNA from fired cartridge cases and exploded pipe bombs (3). This study concluded that if a sufficient quantity of DNA was present on the cartridge case prior to firing, recovery of nuclear DNA was possible

after firing. The same conclusion was reached with regard to pipe bombs post deflagration. In this particular study, none of the pipe bombs were able to yield a full DNA profile post-deflagration, but some alleles were detected. It is of importance to note that this study was only preliminary in nature, very few repeats of this test were conducted (5 or less).

MATERIALS AND METHODS

Decontamination of Pipe Bomb Components

The components for a total of 27 1-inch diameter pipe bombs, 13 PVC and 14 galvanized steel pipes and caps were purchased at a local hardware store. Half of the PVC caps and half of the metal caps were drilled to accommodate the fuse. With the handler wearing disposable latex gloves, the pipes, caps, and fuses went through a methodical decontamination procedure to remove any existing DNA. Every pipe and cap (both metal and PVC) were soaked in 10% Bleach water for 10 minutes, rinsed with distilled water, and blotted dry with paper towels (8). Next, each piece was placed in the UV hood (P.C.R. Chamber By PLAS LABS), and rotated every 15 minutes for a total exposure time of 45 minutes to ensure all areas of the pipes and caps were exposed directly to the UV light (8).

The time fuses were each cut to a length of 6 feet. To avoid getting the ends wet (which would render the fuse useless), the ends were covered with masking tape before being wiped down with 10 % Bleach water (8).

The "odd" galvanized steel pipe bomb (#14) was not deflagrated, but used as a positive control to ensure skin cells were being deposited on the pipe bombs. One of the ten subjects was chosen randomly to handle this additional bomb for this task. All decontaminated pipes, caps, and fuses were stored in paper evidence bags numbered 1 – 13, one set metal and one set PVC per bag. Each of the ten subjects were given one bag and assigned that number for the remainder of the study. The remaining three bomb pairs served as negative controls.

Sample Collection

Ten Caucasian volunteers, four men and six women participated in this study. Seven of the ten subjects washed and dried their hands prior to handling the pipe bomb components. Hand washing was advocated because a previous study has shown that DNA can be detected from two people, if for example these two people shook hands and a swab was taken from one persons hand (38). It was determined part way through subject participation that eliminating this possible variable of secondary DNA contamination on the subjects' hands would be desirable for this study.

Following decontamination of the pipe bomb constituents, each subject first handled the cap without the drilled hole using a twisting motion with their hands for 10 seconds. Second, the subjects handled the fuse by sliding their hands down the length of the fuse two times. Next, each individual took the cap with the drilled hole and threaded the fuse approximately three inches into the pipe and used masking tape to secure the fuse in place. Then, the subjects twisted this cap onto the pipe handling it in this twisting motion for 10 seconds. The above procedure was followed for both the metal and PVC pipe bombs. However, an additional step was required for the PVC bombs as there were no threads to twist the caps on. Instead, Oatey Regular/Clear PVC Cement was utilized cementing the cap with the fuse threaded through to the pipe body. The subjects still used a twisting motion on these caps as well as pressure for the 10 seconds to secure the caps on the pipe.

Once all the pieces had been handled and the pipe bombs partially assembled as described above, they were placed in their numbered bags, sealed shut, and stored at

room temperature to await deflagration. The pipe bombs were decontaminated November 14, 2001 and handled by the study volunteers on November 15.

Bomb Assembly and Deflagration

For safety reasons, the bombs were transported unfinished to the demolition site (a rock quarry) near Ann Arbor, MI. At the quarry, two bomb squad members (identified as Investigator 2 and 3) added gunpowder to the previously assembled bombs one bomb-pair at a time. Using gloves, they removed the one-inch diameter pipe with attached cap and fuse from the bag, removed the outer coating of the fuse inside the pipe only, and funneled in the smokeless powder until the fuse tip was immersed in the powder. Enough powder was used to ensure the powder would not shift away from the exposed time fuse. In this study, IMR$_{TM}$ powder SR 4756 by DuPont was utilized. This powder is a single-base extruded powder (10). It was chosen by the Michigan State Police Bomb squad for this project simply because they had a large supply on-hand. Once the powder was added, the second cap was then secured (the metal cap was twisted on and the PVC was glued on).

Prior to the final bomb assembly, two small holes were dug at the blast site approximately 6 – 12 inches deep and about 6 inches in diameter. The metal and PVC pipes were placed in separate holes with the fuse end up. A large rock was placed on top of each hole to keep the fragments contained within each hole. This was done for two reasons: first, to make the fragments easier to find, and second, to ensure cross contamination of pieces from different bombs would not occur. In some instances,

fragments did escape the holes but were not collected for analysis to avoid possible cross contamination between bombs.

Once the bombs were deflagrated, the fragments from each hole were collected (with gloves) by two of the investigators in new and separate paper bags and sealed. The above procedure was performed a total of 13 times, once for each bomb pair. This portion of the project took two days in the field to complete. Bomb pairs numbered 1,3,5,6, and 7 were deflagrated on December 7, 2001; Bomb pairs 2,4, and 8 – 13 were deflagrated on December 12.

Laboratory Sample Collection

In order to document the degree of fragmentation and to show the variation in amount of fragments recovered, each bomb was photographed at the lab prior to swabbing (Appendix A, B). Once photographed, the pieces were swabbed using a method known as the double swab technique (34). The first of two swabs was moistened with distilled water and rubbed over an entire fragment. Then, a second dry swab was used in the same manner. This technique was repeated with the same two swabs over as many fragments as possible until the amount of soil became so great that the swabs were saturated with soil and not collecting any more debris. Both swabs were allowed to air dry, then the cotton tips from both swabs were removed, cut up, and placed directly into an extraction tube. The tubes were given a number designation corresponding to the person who handled the bomb, and were labeled as metal or PVC. The letter "A" represented pre-deflagration samples and the letter "B" represented post-deflagration samples.

Prior to exploding the pipe bombs, all three pairs of negative controls were swabbed (also using the double swab technique) to ensure the decontamination procedure worked correctly. In addition, if contamination were to be seen in a negative control post-deflagration but not pre-deflagration, an idea of where the contamination occurred could also be determined. Likewise, a positive control was required to ensure that skin cells were transferred to the pipe bombs. One subject (#13) was given pieces for a third pipe bomb to handle in the same manner as the other bomb samples; this bomb was labeled 14 Metal A. These components were then swabbed, but not deflagrated.

Organic Extraction

As mentioned previously, the cotton tips from both swabs from each sample were removed with a scalpel, cut up, and placed in an extraction tube. Next, 600µl of stain extraction buffer (Tris/EDTA/NaCl/SDS solution) was added to each tube followed by 30µl of Protinase K. These solutions act to digest the proteins that are attached to the DNA molecule. Each sample was then vortexed to mix the components, and a quick spin in an Eppendorf 5417C Centrifuge for 10 seconds was performed. The samples were then placed in an incubator set at 56°C for 3 hours.

Following the incubation, the tubes were spun down briefly (10 seconds) to remove condensation from the lids. Then the cotton swabs were removed from the extraction solution and placed in a spin basket, which was then inserted back in the extraction tube. The tubes were placed in the centrifuge for 5 minutes at 10,000 rpm to remove excess liquid from the cotton swabs. When accomplished, the spin baskets were removed and discarded.

Next, 630µl of Phenol / Chloroform / Isoamyl Alcohol was added to each sample, and the tubes were vortexed until a milky emulsion was achieved (approximately 10 seconds). The phenol portion denatures proteins and dissolves lipids, the chloroform denatures proteins and facilitates the separation of layers, and the alcohol controls foaming (8). The tubes were spun in the centrifuge for 5 minutes at 10,000 rpm to allow separation of the layers. Meanwhile, Amicon Centricon®-100 concentrators were being labeled and assembled for the next step. Centricon®'s (and Microcon®'s) have a filter that collects molecules equal to and greater than 100,000 Daltons (18). DNA will collect on the filter and the denatured proteins and other waste will pass through the filter into the waste reservoir. The DNA is purified and concentrated by this step. 100µl of TE^{-4} Buffer was added to each Centricon® filter and spun through at 500 xg for 5 minutes. As soon as the Centricon®'s were prepared, the top layer from the extraction tubes, referred to as the DNA extract, was removed and added to the centricon, along with 1ml of TE^{-4}. Once the TE^{-4} Buffer and DNA extract was added to the Centricon® filters, they were spun down for 30 minutes at 500 xg.

To wash the DNA on the Centricon® filters, 1 ml of TE^{-4} was added to each and spun down at 500 xg for 30 minutes. After this wash step, the Centricon® filter was removed from the reservoir and inverted into the attached vial. These were spun at 1000 xg for 3 minutes to remove the DNA from the filter. The newly purified DNA extract was then removed from the vial and placed into a newly labeled microcentrifuge tube and stored at –20°C until the next day.

Yield Gel

An agarose yield gel is the first of two methods utilized by the Michigan State Police Crime Laboratory to quantify extracted DNA from each sample. Yield gels also indicate the quality of the sample DNA. Smearing denotes degraded DNA, and a single clear band indicates high molecular weight DNA. The agarose gel was prepared ahead of time and was composed of 0.25g agarose in 25ml TAE Buffer with 2.5µl ethidium bromide. It had a total of 28 wells divided equally into two rows of 14. Once the gel was immersed in the running buffer (180ml of TAE), the samples were loaded with a 10µl pipette.

The first well in both rows (1 and 2) of the gel is reserved for the visual marker or Ladder, Lambda Hind III/Eco Rl; 3µl of this ladder was loaded into each of the two wells. The next six wells (#2-7) are reserved for the quantitation standards to which the DNA samples are compared; 6µl of each standard was loaded in the following order: 500ng, 250ng, 125ng, 63ng, 31ng, and 15ng. The test samples were prepared in the following manner: 4µl of DNA extract was added to 2µl of loading buffer (bromophenyl blue/glycerol) for a total of 6µl. Then, 6µl of each sample mixture was loaded into each remaining well. Electrophoresis was carried out at 175 volts for approximately 12 minutes using a Horizon 58®, Life Technologies™, GIBCO BRL Horizontal Gel Electrophoresis Apparatus.

The ethidium bromide that was added to the agarose gel intercolates the DNA strands as they migrate through the gel. Keeping in mind that ethidium bromide glows in the presence of Ultraviolet (UV) light, we utilized this property to visualize the DNA in the gel lanes. A Fotodyne Foto® / Phoresis unit was used to shine UV light on the gel

and a photograph of each gel was taken to interpret the gel. The quantity of each sample was determined by visually comparing the standard it most closely resembled and assigning it that concentration.

QuantiBlot®

The second more sensitive quantification method utilized by the Michigan State Police Crime Laboratory is called a QuantiBlot® (QB). DNA quantification is critical to obtain optimal amplification results, particularly with Profiler Plus™ kits where the goal is to add 1.0 – 2.5ng of human DNA to the reaction. With such a narrow "optimal" range, a more specific quantification method is required beyond the yield gel, which only has a detectable range of 125ng – 3.75ng of DNA (2). Thus, the Perkin Elmer / Applied Biosystems QuantiBlot® Kit was utilized to obtain a more sensitive quantification reading. QuantiBlot's® have quantitation standards that range from 2ng of DNA per µl to 0.03125ng of DNA per µl, although readings below the lowest standard can also be estimated. An additional significant advantage of this method is that the probe is primate specific (2). Any positive result indicates the presence of human DNA.

Similar to a yield gel, there are 7 known standards expressed in ng/µl. However, in a QuantiBlot® there is also a blank, and two calibrators included in the kit to ensure the other standards are balanced and working properly. Based on the yield gel results, if the DNA is too concentrated, a dilution with Milli Q water can be prepared. Otherwise, 5µl of straight extract is added to 150µl of spotting solution (0.4N NaOH, 25mM EDTA, 0.00008% Bromothymol Blue). The 155µl mixture is pipetted into each corresponding slot, as specified in the QB Kit protocol. This same protocol (printed in the product

insert) was then followed to completion and the chemiluminescent detection method chosen. The membrane was exposed to x-ray film for 45 minutes. The autoradiograph results were interpreted by comparing the signal intensity of the known size standards to the signal from the test samples (2).

Polymerase Chain Reaction (PCR)

DNA Amplification was accomplished using the PE Applied Biosystems AmpF*l*STR® Profiler Plus™ PCR Amplification Kit as well as the PE Applied Biosystems GeneAmp™ 2400 PCR Instrument System. As mentioned previously, the goal is to add 1.0 – 2.5ng of human DNA to the amplification reaction. The Michigan State Police Laboratory in Northville prepares a solution of approximately 1.25ng DNA in 10µl of volume. For samples exceeding the optimum amount of input of DNA (approximately 1.0ng input or 0.1ng/µl) Milli-Q (ultra pure) H_20 is used to prepare a dilution for 10µl volume. In some instances, the quantity of the extract is less than 1.25ng/10µl (there may even be no signal at all). In this situation there are two possible approaches that can be taken for such a sample: (1) attempt amplification with an addition of 10µl of extract, or (2) concentrate the extract to a smaller volume using a Centricon®-100 or Microcon®-100 before amplification in order to add the maximum amount of DNA recovered during the extraction process (2). Both approaches were attempted in this study. In the first round of DNA amplification, 10µl of straight extract were used for each test sample.

In addition to the input DNA, every sample also requires 15µl of "master mix". The master mix, which is prepared in a separate microcentrifuge tube, contains 10.5µl of

reaction mixture, 5.5µl of primers, and 0.5µl of AmpliTaq Gold™ per sample to be amplified. PCR was then performed using the parameters listed in Table 1.

TABLE 1: Thermal Cycling Times and Temperatures

Steps in PCR Process	*Temperature (°C)*	*Time*
1. Initial Incubation Step (Hot Start)	95°C	11 minutes
2. Step Cycles (repeated 28 times) : (1) Denature	94°C	1 minute
(2) Anneal	59°C	1 minute
(3) Extend	72°C	1 minute
3. Final Extension	60°C	45 minutes
4. Final Step	25°C	hold

The samples can be stored in a -20°C freezer until needed or may be used immediately following amplification.

Microcon®100

In the first round of PCR amplification, 10µl of straight extract was used for each bomb sample as well as each control. Although some results were observed with this method, the Microcon® was utilized to concentrate the original extract in order to add more DNA to the PCR reaction without the large volume of liquid TE^{-4} in which the DNA was solubilized. Each sample had between 80 - 140µl of extract to start; the objective was to concentrate the volume to 10µl or less for the bomb samples and negative controls.

The membranes were rinsed with 100µl TE^{-4} and centrifuged at 500 xg for 5 minutes in the microcentrifuge. Then, each extract was pipetted into a sample reservoir and spun at 500 xg for 10 minutes. The samples were considered ready when the

membranes were still wet around the edges, but appeared dry throughout most of the center. Many samples had to be spun an additional 10 minutes to achieve this appearance. Lastly, each sample reservoir was inverted into a new microcentrifuge tube and spun at 1000 xg for 3 minutes to transfer the concentrated DNA into the tube. Each sample had 4-10µl of concentrated extract.

Next, the PCR process (as described above) was repeated with the newly concentrated samples. All samples below 10µl were brought up to 10µl with Milli Q H_2O. For this final amplification all the remaining extract was used.

Capillary Electrophoresis

Once DNA amplification was complete, capillary electrophoresis was performed on the samples using the ABI Prism 310 Genetic Analyzer™. As with PCR, a "master mix" was prepared in a microcentrifuge tube with 24µl of deionized formamide and 1µl of GS500 ROX internal size standard for each sample, for a total volume of 25µl. The formamide denatures the DNA and prevents the complimentary strands from binding during electrophoresis, thus improving resolution (8). Once the master mix was pipetted into each tube, 3µl of the amplified DNA product was added to each. The Profiler Plus™ allelic ladder was prepared in the same manner, with 25µl master mix and 3µl of the ladder; a master mix blank was also prepared with just the formamide and ROX. The positive controls were also prepared with 25µl master mix, and only 1µl of amplified product to ensure the data would not be off scale. Using a Fisher Scientific Dry Bath Incubator, the samples were incubated at 95°C for 3 minutes. Next, the samples were

transferred to two bench top coolers (from -20°C freezer), also for 3 minutes. Heating and then cooling also aids in keeping the DNA molecules single stranded (8).

A sample sheet and injection list was prepared for the sample run with the parameters as listed in Table 2.

TABLE 2: ABI Prism 310 Run Parameters

POLYMER/FILTER	GS STR POP4 F
Injection Time	5 seconds
Injection kV	15.0
Run kV	15.0
Run Temperature	60.0°C
Run Time (per sample)	24 minutes
Matrix File	031001
Auto Analyze	Yes
Size Standard	GS ROX 500
Analysis Parameters	Analysis Default

Once the run was complete, the matrix was applied. The matrix is a mathematical or software filter that allows the fluorescent signals emitted by the tagged DNA fragments to be distinguished from one another. The matrix allows DNA fragments of the same size to be run at the same time in the same capillary by labeling them with different fluorophores (dyes) (8). GeneScan™ 2.0 and Genotyper™ 2.1 software were used to generate electropherograms from the 310 data. The guidelines utilized by the Michigan State Police DNA/Biology unit for casework samples were utilized when interpreting this data. A modified list of these guidelines are listed below (2, 26).

1. Alleles of a genetic profile with an RFU value of 150 – 4500 were declared reportable.

2. Alleles of a genetic profile with an RFU value of 50 – 149 were declared active. Active alleles are not reportable and may not be used in statistical calculations, but may still be useful for investigative purposes such as excluding a suspect who could not have contributed a particular set of alleles.
3. Alleles of a genetic profile with an RFU value below 50 were declared undetectable. Undetectable alleles are not reportable.
4. Stutter products are one repeat unit (4 base pairs) shorter than the main allele, and are expected artifacts produced from the PCR process. As depicted in Figure 8, stutter occurs when complimentary DNA strands breath apart during DNA synthesis causing one repeat unit to bubble out so it is not copied (39). Stutter bands are reproducible artifacts, and occur in a predictable ratio for each locus. Table 3 was used to interpret the stutter products for the maximum percent stutter allowed at each locus. In order to be called stutter, the peak height ratio cannot exceed the maximum percentage in table 3; although elevated stutter can be suspected with certain samples.
5. In regards to heterozygous alleles at a particular locus, the heterozygote peak ratio percentage is determined by dividing the smallest RFU value by the largest RFU value and multiplying by 100. Validation by the Michigan State Police Crime Laboratory determined the heterozygote peak ratio to be ≥70%. If less than 70%, a heterozygote imbalance exists and must be interpreted with caution as it may indicate several possible problems. A heterozygote imbalance can be indicative of a preferential amplification or a mixture of more than one person's DNA.

6. A condition exists where some individuals have 3 alleles at a particular locus. Since all individuals taking place in this study were known to have only one or two alleles at each locus, a single source sample was declared if only one or two alleles were present at all loci examined.
7. A sample was considered to be a possible mixture (originating from more than one source) if more than two alleles were present at two or more loci, and the peak height ratios for heterozygous loci were not in the expected proportions.
8. For the purposes of this study, only 10 of the possible 13 STR loci were examined. A full reportable genetic profile was declared if all the loci examined (10 loci in this study) had alleles with RFU values between 150-4500, and heterozygote peak ratios were acceptable.
9. A partial reportable profile was declared when a full reportable profile could not be declared. At least 3 of the STR loci had alleles with RFU values between 150-4500, and heterozygote peak ratios were acceptable.
10. An active profile was declared when one or more loci exhibited alleles with an RFU value above 50 and did not meet the guidelines for a full or partial profile type. Also included were heterozygous loci that showed extreme imbalances and were thus not reportable and/or one of the alleles was reportable and the other allele was active.
11. An undetectable profile was declared when all alleles had an RFU value below 50.

FIGURE 8: Slipped Strand Mispairing Model

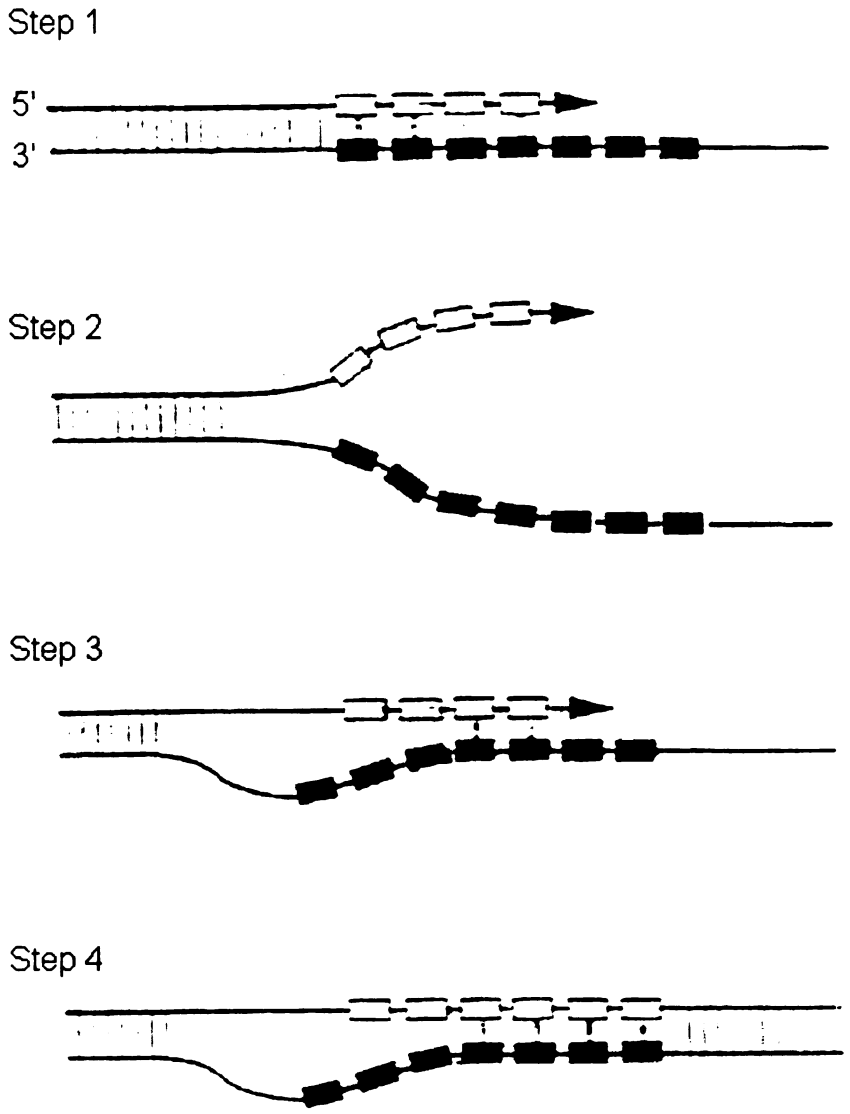


TABLE 3: Profiler Plus™ Stutter Percentages

Locus	*% Stutter*	*Dye/Color*
D3S1358	15	5 FAM/Blue
vWA	15	5 FAM/Blue
FGA	15	5 FAM/Blue
D8S1179	12	JOE/Green
D21S11	15	JOE/Green
D18S51	18	JOE/Green
D5S818	12	NED/Yellow
D13S317	12	NED/Yellow
D7S820	12	NED/Yellow

Quality Assurance /Quality Control

The Michigan State Police Crime Laboratory is an ASCLD certified laboratory, meaning they abide by the highest standards of quality in their facility. The Northville Laboratory scientists use the same equipment and procedures that were also used in this study. Thus, to meet compliance with the Michigan State Police QA/QC procedures, a stain blank, ILC, amplification positive control (9947A), amplification negative control, and a master mix blank were subjected to the same procedures as the samples to ensure the quality of the testing results.

RESULTS

In summary, the ten subjects handled 20 of the 26 pipe bombs prior to deflagration in this study; 6 were negative controls. Each subject handled 2 bombs (1 galvanized steel and 1 PVC). The bombs were deflagrated and the pieces collected in new separate bags. The bags were transported back to the Northville Laboratory and the exploded bombs photographed to document the degree of fragmentation and recovery (Appendix A, B).

Yield Gel Results

With the exception of the internal lab control sample that had a clear and distinct band, the remaining samples where either ruled as NV (nothing visible) or <<3.75 ng/µl. Those samples with some visible DNA on the yield gel were very faint and smeared, suggesting degradation. The control samples and quality control blanks rendered acceptable results.

QuantiBlot Results

Again, with the exception of the internal lab control sample, every sample was observed to be less than the smallest QuantiBlot standard of 0.15625 ng/5µl (or 0.03125ng/µl) (Table 4). Interestingly, in the post-deflagration first negative control (1 Metal B and 1 PVC B) and in the third negative control (4 PVC B) human DNA was also present, although at a very low quantity (<<0.03125ng/µl) (Table 4). No DNA was observed in the remaining negative control samples (Table 4). As a result of the

contamination observed on some of these negative controls, a contamination study was performed.

TABLE 4: QuantiBlot Results

DNA in ng/µl	*Sample ID*
2.0	ILC121401
0.25	ILC110801
0.03125	14 Metal A 14 PVC A
< 0.03125	3 PVC B 5 PVC B 7 Metal B 7 PVC B 8 Metal B 12 PVC B 13 Metal B 13 PVC B
<< 0.03125	1 Metal B* 1 PVC B* 3 Metal B 4 PVC B* 6 Metal B 9 Metal B 11 Metal B 11 PVC B
NV (nothing visible)	S110801* S121401* 1 PVC A* 1 Metal A* 2 PVC A* 2 Metal A* 4 PVC A* 4 Metal A* 2 Metal B* 2 PVC B* 4 Metal B* 5 Metal B 6 PVC B 8 PVC B 9 PVC B 10 Metal B 10 PVC B 12 Metal B

* Negative Control

Contamination Study

At the completion of the main study, a contamination study was undergone in attempt to explain some contamination observed in the results. The contamination study samples consisted of QA/QC samples: a stain blank, the internal lab control (ILC), an amplification positive control (9947A), an amplification negative control, and a master mix blank (formamide and GS500 ROX). A pipe negative control sample, a pipe positive control sample, and an open tube (cough) sample were the main contamination study samples.

To clarify, a metal pipe the exact size used by the participants in this study was subjected to the same decontamination procedure previously explained. The pipe was swabbed to ensure the decontamination procedure worked correctly. Then, the author (Investigator 1) handled the pipe for 10 seconds in the same manner as the subjects in the study did, which was described previously. The pipe was then swabbed after handling also using the double swab technique (34). The last sample, termed the open tube cough sample, is accurately described as such. One tube was placed far away from the other samples and Investigator 1 coughed several times near the tube. This was done to simulate the cold the author had at the time of extracting the test samples. Then, the swabs, QA/QC, and open tube samples were extracted using the same organic extraction protocol described above.

As mentioned previously, the main study samples were quantified, amplified, and electrophoresed without being concentrated during the first 310 run. To improve results, a second run was performed where the extracts were concentrated to a smaller volume and then re-amplified and electrophoresed. Since the best results were obtained using a

concentrated extract, the contamination study samples used this protocol so all the extracts were immediately concentrated after extraction using the above-described Microcon®-100 procedure, to a volume of 20µl. If it was less than this volume, TE^{-4} was added to increase the volume to 20µl. They were quantified by a yield gel, followed by the QB, then amplified by the same PCR process, and subjected to electrophoresis on the ABI 310. The same kit lots and the same reagents were used in the contamination study as on the main study samples.

Contamination Study Yield Gel Results

In the contamination study, the yield gel results were analogous to the main study results. The ILC sample had a clear distinct band at approximately <<3.75ng/µl; the remaining samples were ruled NV.

Contamination Study QuantiBlot® Results

In the contamination study, the stain blank and the pipe negative control (the pipe was swabbed after decontamination) were ruled NV. The pipe positive control (the pipe the author handled) contained 0.25ng/µl of DNA, and the ILC had 2.0ng/µl DNA. The tube the author coughed into had a faint line, thus was estimated <<0.03125ng/µl, much less than the smallest standard (Table 5).

TABLE 5: Contamination Study QuantiBlot Results

DNA in ng/µl	*Sample ID*
2.0	ILC011802
0.25	KE pipe positive control
<<0.03125	KE cough
NV (nothing visible)	S011802 KE pipe negative control

STR Results

The exploded bombs were placed in 1 of 3 categories based on the degree of fragmentation. Category 1 was for bombs where the majority of the device was intact, category 2 was for highly fragmented or mangled bombs where many pieces were recovered, and category 3 was for highly fragmented or mangled bombs but where few pieces were recovered. This information was then compared to the type of DNA profile generated from the recovered explosive devices using unconcentrated DNA extract (Table 6), using concentrated DNA extract (Table 7), and a combination of both runs (Table 8).

Table 6 includes the type of profiles generated from unconcentrated DNA extracts, whereas Table 7 contains the data from the concentrated DNA extracts that used the Microcon®100's. Focusing exclusively on the 20 subject-handled bombs, the unconcentrated extract generated 1 partial genetic profile, 6 active profiles, and the remaining 13 profiles were undetectable (Table 6). Using concentrated extract, 1 bomb generated a full profile, 1 gave a partial profile, 5 were active, and 12 rendered undetectable profiles (Table 7).

TABLE 6: Types of Profiles Generated from Unconcentrated DNA Extract

Profile Type	*Sample ID*
Partial	**13 PVC B**
Active	**6 Metal B, 6 PVC B,** 7 PVC B, 8 PVC B, **10 PVC B, 12 PVC B**
Undetectable	**3 Metal B,** 3 PVC B, **5 Metal B,** 5 PVC B, 7 Metal B, 8 Metal B, *9 Metal B*, 9 PVC B, 10 Metal B, 11 Metal B, *11 PVC B*, 12 Metal B, 13 Metal B

KEY: **BOLD – Majority Intact**
REGULAR – Highly Fragmented/Mangled (Many pieces recovered)
ITALICS – Highly Fragmented/Mangled (Little recovered)

TABLE 7: Types of Profiles Generated from Concentrated DNA Extract

Profile Type	*Sample ID*
Full Reportable	**6 Metal B**
Partial Reportable	**3 Metal B, 6 PVC B**
Active	3 PVC B, **5 Metal B,** 5 PVC B, 12 Metal B, **13 PVC B**
Undetectable	7 Metal B, 7 PVC B, 8 Metal B, 8 PVC B, *9 Metal B*, 9 PVC B, 10 Metal B, **10 PVC B,** 11 Metal B, *11 PVC B*, **12 PVC B,** 13 Metal B

KEY: **BOLD – Majority Intact**
REGULAR – Highly Fragmented/Mangled (Many pieces recovered)
ITALICS – Highly Fragmented/Mangled (Little recovered)

Many samples had different profile types when using the unconcentrated versus the concentrated DNA extract. Thus, when combining the data, the most "active" profile was used for each bomb. For example, sample 6 Metal B generated an active profile with unconcentrated extract (Table 6), but a full reportable profile with concentrated extract (Table 7), so that sample was given the designation of a full reportable profile (Table 8). On the other hand, sample 13 PVC B gave a partial profile with unconcentrated extract (Table 6), but only an active profile with concentrated extract (Table 7). Using the

standard of the most active profile, the partial profile generated with the unconcentrated extract was used in the final tabulation (Table 8).

In combining the data from the 310 runs (unconcentrated and concentrated DNA extract), there were 7 subject-handled bombs in category 1; 1 generated a full profile, 3 generated partial profiles, and the remaining 3 gave active profiles (Table 8). 2 of the 3 partial profiles gave statistically useful profile types using the FBI's Caucasian population data allele frequencies (Appendix E). 3 of the 7 category 1 subject-handled bombs were noticeably burned from the smokeless powder; none of these 3 generated full profiles, although two partials and one active profile was still generated (Table 8).

The majority of the subject-handled bombs, 11, fell into category 2. 5 of these were active, and 4 of these 5 had two or more active loci. The remaining 6 bombs rendered undetectable profiles (Table 8). Lastly, 2 of the 20 bombs fell into category 3. As might be expected, these fragments gave undetectable profiles (Table 8).

Besides observing the fragmentation pattern, another variable, metal versus PVC, was compared to the type of profiles generated in the combined 310 runs (Table 8). Metal bombs generated 1 full profile, 1 partial profile, and 2 active profiles, whereas PVC bombs generated 2 partial profiles and 6 active profiles.

TABLE 8: Summary Types of Profiles Generated from both Unconcentrated and Concentrated DNA Extract

Sample ID	*Profile Type*	*Comments*
3 Metal	Partial	5 loci observed – All match known profile. 3 reportable, 2 active.
5 Metal	Active	8 loci observed – 7 match known profile.). 1 reportable, 7 active. D3 locus has 3 peaks (most likely elevated stutter).
6 Metal	Full Reportable Profile	10 loci observed – All match known profile. 10 reportable loci. FGA has a heterozygote peak imbalance.
6 PVC*	Partial	10 loci observed – All match known profile. 8 reportable, 2 active
10 PVC	Active	1 locus observed – match known profile. 0 reportable, 1 active (XY) - 56 RFU's
12 PVC*	Active	8 loci observed – 7 match known profile. 1 reportable, 7 active. D8 locus has 3 peaks, appears to be contamination from the bomb squad member who assembled the device.
13 PVC*	Partial	10 loci observed – All match known profile. 7 reportable, 3 active.
3 PVC	Active	4 loci observed – All match known profile. 1 reportable (XY), 3 active.
5 PVC	Active	1 locus observed – match known profile. 0 reportable, 1 active (XY)
7 Metal	Undetectable	No loci observed
7 PVC	Active	2 loci observed – match known profile. 0 reportable, 2 active (D3, and XY)
8 Metal	Undetectable	No loci observed
8 PVC	Active	2 loci observed – match known profile. 0 reportable, 2 active loci (D8, and XY)
9 PVC	Undetectable	No loci observed
10 Metal	Undetectable	No loci observed
11 Metal	Undetectable	No loci observed
12 Metal	Active	8 loci observed – 5 match known profile. 0 reportable, 8 active. VWA and D8 show evidence of elevated stutter, D3 has a 3rd peak – possible mixture.
13 Metal	Undetectable	No loci observed
9 Metal	Undetectable	No loci observed
11 PVC	Undetectable	No loci observed

KEY: **BOLD** – **Category 1**
REGULAR – Category 2
ITALICS – *Category 3*
* – Noticeable burning and charring present.

The results of the 310 runs for the negative controls are as follows. There were 6 negative control bombs (3 Metal, 3 PVC). Each was swabbed pre-deflagration and designated the letter "A", and then swabbed post-deflagration like the subject-handled bomb fragments and designated the letter "B". They were treated in the same manner as the other bombs throughout the entire experiment; thus they also used unconcentrated DNA extract in the first 310 run, and a second 310 run used concentrated DNA extract. Unconcentrated extract revealed that all negative controls both "A" and "B" were undetectable, with the exception of sample 4 PVC A, which had 2 reportable loci (Amelogenin [XY] and D21), with the remaining loci being active (Table 9). After reviewing a table of known DNA profiles, both subjects and investigators 1,2, and 3, it was discovered that these spurious alleles all matched the known DNA profile of the author, labeled as Investigator 1 (Table 10, 11).

When the extraction volumes were concentrated for the second 310 run, the STR results showed more activity. Rather than 1 "A" control showing activity, 5 out of 6 "A" controls showed some activity, albeit low, and only 1 remained undetectable (Table 9). 4 PVC A became a full reportable profile that again matched the author's known genetic profile (Table 10, 11).

The "B" (post-deflagration) negative controls also showed some activity when concentrated. 1 Metal B had one active allele at the FGA locus (54 RFU's) that matched the author's known genetic profile (Table 10, 11), and 4 PVC B had 1 active locus, XY, at 54 RFU's.

As mentioned previously, to meet compliance with QA/QC procedures, a stain blank, ILC, amplification positive control (9947A), amplification negative control, and a

master mix blank were subjected to the same procedures as the samples to ensure the quality of the testing results. All results were acceptable in both 310 runs, although allele dropout was observed for the amplification positive control (9947A) in the second 310 run when concentrated extracts were used (Table 9).

TABLE 9: QA/QC and Control Sample Results from Both 310 Runs

Profile Type	*Unconcentrated DNA Extract*	*Concentrated DNA Extract*
Full Profile	ILC121401, 14 Metal A, 9947A	4 PVC A
Partial Profile	4 PVC A	9947A*
Active		1 PVC A, 1 Metal B, 2 Metal A, 2 PVC A, 4 Metal A, 4 PVC B
Undetectable	S110801, S121401, Amp Neg, MMBlank, 1 Metal A, 1 Metal B, 1 PVC A, 1 PVC B, 2 Metal A, 2 Metal B, 2 PVC A, 2 PVC B, 4 Metal B, 4 Metal B, 4 PVC B	S121401, Amp Neg, MMBlank, 1 PVC B, 1 Metal A, 2 Metal B, 2 PVC B, 4 Metal B

* Allele dropout observed at FGA, D18S51, and D7S820.

When viewed separately, the QB and STR results appear very straightforward, but there are a couple of underlying patterns in the data which will be highlighted here, and then discussed in the subsequent section. First, viewing select subject-handled pipe bomb samples, 5 Metal B, 6 PVC B, and 12 Metal B all registered as nothing visible (NV) for the QB. However, when the extracts were concentrated and run on the 310, each of these samples generated at minimum, active profiles with a range of 8 – 10 loci, each matching the known subject profiles (Table 12). Conversely, subject handled bomb samples 7 Metal B, 13 Metal B, 9 Metal B, 11 Metal B, and 11 PVC B all registered <0.03125 (or <<0.03125) on the QB, yet no active alleles were generated for any of these samples (Table 12).

TABLE 10: Known DNA Profiles

Sample ID	*D3S1358*	*vWA*	*FGA*	*Amelogenin*	*D8S1179*	*D21S11*	*D18S51*	*D5S818*	*D13S317*	*D7S820*
Known 3	15,19	16,17	20,22	X,X	11,13	31.2,31.2	12,15	11,11	10,11	10,12
Known 5	16,17	14,16	19,22.2	X,X	13,14	30,30	17,18	9,11	10,11	10,12
Known 6	16,18	16,16	20,21	X,Y	13,13	32.2,33.2	14,19	11,13	11,11	9,11
Known 7*	14,16	14,16	19,22	X,X	14,14	30,33.2	12,14	11,13	8,11	9,10
Known 8	15,16	17,18	22,24	X,X	11,13	30,30.2	12,14	10,11	11,13	8,10
Known 9	15,18	18,19	22,24	X,Y	12,12	28,33.2	15,15	11,12	11,12	11,14
Known 10	16,16	18,19	22,23	X,X	8,11	29,30	12,13	12,13	11,12	9,13
Known 11	17,18	17,19	19,22	X,X	11,15	28,30	20,20	12,12	9,13	9,11
Known 12	15,16	14,17	21,24	X,Y	14,16	30,30	16,18	10,13	10,10	8,10
Known 13* & 14	18,18	17,17	21,22	X,Y	9,14	29,31.2	13,14	10,12	11,11	8,11
Investigator 1	15,17	17,17	21,22	X,X	11,14	30,30	15,22	10,12	11,12	11,12
Investigator 2	18,19	15,17	24,25	X,Y	12,12	30,30.2	10,12	10,12	9,13	9,12
Investigator 3	16,17	17,18	19,22	X,Y	11,14	31,32.2	17,19	9,12	8,14	8,11
ILC	15,15	17,19	22,23	X,X	10,13	26,31.2	13,18	11,12	12,14	9,11
9947A	14,15	17,18	23,24	X,X	13,13	30,30	15,19	11,11	11,11	10,11

*Known DNA Profiles established for this project. (Remaining Profiles were previously established.)

TABLE 11: Profiles Generated from Pipe Bomb Fragments and Control Bombs

Sample ID	*D3S1358*	*vWA*	*FGA*	*Amelogenin*	*D8S1179*	*D21S11*	*D18S51*	*D5S818*	*D13S317*	*D7S820*
3 Metal B	15^{A},19^{A}	16,17	NR	X,X	11,13	NR	NR	11,11	NR	NR
3 PVC B	NR	NR	NR	X,X	NR	31.2^{A}	NR	11^{A}	11^{A}	NR
5 Metal B	15^{A},16^{A}, 17^{A} **	$14,16^{A}$ *	19^{A}	X,X	$13,14^{A}$ *	30^{A}	NR	9^{A},11^{A}	10^{A}	NR
5 PVC B	NR	NR	NR	X^{A}	NR	NR	NR	NR	NR	NR
6 Metal B	16,18	16,16	20,21*	X,Y	13,13	32.2,33.2	14,19	11,13	11,11	9,11
6 PVC B	16,18	16,16	20,21	X,Y	13,13	32.2,33.2	14^{A},19^{A} *	11,13	11,11	9^{A},11^{A} *
7 PVC B	16^{A}	NR	NR	X^{A}	NR	NR	NR	NR	NR	NR
8 PVC B	NR	NR	NR	X^{A}	13^{A}	NR	NR	NR	NR	NR
10 PVC B	NR	NR	NR	X^{A}	NR	NR	NR	NR	NR	NR
12 Metal B	$15,16^{A}$,17^{A} **	14^{A},17*	NR	X,Y*	14^{A},15^{A}, 16^{A} **	30	NR	10^{A},13^{A} *	10^{A}	NR
12 PVC B	15,16	14,17	21,24	X,Y	12,14,16	30	NR	10,13	10	NR
13 PVC B	18,18	17,17	21,22	X,Y	9,14*	$29,31.2^{A}$ *	13^{A},14^{A}	$10,12^{A}$ *	11,11	8^{A},11^{A} *
14 Metal A	18,18	17,17	21,22	X,Y	9,14	29,31.2	13^{A},14^{A}	10,12	11,11	8^{A},11^{A} *
1 PVC A	NR	17^{A}	21^{A}	X,X	14^{A}	NR	NR	NR	NR	NR
1 Metal B	NR	NR	21^{A}	NR	NR	NR	NR	NR	NR	NR
2 Metal A	15^{A}	NR	NR	X^{A}	13^{A}	NR	13^{A}	NR	11^{A}	NR
2 PVC A	15^{A}	17^{A}	NR	X^{A}	NR	30^{A}	NR	NR	NR	NR
4 Metal A	17^{A}	NR	19^{A}	X,X	11^{A}	NR	NR	10^{A}	NR	NR
4 PVC A	15,17	17,17	21,22	X,X	11,14	30,30	15,22	10,12	11,12	11,12
4 PVC B	NR	NR	NR	X^{A}	NR	NR	NR	NR	NR	NR

*Heterozygote Ratio falls below 70%

**Shows possibility of mixture, and/or elevated stutter

^{A} Active Allele (50 – 149 RFU'S)

NR – No Results

TABLE 12: Comparison of QB and STR results in subject-handled bomb samples

Sample ID	*QB (ng/μl)*	*Profile Type*
5 Metal B	NV	Active
6 PVC B	NV	Partial
12 Metal B	NV	Active
7 Metal B	<0.03125	Undetectable
13 Metal B	<0.03125	Undetectable
9 Metal B	<<0.03125	Undetectable
11 Metal B	<<0.03125	Undetectable
11 PVC B	<<0.03125	Undetectable

Similar patterns are also observed with the negative control pipe bomb samples. 3 controls rendered positive QB results, yet 2 of 3 generated just one active locus and the third resulted in an undetectable profile (Table 13). On the other hand, 5 negative control samples registered as NV for the QB, yet generated at minimum active alleles at 3 or more loci (Table13).

TABLE 13: Comparison of QB and STR results in negative control bomb samples

Sample ID	*QB (ng/μl)*	*Profile Type*
1 Metal B	<<0.03125	Active (1 locus)
4 PVC B	<<0.03125	Active (1 locus)
1 PVC B	<<0.03125	Undetectable
1 PVC A	NV	Active
2 Metal A	NV	Active
2 PVC A	NV	Active
4 Metal A	NV	Active
4 PVC A	NV	Full reportable profile

Finally, Table 14 highlights the STR results of the contamination study. The QA/QC samples were all acceptable, although the stain blank did have activity at one locus (Amelogenin). The pipe negative control gave an undetectable profile, the pipe positive control gave a full reportable profile, and the tube Investigator 1 coughed near

gave an active profile (7 active loci). These last two samples (pipe positive control and the cough sample) each matched the author's known DNA profile (Table 10).

TABLE 14: Profiles Generated from Contamination Study

Sample ID	*D3S1358*	*vWA*	*FGA*	*Amelogenin*	*D8S1179*	*D21S11*	*D18S51*	*D5S818*	*D13S317*	*D7S820*
S011802	NR	NR	NR	X^A Y^A	NR	NR	NR	NR	NR	NR
KE Pipe Neg Cont	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
KE Pipe Pos Cont	15,17	17,17	21,22	X,X	11,14	30,30	15,22	10,12	11,12	11,12
KE Cough	17^	17^	21^	X^	NR	30^	15^,22^ *	NR	NR	11^
Amp Neg	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
MMBlank	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
ILC	15,15	17,19	22,23	X,X	10,13	26,31.2	13,18	11,12	12,14	9,11
9947A	14,15	17,18	23,24	X,X	13,13	30,30	15,19	11,11	11,11	10,11

*Heterozygote Ratio falls below 70%

^ Active Allele (50 – 149 RFU'S)

NR – No Results

DISCUSSION

As mentioned previously, the purpose of this research was to discover if obtaining usable genetic profiles from exploded pipe bomb devices was possible. The results verify that recovering DNA from the individual who handled the bomb prior to deflagration occurred in several instances and the subsequent generation of usable profiles was ultimately successful. Of the twenty bombs handled by subjects, four bombs gave reportable results that matched the known genetic profiles (Table 8, 10). Using population statistics, 3 of these 4 bombs gave useful estimates of how often these profiles would expect to be seen ranging in value between 1 in 4 million up to 1 in 7 billion (Appendix E).

To summarize, three usable profiles from a total of 20 possible bombs are a 15% success rate. As will be discussed in this section, suggested improvements in the method could potentially double this success rate to approximately 30%. Swabbing bomb fragments for DNA may provide law enforcement with another tool for apprehending bombers. This is an exciting result of this study, and should be explored further with additional research. Although there were some contamination problems that arose during this study, suggestions for improving the success rate as well as prevention of these problems will be discussed.

The importance of yield gels lie in their ability to give information regarding DNA quality and quantity. If the band is solid, that indicates the DNA is of a high molecular weight quality, whereas if there is smearing, the DNA is degraded (fragmented) to some degree (2, 8). One way degradation of DNA occurs is through the introduction of bacteria in a sample. The pipe bombs in this study were detonated in holes

dug in the ground at a rock quarry. The soil was moist from recent rainfall, and upon recovery each pipe bomb was coated in soil. Numerous bacteria inhabit soil; in fact, it is estimated that the top eight meters of soil carry as many as 26 x 10^{28} bacteria (37). As described by Inman and Rudin, soil quickly degrades human DNA due to the large amount of bacteria present (14). It stands to reason then, that the DNA from the sloughed skin cells of the subjects became intermixed with the bacteria from the soil, degrading the sample DNA, which explains the smearing in the majority of the sample lanes in the yield gel photographs (14, 31). In addition, yield gels are not specific for human DNA (2). Thus, when the negative control bomb samples exhibited smearing in the photographs, it was attributed to low-molecular weight bacterial DNA introduced through the soil, rather than contamination from human DNA.

DNA quantification is critical to obtain optimal results. Specifically, the goal is to add an optimum amount of approximately 1.25 ng of input human DNA in 10µl of volume to the amplification reaction. Reviewing the QB results, no subject-handled pipe bomb sample comes close to yielding that amount of DNA. Each sample was less than the lowest QB standard of 0.03125ng/µl of DNA. Even if all the samples were equal to the lowest quantitation standard and 10µl of this extract were added to the PCR reaction, that is only 0.3125ng of DNA, much less than is recommended by the manufacturer for optimum results.

The STR results from the first 310 run reflect the small amount of DNA that was added to the PCR reaction (Table 6). No full reportable genetic profiles were generated, and in fact, only one partial profile was generated in this run. However, upon concentration of the DNA extract, which allowed more DNA to be added to the PCR

reaction, one full reportable profile and two partial profiles were generated. Thus, concentrating the DNA extract increased the success of the PCR process. Despite the advantages of concentrating the extract, it created some problems as well.

Although a second QB was not performed after concentrating the extract to determine how much DNA was present, it was suspected that the amount of DNA added to the PCR reaction was still below the manufacturer's recommendation of 1 – 2.5ng of DNA. In other words, these are low copy numbers (LCN) of DNA molecules that are being added to the PCR reactions (11,12). This is significant because previous studies have shown that all methods designed to analyze low amounts of DNA have several inherent disadvantages that are "primarily derived from stochastic variation." (8, 11). In other words, when increasing the sensitivity of the method to detect small amounts of DNA, and there are few copies of the DNA fragments to begin with, any allele(s) can be amplified early in the PCR process. It is then likely that these alleles will be preferentially amplified thereby causing some unavoidable conditions. According to Gill these conditions include (11):

(1) Allele dropout or heterozygote imbalance because one allele of a heterozygote locus can be preferentially amplified;

(2) *Stutters, otherwise known as false alleles, may be preferentially analyzed; and*

(3) *The method is prone to sporadic contamination – amplifying alleles that are unassociated with the crime stain or sample.*

All of these problems are observed in several of the generated electropherograms in this study. In fact, each sample listed in Table 11 (profiles generated from pipe bomb fragments and control bombs) has either allele dropout, heterozygote imbalance, elevated

stutter, or contamination; and some samples have two or three of these problems. It is likely these conditions arose due to the combination of working with LCN DNA and increasing the sensitivity of the PCR method.

Gill and his colleagues explain in these studies that regardless of the cleanliness of the laboratory and the stringent conditions employed, laboratory based contamination cannot be completely avoided when the sensitivity of the method is increased for LCN DNA. Many of their negative controls showed low levels of spurious alleles, and many were at the lower molecular weight loci, but not in all instances (12). The same was true of the negative controls in this study. Before the extracts were concentrated only one control sample (4 PVC A) showed evidence of gross extraneous contamination; the remaining control samples showed no evidence of contamination. However, when increasing the sensitivity of the method by concentrating the extracts, six additional samples showed evidence of spurious alleles with low RFU values (Table 9).

Their recommendation for dealing with contamination in negative controls when dealing with LCN DNA is to re-amplify the extracts to determine if the laboratory-based contamination can be reproduced. If the alleles cannot be replicated after re-amplifying the extract, there is no cause for concern that these alleles have contaminated the other extracted samples (12).

Unfortunately, in this study, re-amplifying the original extracts was not possible. When the extracts were concentrated the volume obtained was only enough for 1 round of PCR amplifications. Instead, a contamination study was performed to try and re-create the conditions imposed on the samples. In addition, the author's DNA was often

observed to be the contaminating source, so it was desirable determine how easily the analyst may slough skin cells on handled objects.

As expected, the pipe bomb the author handled and then swabbed gave positive QB results (Table 5) and generated a full reportable genetic profile that matched the author's known DNA profile (Table 10,14). These results confirmed that the author does shed skin cells easily and abundantly; thus, there is a need to be even more careful about following aseptic protocols, such as changing gloves frequently. In addition, the tube the author coughed near also gave positive QB results and generated an active profile that matched the author's known DNA profile (Table 10,14). These results again reveal the sensitivity of the PCR method, and indicate the importance of a sterile environment. In general, the sensitivity also reinforces the importance for law enforcement and laboratory employees to exercise extreme care and caution when collecting or handling evidence to avoid contamination. These results also suggest that one should wear a mask and change gloves frequently when infected with a head cold to avoid contaminating samples.

The contamination study also demonstrated that the decontamination procedure used on the pipe bombs was successful; no DNA was detected on the QB from the decontaminated control and no detectable genetic profiles were generated (Table 5,14). Lastly, this study provided further evidence that contamination cannot be completely avoided with procedures designed for LCN DNA. The stain blank in this study gave no evidence of human DNA contamination throughout the DNA process. However, when a profile was generated an active locus was discovered at Amelogenin (Table 14). What was odd about this finding was that there was an X and Y allele present. All females are X, X at Amelogenin and all males are X, Y. No male samples were run at the same time,

and no males were near these samples in the laboratory at any time. Also, the Northville DNA unit was comprised entirely of female employees at the time of this study. In other words, there is no explanation for the origin of these spurious alleles, which lends further support to the findings by Gill and his colleagues.

Logically, one would expect a sample that renders a QB result would also generate some type of genetic profile on the 310. However, there were five subject-handled bomb samples and 1 negative control bomb sample that had a positive QB result, yet not a single active allele was generated in the profiles (Table 12,13). Furthermore, one would also reasonably expect to find that if a profile is generated for a sample, one should be able to refer back to the QB results and find a positive result. Yet, 3 subject-handled bomb samples and 5 negative control bomb samples had a QB rating of nothing visible (NV), yet they generated at least an active profile (Table 12,13).

A couple of possibilities exist to justify this result. For the samples that had a positive QB result but no profile was generated, this could be due to degradation of the DNA (8). For the samples that had a negative QB result but generated some type of genetic profile, it is possible for the input DNA to be low enough that the QB could not detect it and still have enough input DNA to generate at least an active profile (8).

Another explanation for the majority of the samples is a statistical problem known as sampling error. Keeping in mind that all the QB results in question were performed on the original large volume extracts, it becomes apparent how sample error can occur. If a large volume of liquid contains a very small amount of DNA, where the DNA is not mixed homogeneously throughout, it is almost impossible to remove a small sample that is representative of the entire volume. Two possibilities then exist in this scenario. One

possibility is that the portion of the liquid removed for the QB removes all or most of the DNA and very little DNA (if any) is left for the PCR amplification reaction. This approach would explain the samples that had a positive QB result with no genetic profile. The second possibility is that the portion of liquid removed for the QB contains TE^{-4} only, and then enough DNA happens to be removed in the next step for successful PCR amplification. This approach would explain how the samples with a negative QB result could generate a genetic profile.

Additionally, these scenarios explain how results between the two 310 runs could vary from sample to sample. One would expect that concentrating the remaining extract would only improve the signal allowing for more alleles to be detected or for alleles to be registering at a greater RFU value. But this premise depends upon equal distribution or equal sampling of DNA throughout the extract. Although DNA has been removed for the first round of tests, it would seem that there should still be plenty of DNA remaining in the original extract for the next time the sample was tested. As hypothesized previously, equal distribution of the LCN DNA throughout the sample did not always occur. In many instances, improved results were observed; however, in some samples concentrating the extract did little to improve the desired result of producing a profile (Table 6, 7).

In addition to the potential problem of the unequal distribution of the DNA, two other possibilities may exist. One explanation for the variation between the 310 runs, is that very little DNA (if any) remained in the original volume of extract after performing the yield gel, the QB, and one round of PCR. In particular, this appeared to be the case if the first round of DNA amplification was successful in generating a profile. Regardless of the amount of DNA removed in the first round of testing, it significantly decreased the

DNA remaining for the second round of testing. Conversely, the second explanation would be that the majority of the DNA remained in the original volume after the first round of testing. Once the extract was concentrated, the generated profile was dramatically improved from the first round because there was more DNA template available for the PCR process.

In hindsight, the extracts should have been concentrated before any testing occurred. The extract volumes were large, greater than 100µl. Each sample could have been concentrated to between 20 and 30µl and the volume would have been sufficient to conduct all of the testing. It is possible that by concentrating the extracts from the start many of the anomalies observed in both in the QuantiBlot®'s and the generated electropherogram profiles may have been eliminated. The possibility of sampling error would have been greatly reduced, as a more equal distribution of the DNA throughout the extract would have been easier to achieve.

In addition to eliminating these problems, it is hypothesized that concentrating these extracts prior to any testing would have also greatly improved the overall results. It is believed that more full and/or partial profiles could have been generated. For example, in reviewing Tables 6 and 7, samples 13 PVC B and 6 PVC B generated partial profiles in one of their runs and active profiles in the other. Instead of having split the DNA between two separate amplifications (two 310 runs), the DNA could have been concentrated for one PCR reaction per sample in order to generate a more complete profile in both samples.

To review, the original question this study desired to answer was whether it was possible to recover enough human DNA from exploded pipe bomb fragments to generate

a genetic profile. Specifically is it possible to recover DNA from the person who handled the bomb prior to deflagration. If the answer to this question was "yes", one additional question needed to be addressed: Is there a difference in the success of recovery of DNA between metal and PVC bomb fragments? Initially, metal bombs were expected to give inferior results. It was hypothesized that the DNA may not survive the heat generated by the explosion, which would be exacerbated by the thermal conductivity of metal, which could cause further degradation of the DNA. However, it was postulated that with plastic, there may be a better chance for the DNA to survive, as plastic does not conduct heat well.

In a 1997 article in the Journal of Forensic Science entitled "Suicidal Terrorist Bombings in Israel – Identification of Human Remains"; this research offered hope that DNA could in fact survive the high temperatures of an explosion. These researchers found that they could extract DNA from tissues of cadavers where the temperatures had reached extremely high temperatures (15). Although specific temperature ranges were not mentioned in this article, a database on gunpowder revealed that the explosion temperature for confined smokeless powder reaches between 2500 – 3000°C (24).

The results of this study indicate that both metal and PVC bombs can successfully yield usable genetic profiles from the individual who handled the bomb prior to deflagration. Additionally, both metal and PVC materials appear to have similar success in regards to generating profiles. The four most complete profiles were detected from two metal bombs and two PVC bombs. A summary of these findings is displayed in Table 15.

Table 15: Successful Profiles Generated by PVC and Metal Bombs and Their Knowns

Sample ID	*Profile*	*D3S1358*	*vWA*	*FGA*	*AY*	*D8S1179*	*D21S11*	*D18S51*	*D5S818*	*D13S317*	*D7S820*
6 Metal B	Full	16,18	16,16	20,21*	X,Y	13,13	32.2,33.2	14,19	11,13	11,11	9,11
6 PVC B	Partial	16,18	16,16	20,21	X,Y	13,13	32.2,33.2	14^{A},19^{A} *	11,13	11,11	9^{A},11^{A} *
6 Known		16,18	16,16	20,21	X,Y	13,13	32.2,33.2	14,19	11,13	11,11	9,11
3 Metal B	Partial	15^{A},19^{A}	16,17	NR	X,X	11,13	NR	NR	11,11	NR	NR
3 PVC B	Active			NR	X,X	NR	31.2^{A}	NR	11^{A}	11^{A}	NR
3 Known		15,19	16,17	20,22	X,X	11,13	31.2,31.2	12,15	11,11	10,11	10,12
13 PVC B	Partial	18,18	17,17	21,22	X,Y	9,14*	$29,31.2^{A}$ *	13^{A},14^{A}	$10,12^{A}$ *	11,11	8^{A},11^{A} *
13 Known		18,18	17,17	21,22	X,Y	9,14	29,31.2	13,14	10,12	11,11	8,11
12 Metal B	Active	$15,16^{A}$,17 ^{A} **	14^{A},17 *	NR	X,Y *	14^{A},15^{A}, 16^{A} **	30^{A}	NR	10^{A},13^{A} *	10^{A}	NR
12 PVC B	Active	15,16	14,17	21,24	X,Y	12,14,16	30	NR	10,13	10	NR
12 Known	Active	15,16	14,17	21,24	X,Y	14,16	30,30	16,18	10,13	10,10	8,10
5 Metal B	Active	15^{A},16^{A}, 17^{A} **	$14,16^{A}$ *	19^{A}	X,X	$13,14^{A}$ *	30^{A}	NR	9^{A},11^{A}	10^{A}	NR
5 Known		16,17	14,16	19,22.2	X,X	13,14	30,30	17,18	9,11	10,11	10,12

NOTE: Only Profiles with 3 or more active loci are displayed.

*Heterozygote Ratio falls below 70%

**Shows possibility of mixture, and/or elevated stutter

^{A} Active Allele (50 – 149 RFU'S)

NR – No Results

Instead of bomb material, the largest factor in regards to DNA typing success appears to be the degree of fragmentation of the bomb. In reviewing Table 8 of the results, every bomb in category 1 (majority intact) has at least one active profile. In addition, every reportable profile, regardless of full or partial was generated by a category 1 bomb. There was also success with bombs in category 2 (Highly Fragmented/Mangled [Many pieces recovered]), but the success was negligible in comparison to bombs in category 1. Five bombs in category 2 registered active profiles, but only 2 of these had 4 or more active loci. Finally, no bombs in category 3 generated a profile. These results suggest that success of generating a genetic profile from the bomb manufacturer depends upon the amount of bomb fragments recovered and how fragmented they are.

Lastly, previous research has shown that DNA recovery on objects is subject" dependent (26, 38). Meaning, each person is different when it comes to their ability to slough skin cells; some people lose skin cells more abundantly and more easily than others do. Logically, the more epithelial cells one leaves behind, the more DNA can be recovered. Some subjects in this study have participated in previous studies where their ability to lose skin cells was established (4, 26). Subjects 5, 12, and 13 were regarded as very good sloughers, subjects 8 and 11 were good sloughers, and subject 10 was not a good slougher. Subjects 3, 6, 7, and 9 had not participated in previous studies; thus their ability to lose skin cells was unknown. In this particular study, subjects 5, 12, and 13 confirmed their ability to shed ample epithelial cells to generate genetic profiles. Subjects 3 and 6 also appear to shed sufficient cells to generate usable genetic profiles.

One variable that can influence the amount of skin cells shed is hand washing (38). This act removes dry or dead surface skin cells as well as minute amounts of

foreign material. If a person handles an object without washing their hands, one would expect that more skin cells would be shed in comparison with handling an object after recently hand washing.

As mentioned in the materials and methods section, 7 of the 10 subjects washed and dried their hands prior to handling the pipe bomb components. Again, hand washing was advocated for this study to eliminate secondary DNA contamination on the hands as a potential contamination source (38). Subjects numbered 5, 12, and 13 did not wash and dry their hands. Reviewing Table 8 in the results, subjects 5, 12, and 13 all generated at minimum one active profile with subject 13 generating one partial genetic profile. In summary, after reviewing the overall results there appears to be a dramatic difference in outcome when hand washing is considered (Table 16). When subjects washed their hands 50% of the bomb profiles were undetectable versus only 16.7% with subjects that did not wash their hands (Table 16). In addition, subjects with unwashed hands generated active profiles 66.8% of the time versus 29% with subjects that did wash their hands (Table 16).

Table 16: Effect of Hand-Washing on STR Results

Profile Type	*Washed Hands*	*Washed %*	*Unwashed Hands*	*Unwashed %*
Full Reportable	1	7.0	0	0.0
Partial	2	14.0	1	16.6
Active	4	29.0	4	66.8
Undetectable	7	50.0	1	16.6

These numbers suggest that hand washing significantly reduced the amount of skin cells (and thus DNA) that is deposited on an object, which lowers the odds of generating a usable genetic profile. On the other hand, unwashed hands strengthen the

chance for more cells to be deposited, and a greater likelihood of generating a genetic profile is achieved. These findings again highlight the importance of hand washing in the laboratory as one method to prevent sample contamination.

Recommendations for Collecting Bomb Fragments for DNA Testing

Using proper procedures and precautions when collecting evidence is of paramount importance. If the explosive device has been deflagrated and is considered safe by the responding bomb squad members, it is the responsibility of the investigating agency to collect the evidence, and then submit it to the laboratory for appropriate testing. When collecting the bomb fragments, investigators should wear clean disposable gloves and a mask to prevent contaminating the evidence, and should change gloves often. If there are bombs in several different locations they should be handled and packaged separately.

If the submitting agency requests DNA testing as well as explosives analysis, the DNA unit should receive the fragments first to avoid laboratory contamination from another unit. Once the fragments have been swabbed for sloughed skin cells, the fragments can be transferred to the appropriate unit for other testing.

Guidelines for DNA tests on bomb fragments

- Collect sloughed skin cells using the double swab technique (34). Water or ethanol can be used to moisten the first swab.
- Perform an organic extraction as described in Budowle, using a Centricon® for precipitating the DNA instead of a Microcon® (6).

- After extraction, use a Microcon® to further concentrate the extracts for all the negative controls and testing samples to a volume of 30 - 40µl. The positive control(s) should not be further concentrated; the amount of DNA after extraction is sufficient for testing.
- Follow PE Applied Biosystems or laboratory protocol for PCR conditions (2). 28 cycles are recommended.
- Use the ABI 310 to perform capillary electrophoresis on the samples. Between 1 and 3µl of amplified product may be added to the 25µl of master mix (formamide and ROX) for each tube, and blanks must be treated the same as the test samples (2).

CONCLUSIONS

In a population of Caucasian males and females, the following conclusions can be drawn regarding the success of DNA recovery on exploded pipe bomb fragments.

1. Enough human DNA from the "bomb manufacturer" can be recovered from exploded pipe bombs, both metal and PVC, to produce reportable genetic profiles. There is no evidence to suggest one surface has more success with DNA recovery than the other.
2. The majority of the pipe bomb must be recovered in order to improve the chance of successful DNA recovery and the subsequent generation of a reportable genetic profile.
3. Successful DNA recovery is also dependent upon the "bomb manufacturer's" ability to slough skin cells on objects they handle; the more cells they shed, the more likely a reportable profile can be generated. If the bomb manufacturer recently washed their hands prior to bomb assembly, it significantly decreases the odds of obtaining a usable genetic profile.
4. Increasing the sensitivity of the method by concentrating the extract is necessary in order to achieve reportable results. However, elevated stutter, allele dropout, heterozygote imbalances, and contamination are common due to LCN DNA and the high sensitivity of the method.
5. Alleles do not necessarily need to be reportable to be of use to law enforcement in an investigation. Active alleles may still be used as a basis for a suspect exclusion, or to continue to include a suspect.

SUGGESTIONS FOR FUTURE RESEARCH

Forensic scientists are continually searching for new and innovative methods to solve the ever-increasing number of crimes that are committed each year. Presently, if a bomb is discovered prior to or after an explosion, law enforcement relies on the components used in its manufacture to help apprehend the criminal. They may have circumstantial evidence that a suspect has the same type of gun powder in his garage as used in the bomb, or they may discover a receipt from a store detailing what types of pipe were bought, which could also match those used in the crime. This type of circumstantial evidence is quite incriminating by itself, but if there is also the suspect's DNA on the bomb, the suspect has almost assuredly sealed his fate.

With advances in technology over the past 10 years, DNA has become a powerful identification tool in law enforcement's arsenal. Sloughed skin cells are just one of many ways that a criminal can leave a trace of his or her DNA behind. By carefully collecting bomb fragments from a crime scene, a DNA scientist may be able to obtain a genetic profile to aid in the search for the perpetrator, especially in situations where there are no suspects.

At the writing of this paper, swabbing bomb fragments for sloughed skin cells to obtain a DNA profile has never been attempted in an actual case, only in this particular controlled research setting. Further research in this area is required to determine how applicable these techniques would be in the present criminal justice system. The following is a list of suggested variables to manipulate to further study the recovery of DNA from explosive devices.

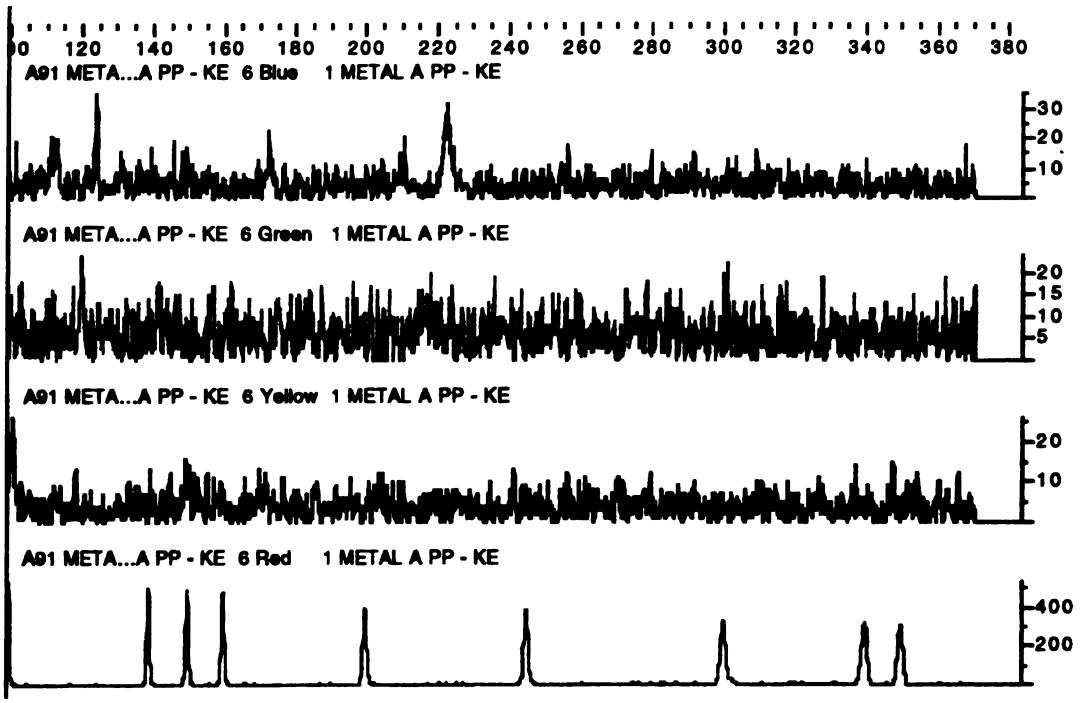
1. A large percentage of planted bombs are discovered before an explosion occurs, such as in the Columbine High School shooting. In these situations, a group of highly trained bomb specialists are called in to render the bomb safe. In a future experiment, rather than explode the bombs, have these professionals perform the procedure to render the bomb safe, and then collect the bomb components for DNA testing. A dated general description for such a procedure is printed in Appendix F, which was taken from Lenz (16). Modern techniques cannot be disclosed for safety reasons.
2. Different types or brands of smokeless powder could be experimented with to determine if some powders inhibit the PCR process.
3. Have the test subjects take the pipe and caps home with them for a week to simulate a person buying and storing the pieces until they are ready to use it. This will allow a more realistic approach to handling time and actual storage conditions.
4. Build other types of improvised explosives. For example, flashlights can be used rather than a pipe.
5. Use a high explosive in the bombs rather than gun powder to determine if DNA can withstand the heat generated by this type of explosion.

APPENDIX A

Appendix A contains electropherograms generated from unconcentrated DNA extracts (First 310 Run) and the corresponding photographs of the recovered pipe bomb fragments.

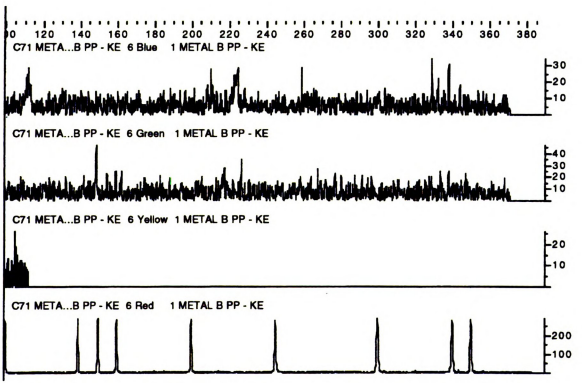
Negative Control Pipe Bomb 1 Metal A

Pre-deflagration, First 310 Run



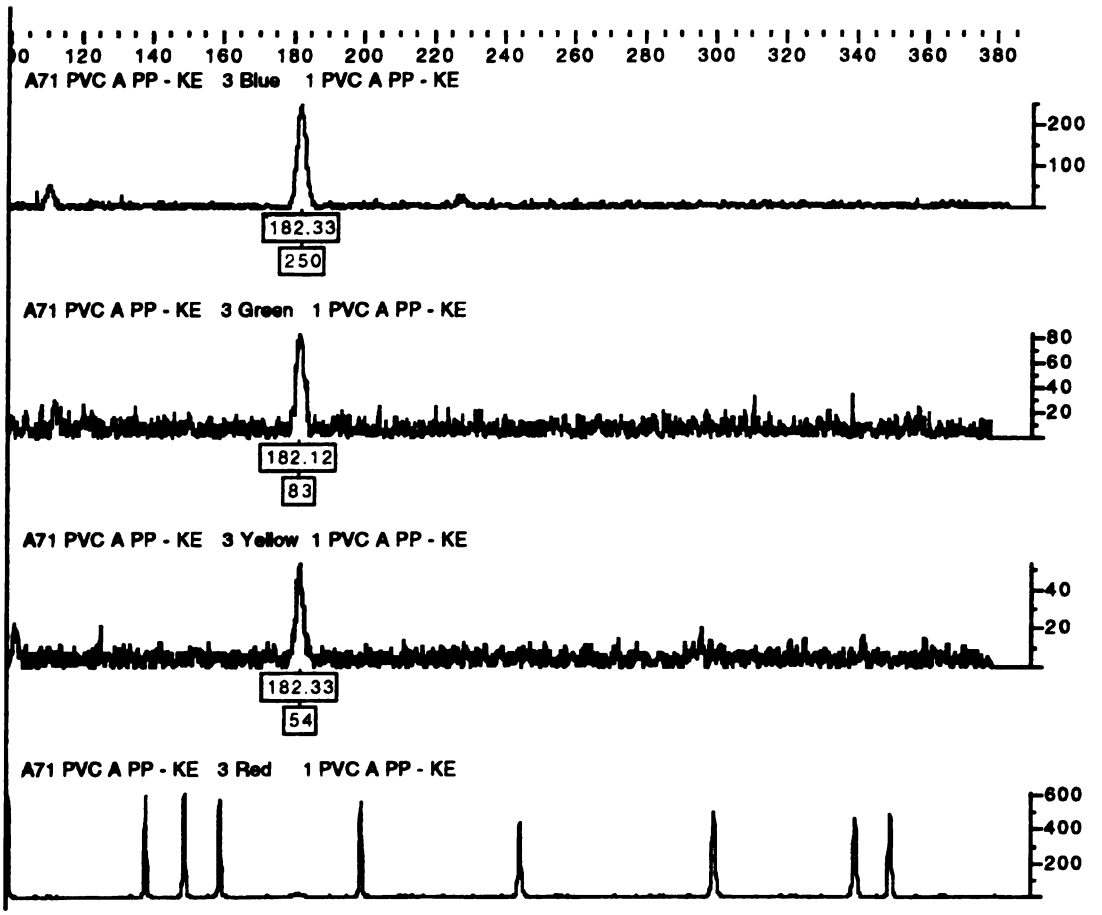
Negative Control Pipe Bomb 1 Metal B

Post-deflagration, First 310 Run



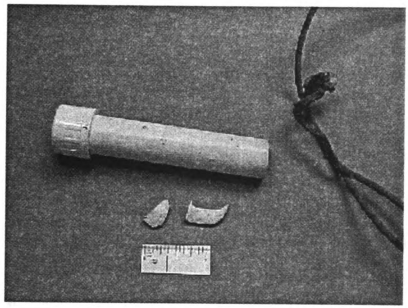
Negative Control Pipe Bomb 1 PVC A

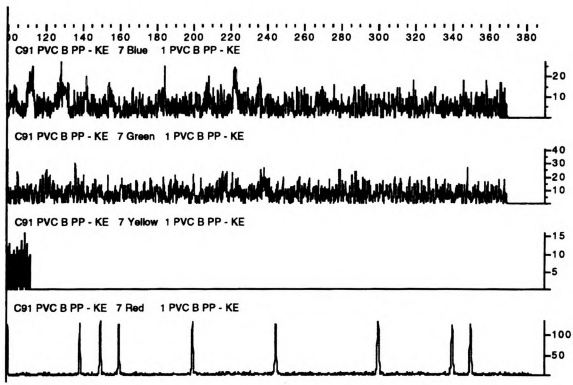
Pre-deflagration, First 310 Run



Negative Control Pipe Bomb 1 PVC B

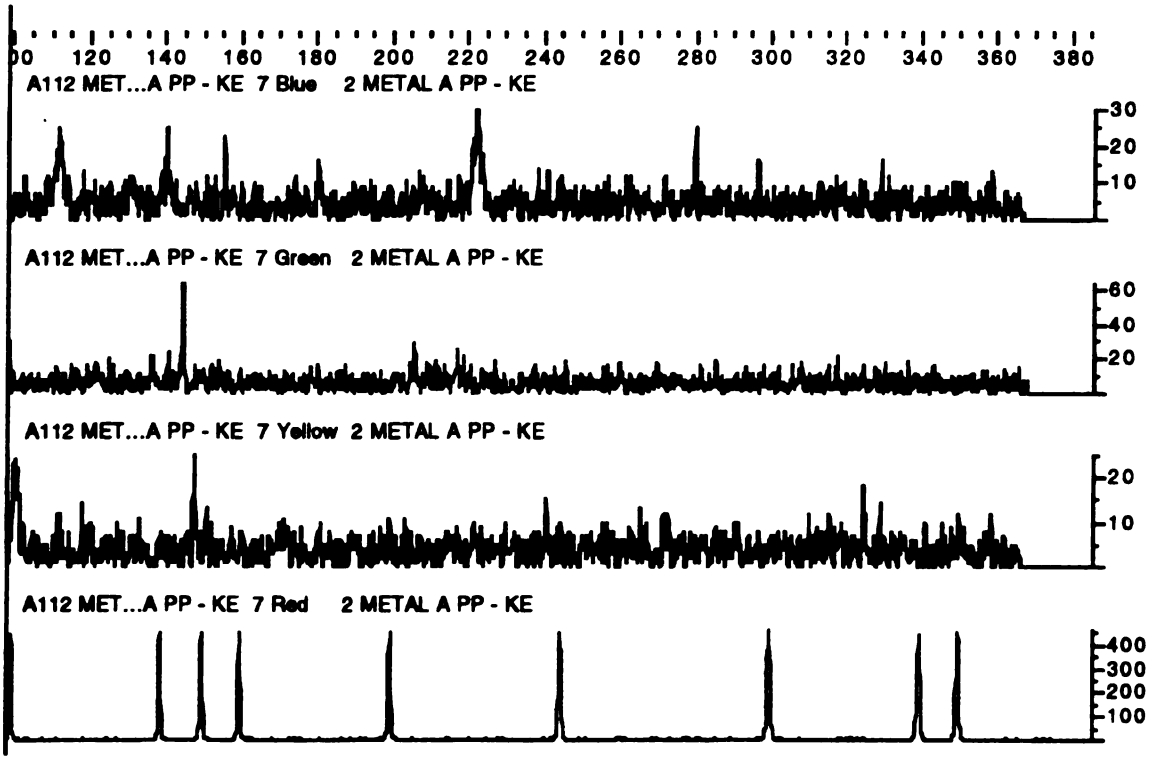
Post-deflagration, First 310 Run





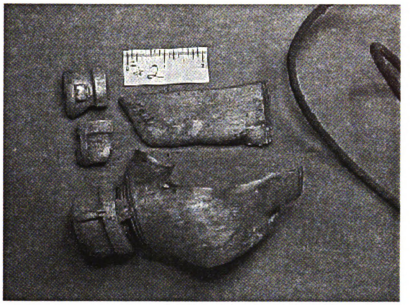
Negative Control Pipe Bomb 2 Metal A

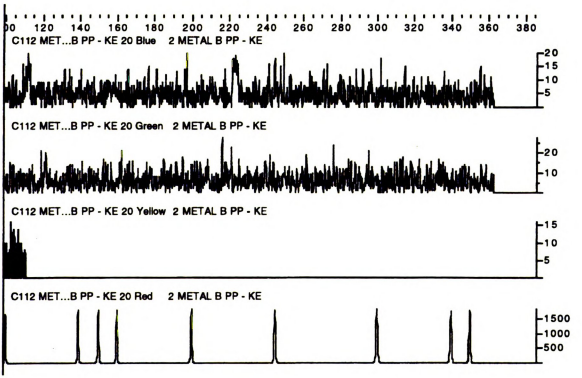
Pre-deflagration, First 310 Run



Negative Control Pipe Bomb 2 Metal B

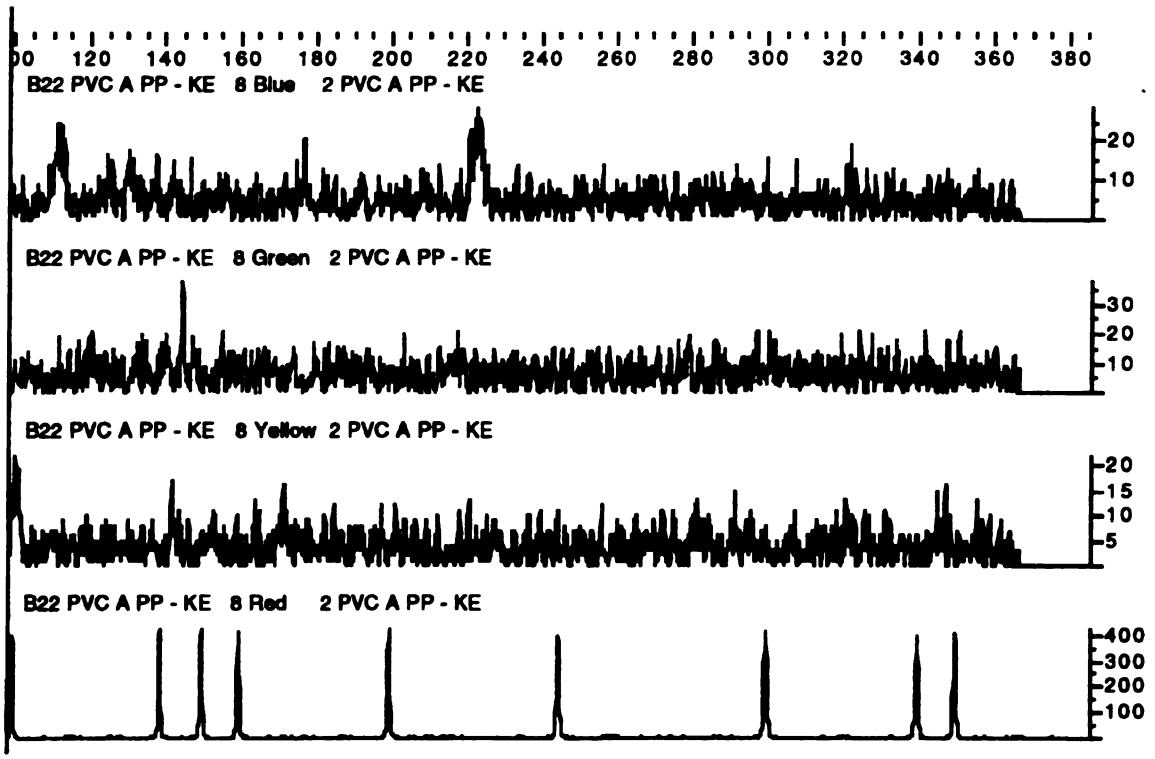
Post-deflagration, First 310 Run





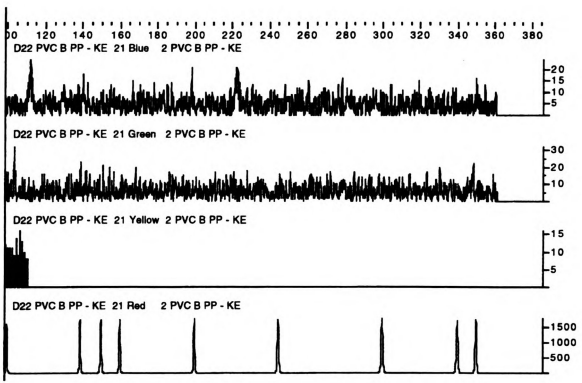
Negative Control Pipe Bomb 2 PVC A

Pre-deflagration, First 310 Run



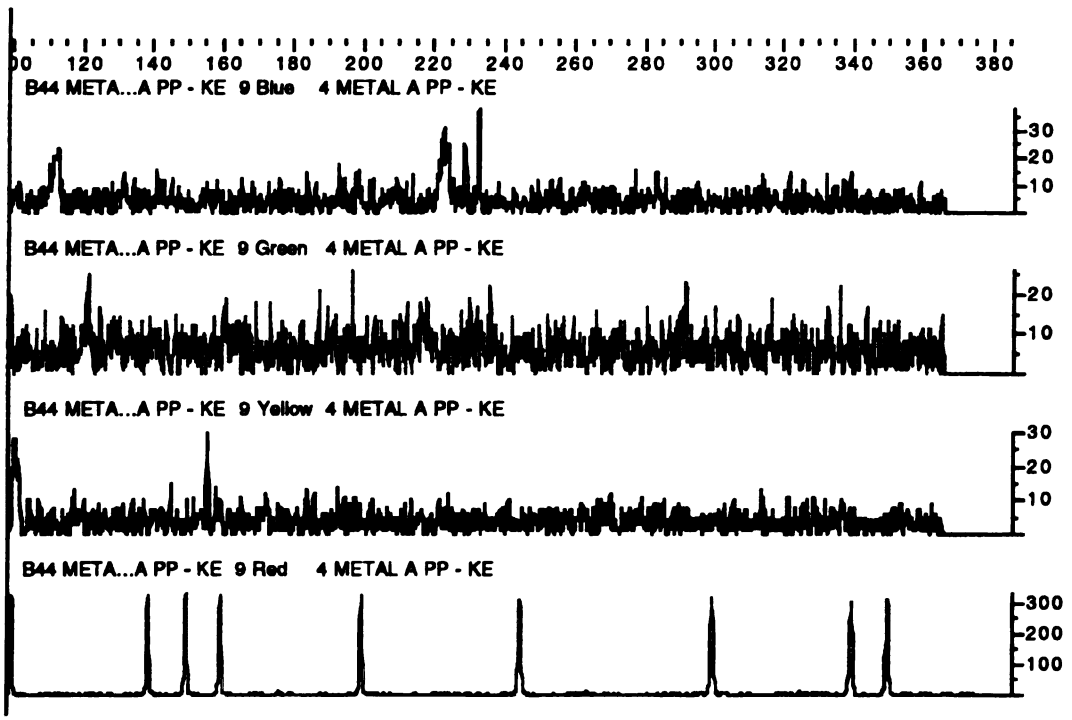
Negative Control Pipe Bomb 2 PVC B

Post-deflagration, First 310 Run



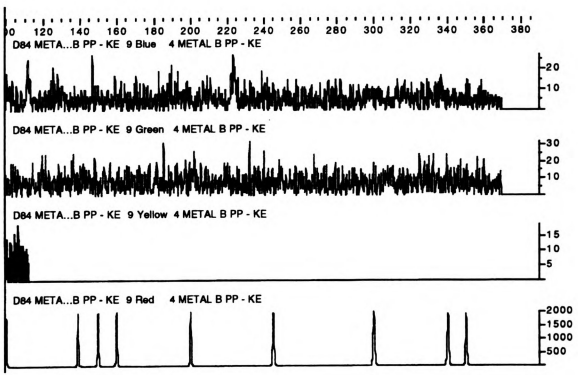
Negative Control Pipe Bomb 4 Metal A

Pre-deflagration, First 310 Run



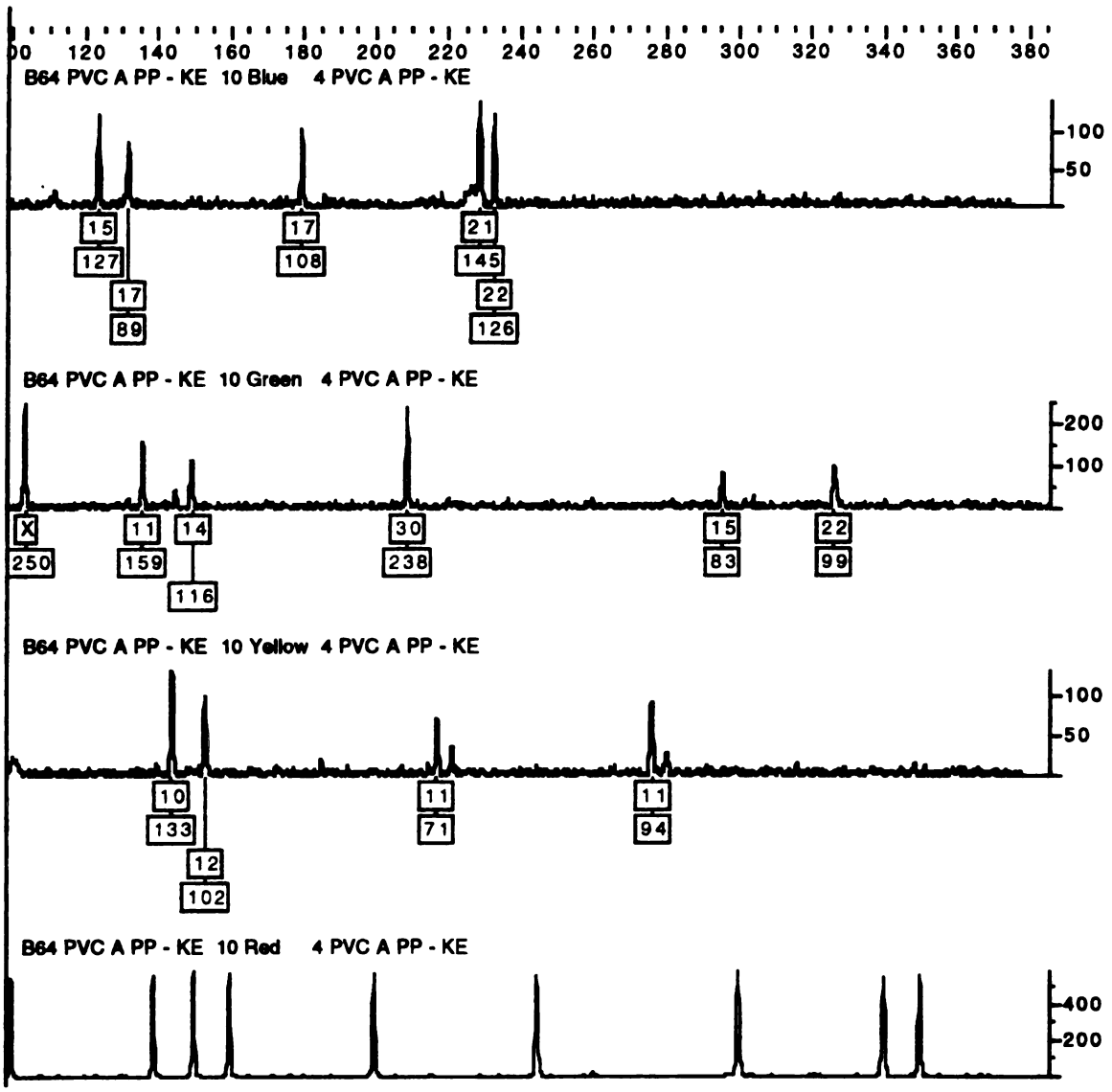
Negative Control Pipe Bomb 4 Metal B

Post-deflagration, First 310 Run



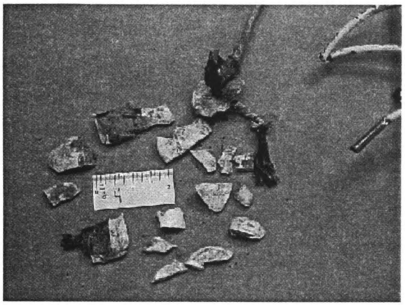
Negative Control Pipe Bomb 4 PVC A

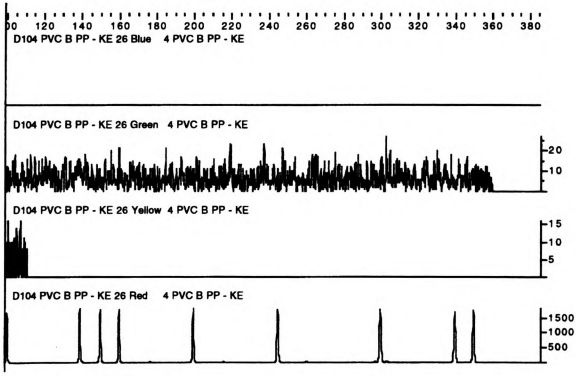
Pre-deflagration, First 310 Run



Negative Control Pipe Bomb 4 PVC B

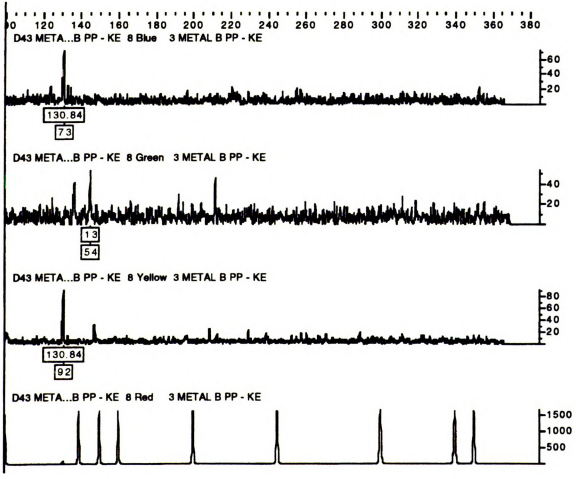
Post-deflagration, First 310 Run





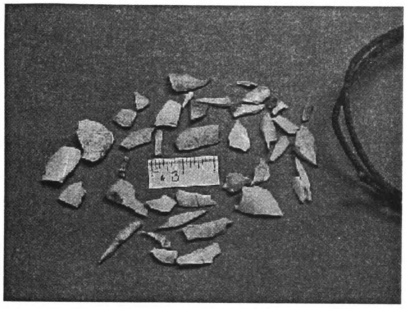
Pipe Bomb 3 Metal B

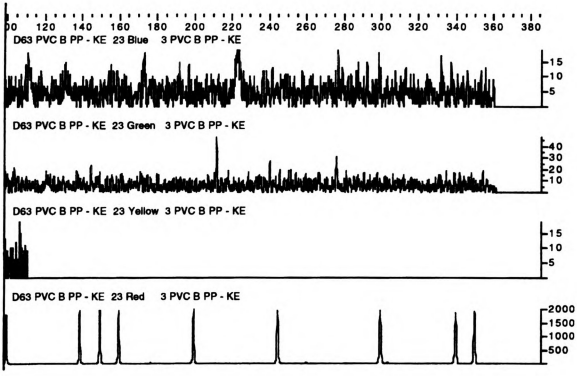
Post-deflagration, First 310 Run



Pipe Bomb 3 PVC B

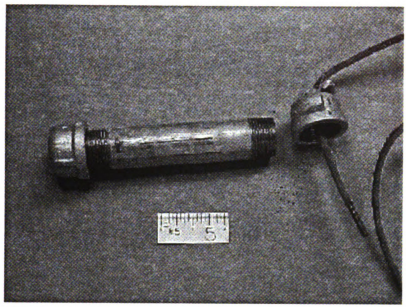
Post-deflagration, First 310 Run

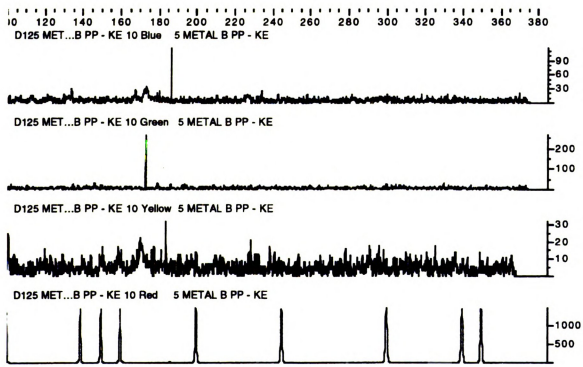




Pipe Bomb 5 Metal B

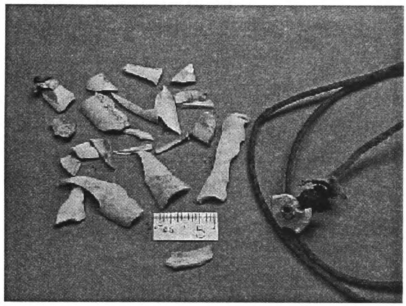
Post-deflagration, First 310 Run

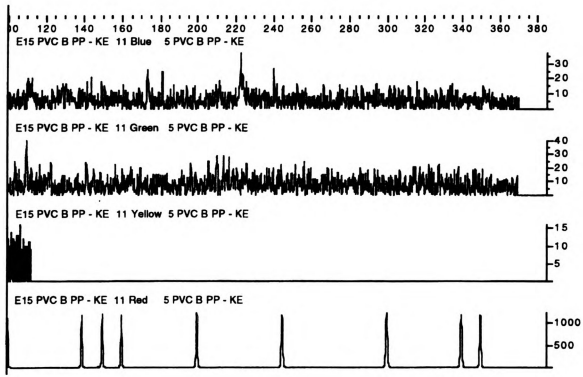




Pipe Bomb 5 PVC B

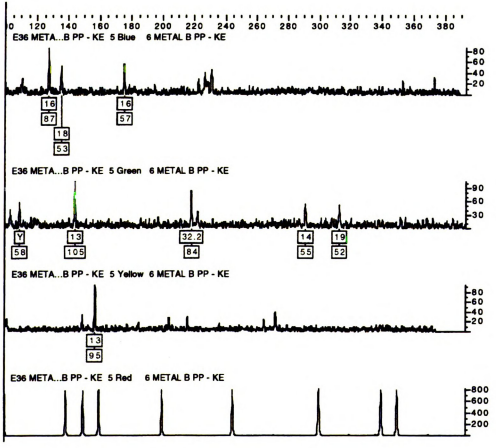
Post-deflagration, First 310 Run





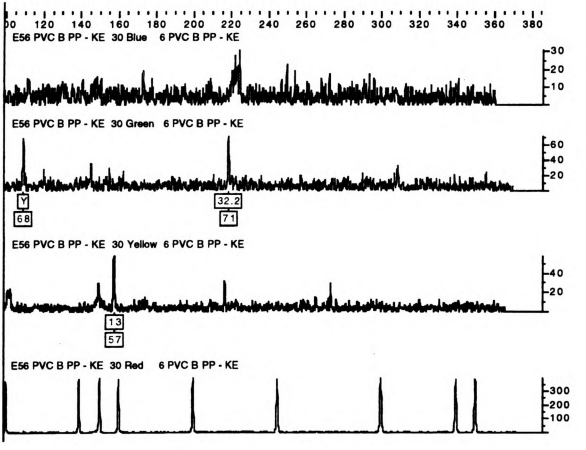
Pipe Bomb 6 Metal B

Post-deflagration, First 310 Run



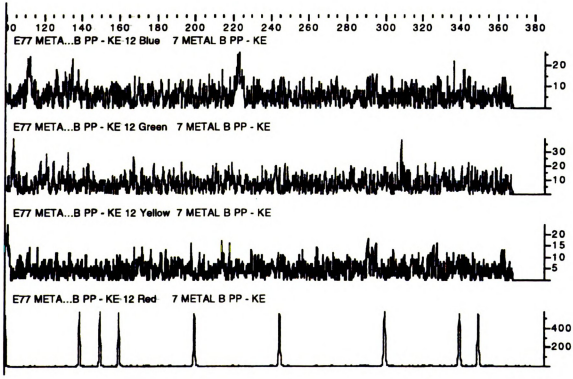
Pipe Bomb 6 PVC B

Post-deflagration, First 310 Run



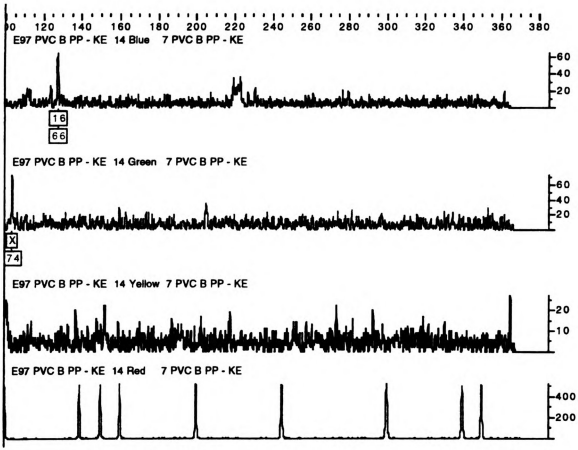
Pipe Bomb 7 Metal B

Post-deflagration, First 310 Run



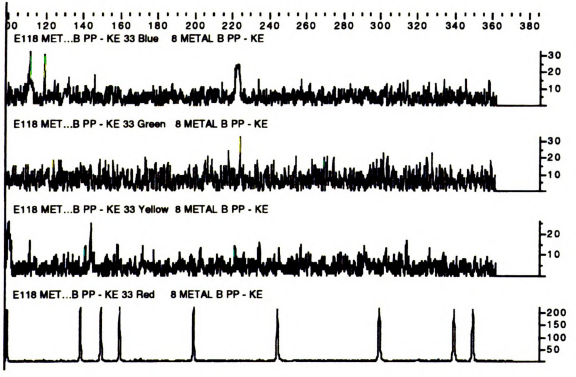
Pipe Bomb 7 PVC B

Post-deflagration, First 310 Run



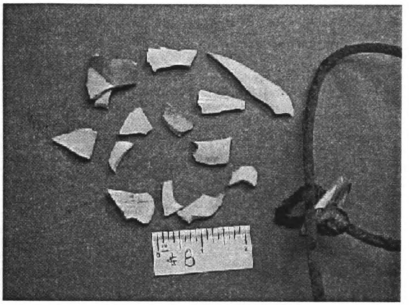
Pipe Bomb 8 Metal B

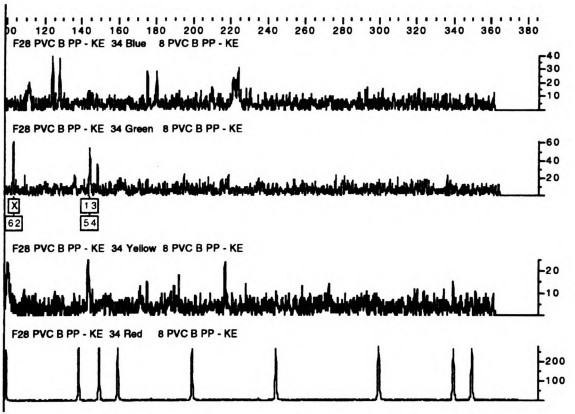
Post-deflagration, First 310 Run



Pipe Bomb 8 PVC B

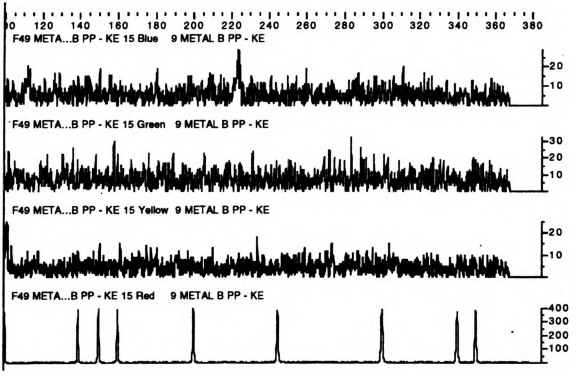
Post-deflagration, First 310 Run





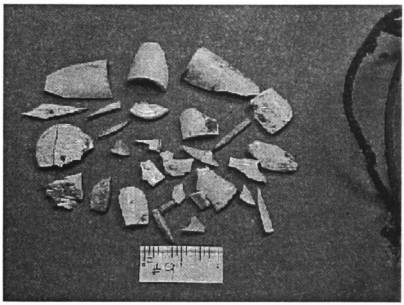
Pipe Bomb 9 Metal B

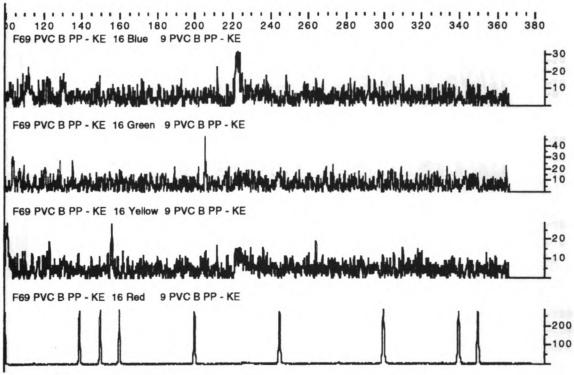
Post-deflagration, First 310 Run



Pipe Bomb 9 PVC B

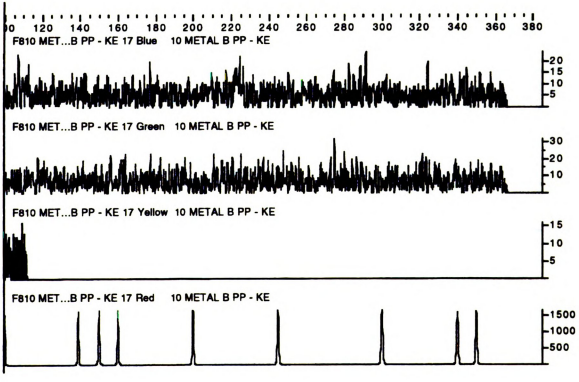
Post-deflagration, First 310 Run





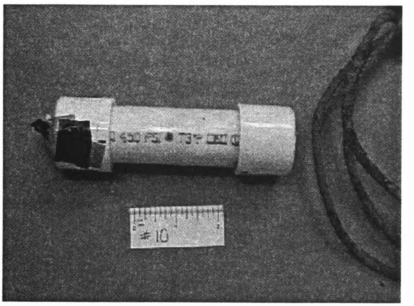
Pipe Bomb 10 Metal B

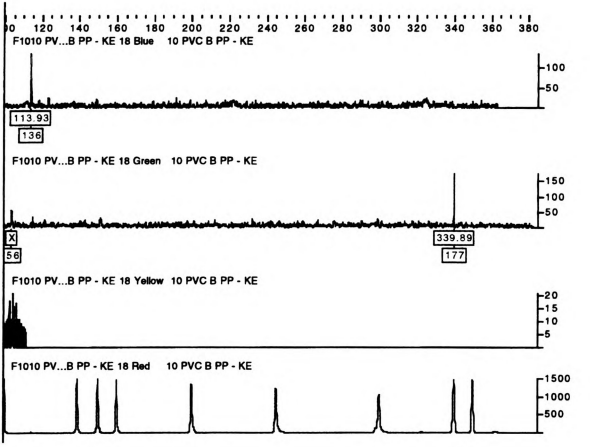
Post-deflagration, First 310 Run



Pipe Bomb 10 PVC B

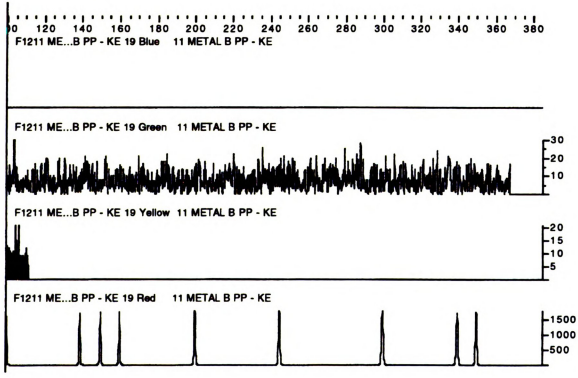
Post-deflagration, First 310 Run





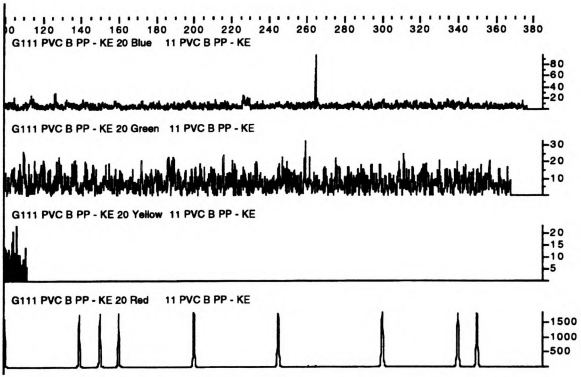
Pipe Bomb 11 Metal B

Post-deflagration, First 310 Run



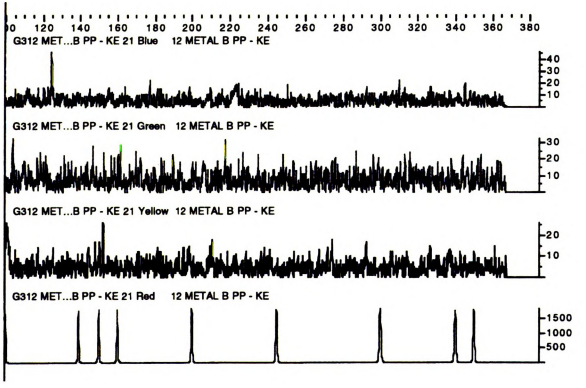
Pipe Bomb 11 PVC B

Post-deflagration, First 310 Run



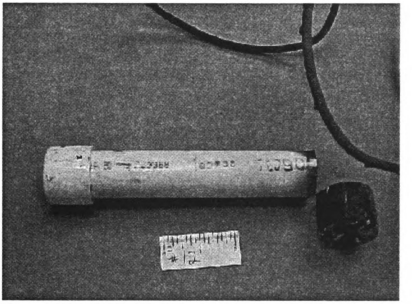
Pipe Bomb 12 Metal B

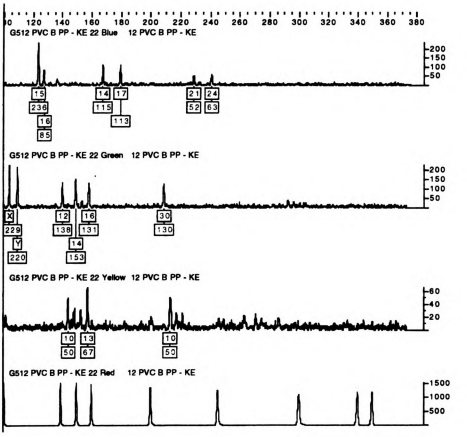
Post-deflagration, First 310 Run



Pipe Bomb 12 PVC B

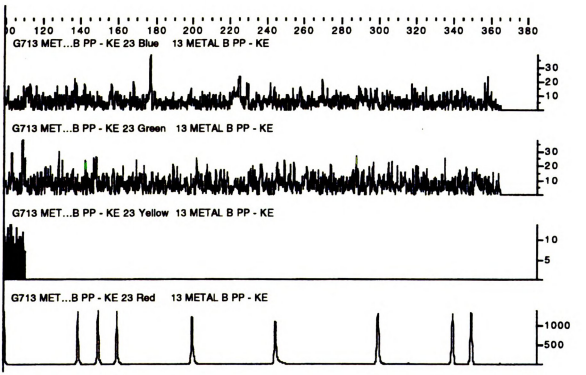
Post-deflagration, First 310 Run





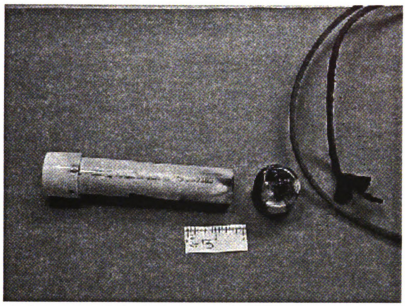
Pipe Bomb 13 Metal B

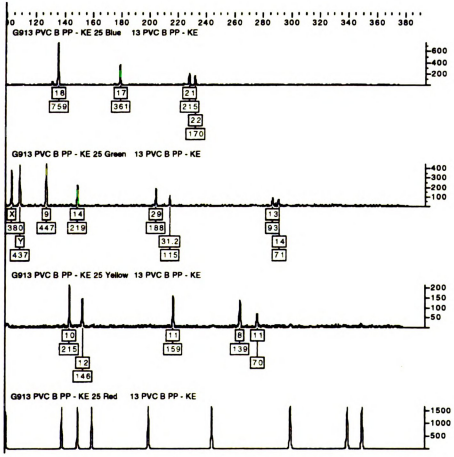
Post-deflagration, First 310 Run



Pipe Bomb 13 PVC B

Post-deflagration, First 310 Run



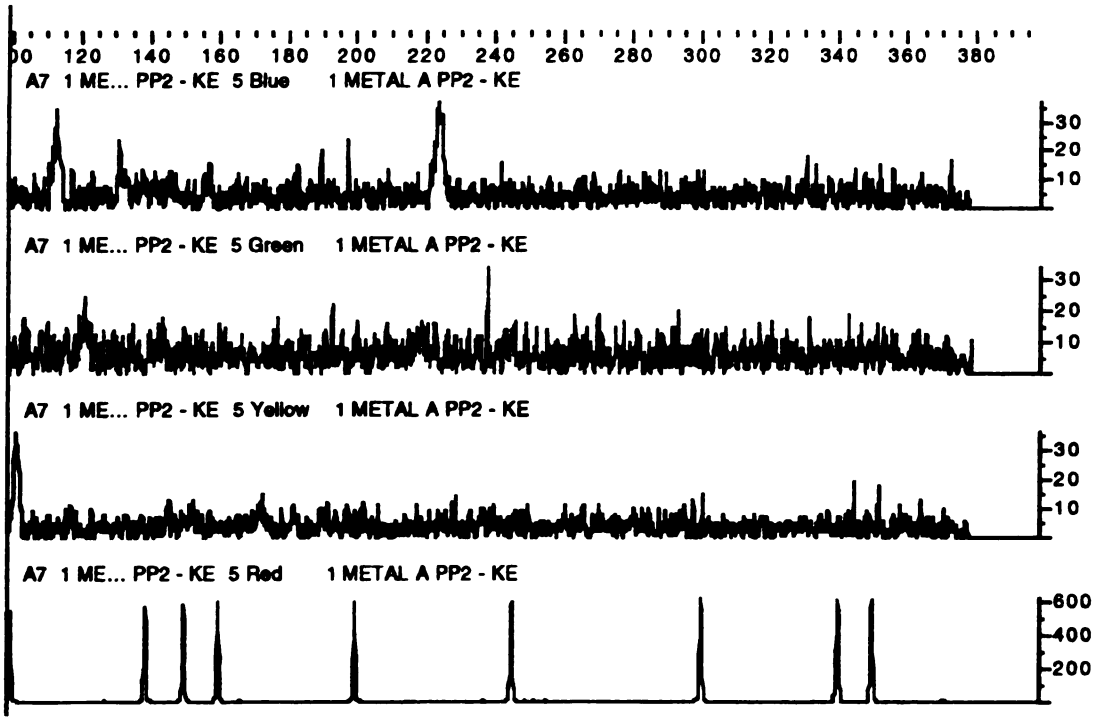


APPENDIX B

Appendix B contains electropherograms generated from concentrated DNA extracts (Second 310 Run) and the corresponding photographs of the recovered pipe bomb fragments.

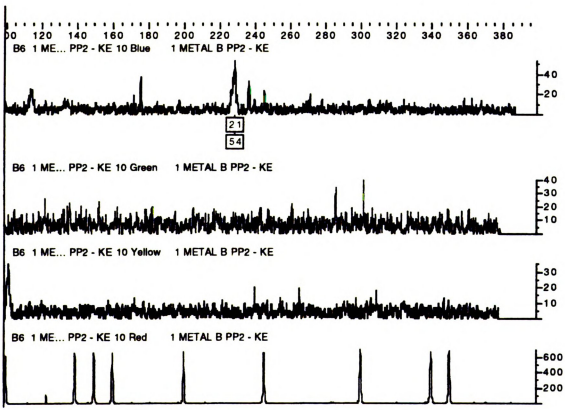
Negative Control Pipe Bomb 1 Metal A

Pre-deflagration, Second 310 Run



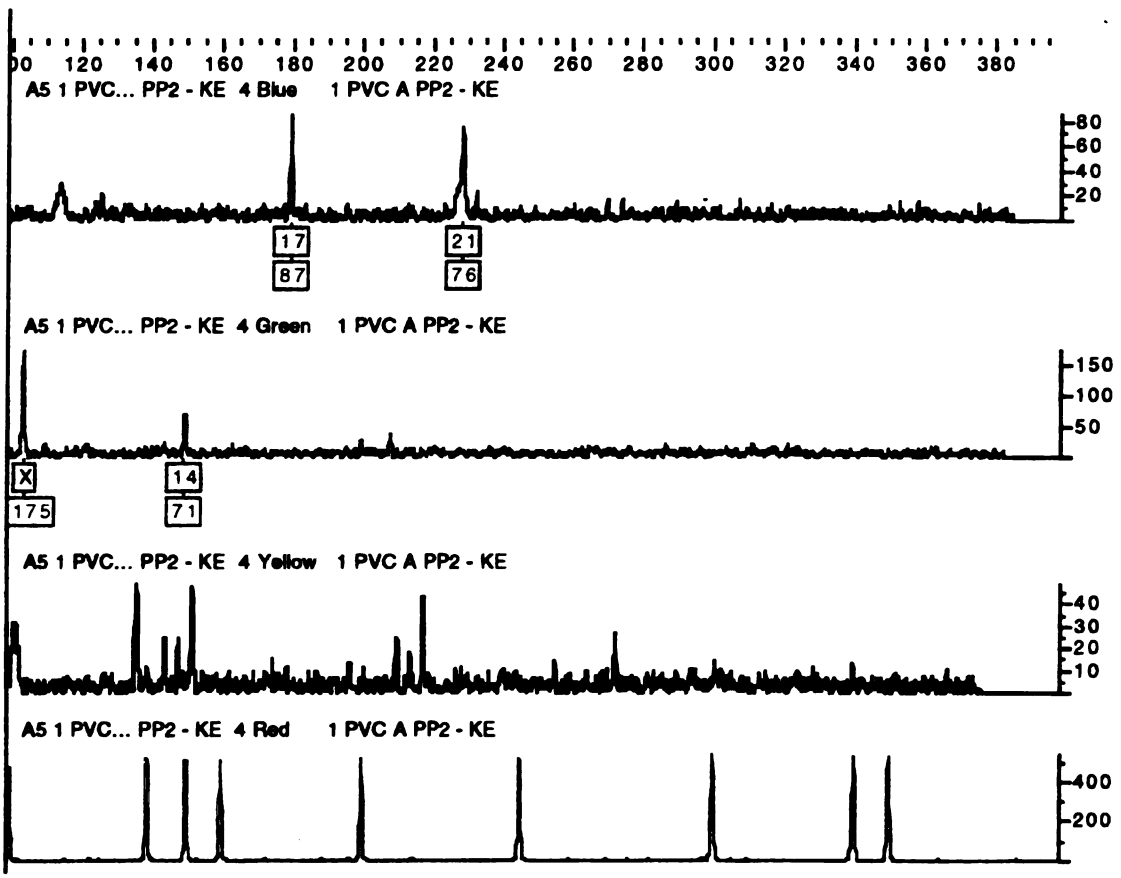
Negative Control Pipe Bomb 1 Metal B

Post-deflagration, Second 310 Run



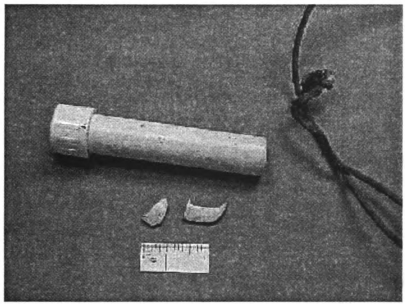
Negative Control Pipe Bomb 1 PVC A

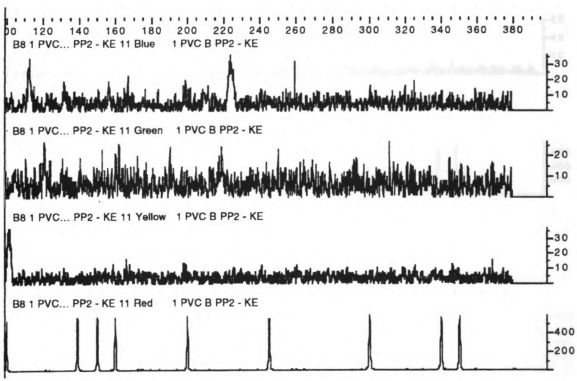
Pre-deflagration, Second 310 Run



Negative Control Pipe Bomb 1 PVC B

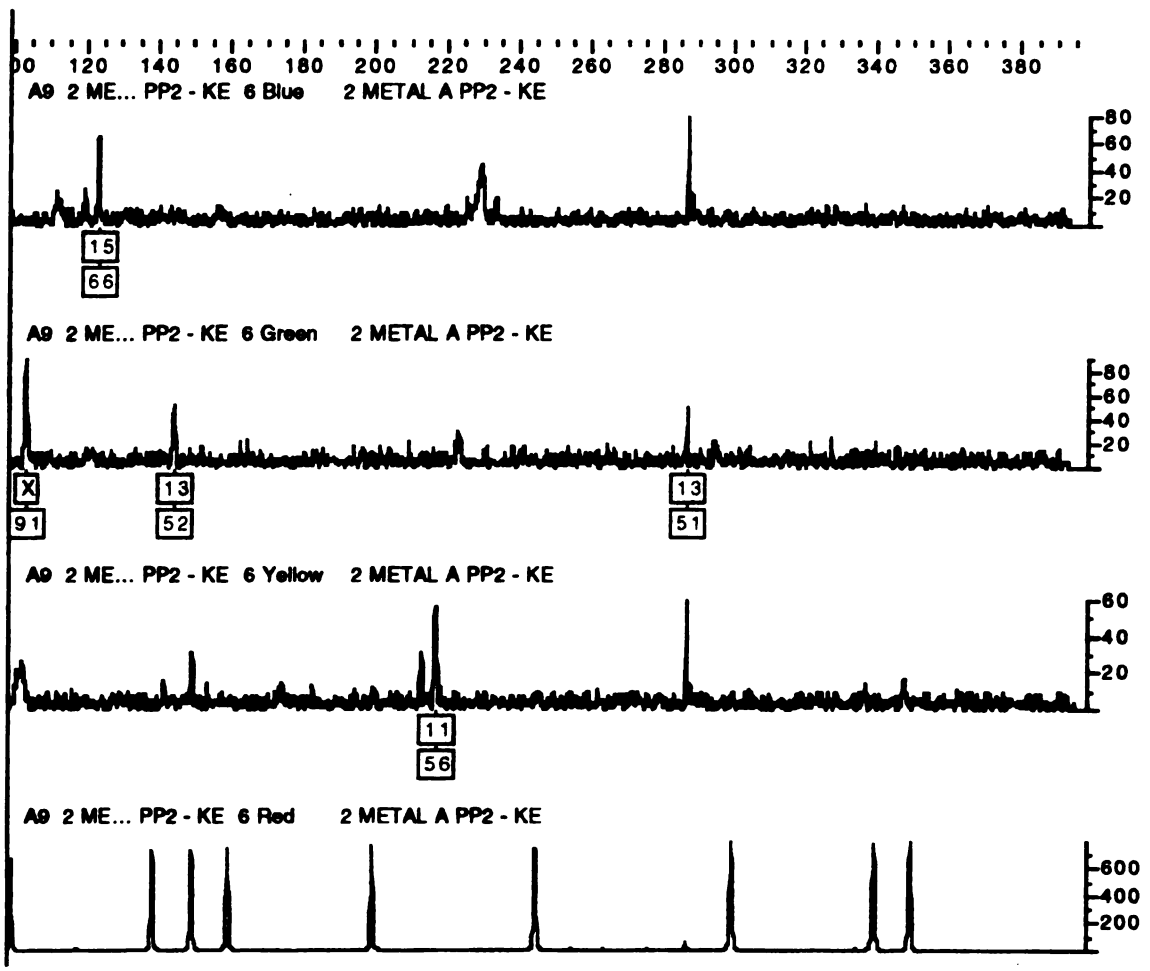
Post-deflagration, Second 310 Run





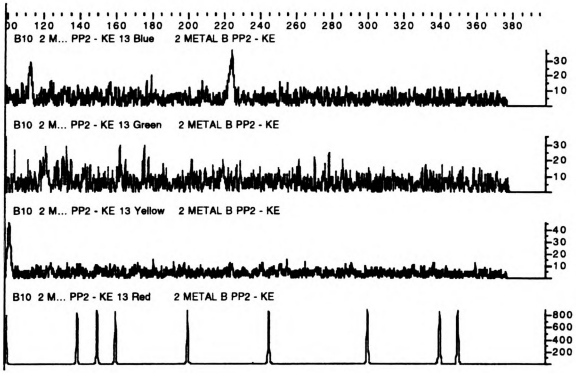
Negative Control Pipe Bomb 2 Metal A

Pre-deflagration, Second 310 Run



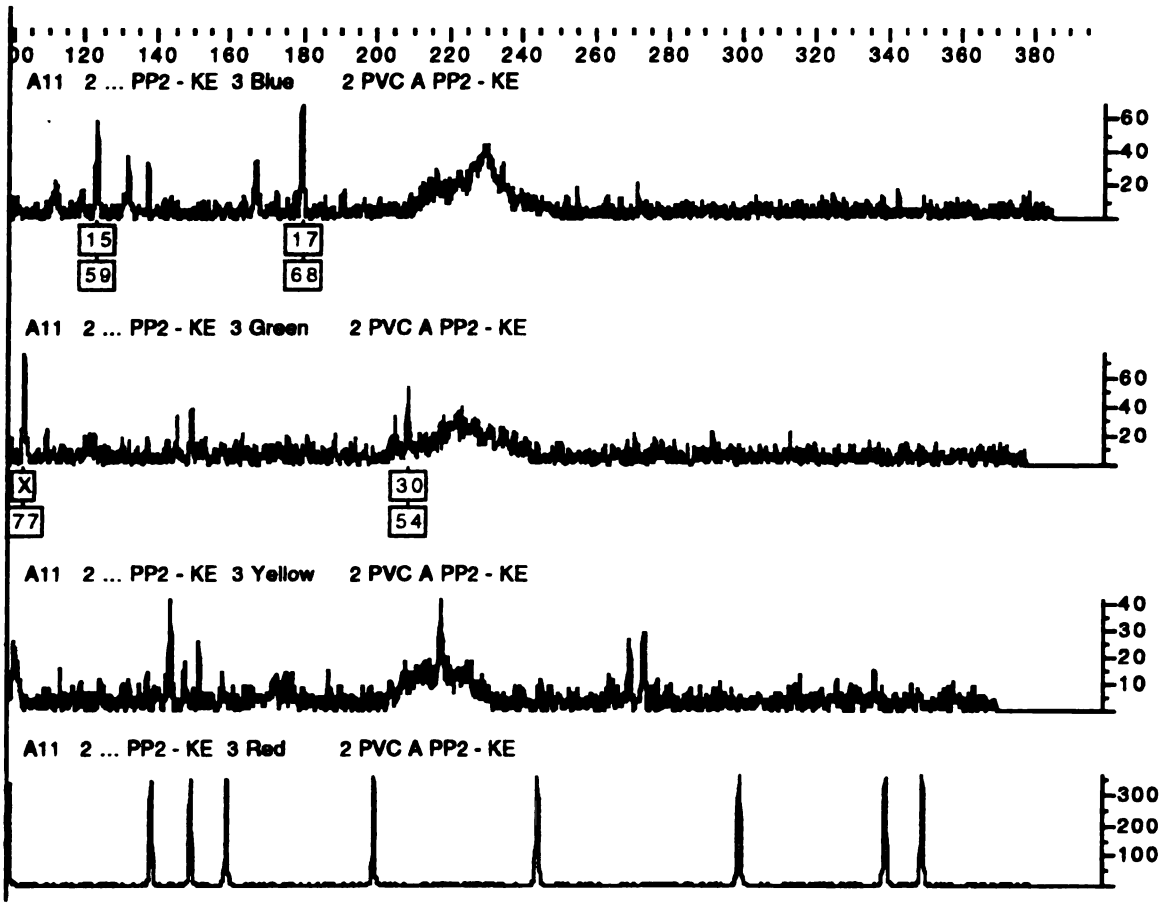
Negative Control Pipe Bomb 2 Metal B

Post-deflagration, Second 310 Run



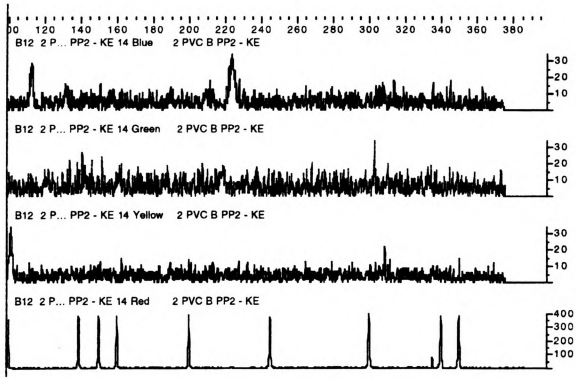
Negative Control Pipe Bomb 2 PVC A

Pre-deflagration, Second 310 Run



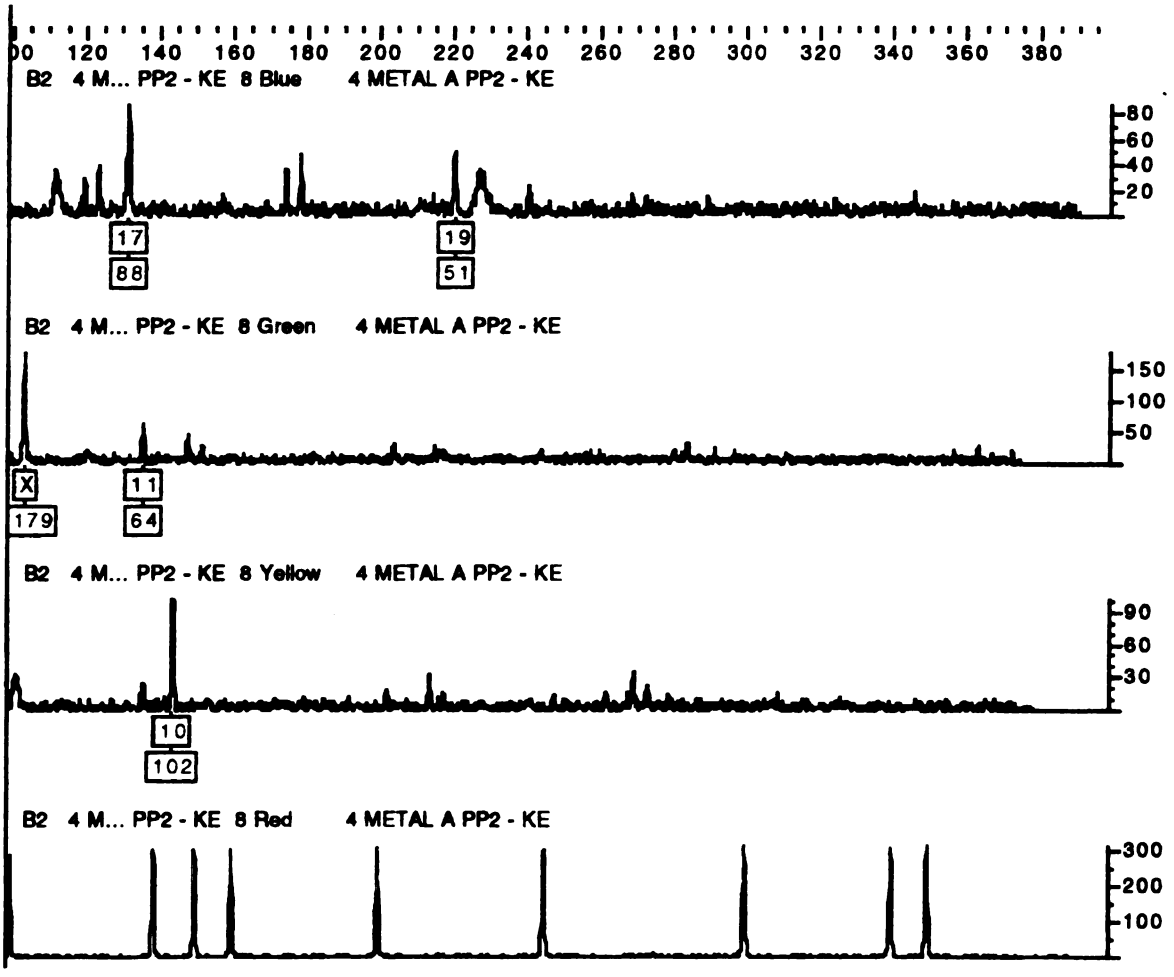
Negative Control Pipe Bomb 2 PVC B

Post-deflagration, Second 310 Run



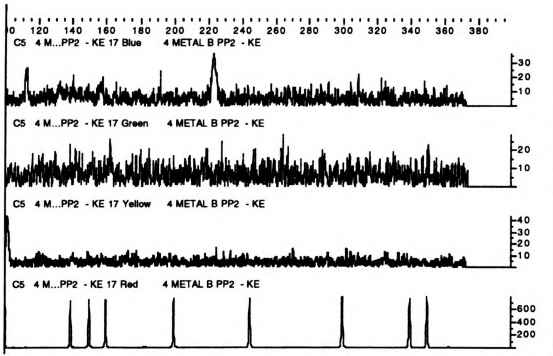
Negative Control Pipe Bomb 4 Metal A

Pre-deflagration, Second 310 Run



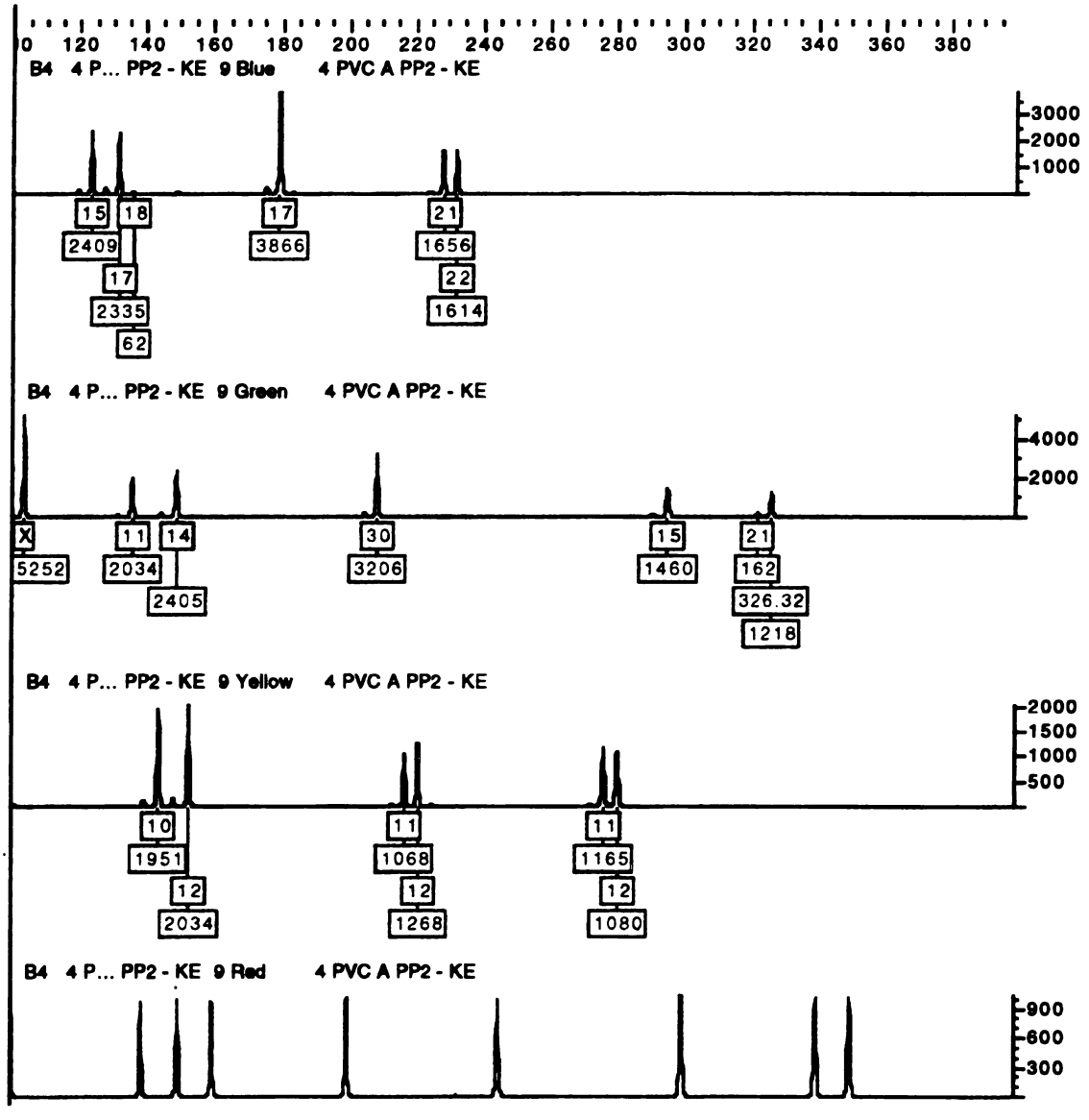
Negative Control Pipe Bomb 4 Metal B

Post-deflagration, Second 310 Run

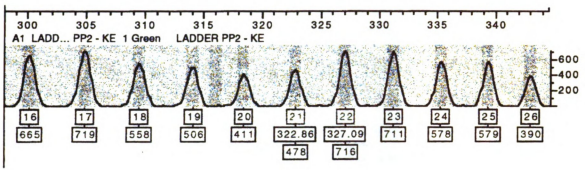


Negative Control Pipe Bomb 4 PVC A

Pre-deflagration, Second 310 Run

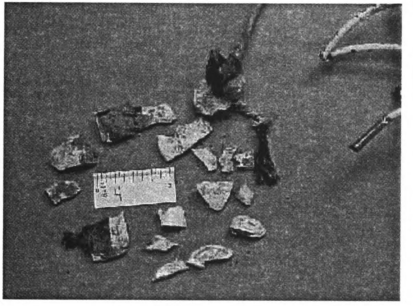


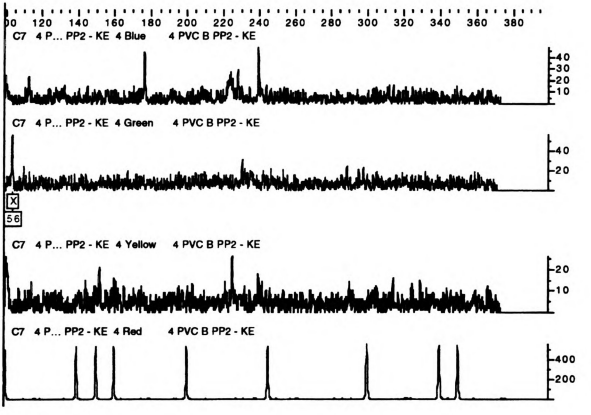
D 21 LADDER


300
305
310
315
320
325
330
335
340
A1 LADD... PP2 - KE 1 Green
LADDER PP2 - KE
600
400
200
16
665
17
719
18
558
19
506
20
411
21
322.86
478
22
327.09
716
23
711
24
578
25
579
26
390

Negative Control Pipe Bomb 4 PVC B

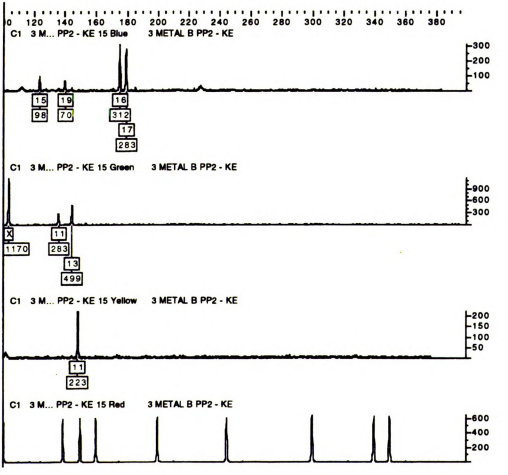
Post-deflagration, Second 310 Run





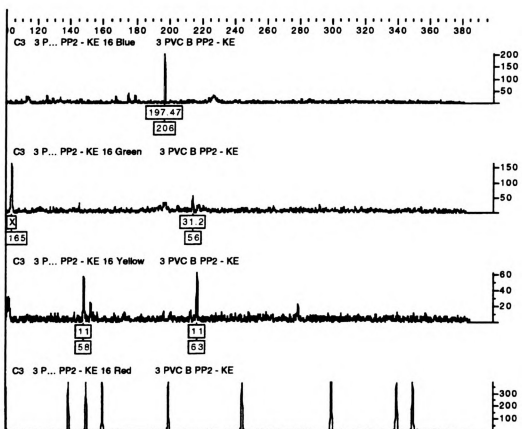
Pipe Bomb 3 Metal B

Post-deflagration, Second 310 Run



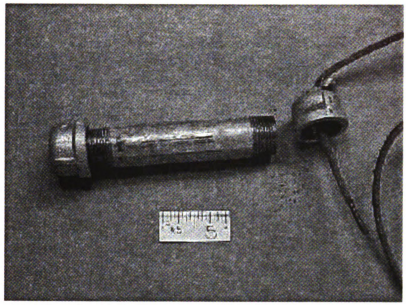
Pipe Bomb 3 PVC B

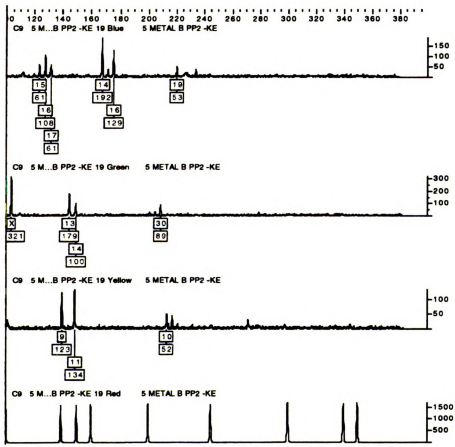
Post-deflagration, Second 310 Run



Pipe Bomb 5 Metal B

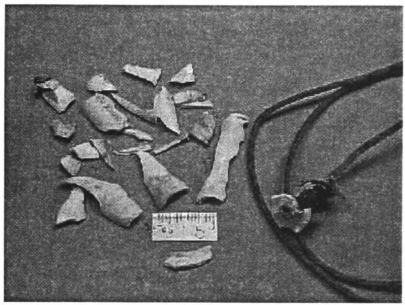
Post-deflagration, Second 310 Run

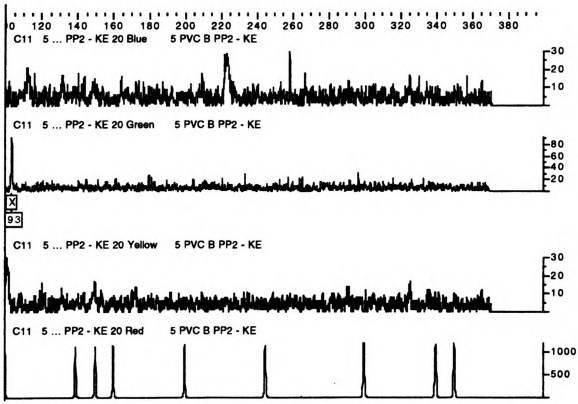




Pipe Bomb 5 PVC B

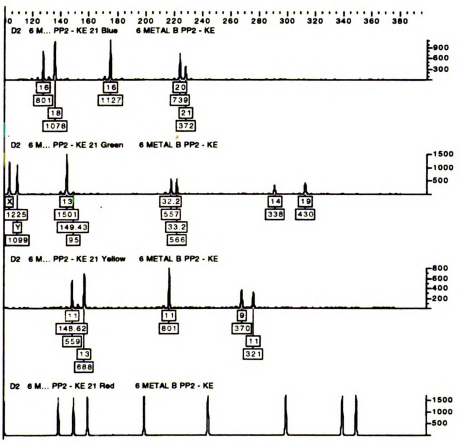
Post-deflagration, Second 310 Run





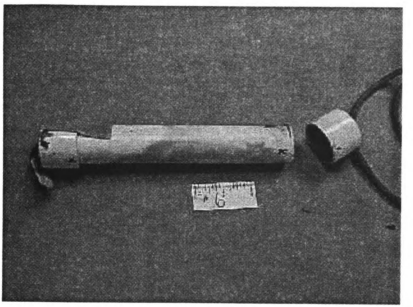
Pipe Bomb 6 Metal B

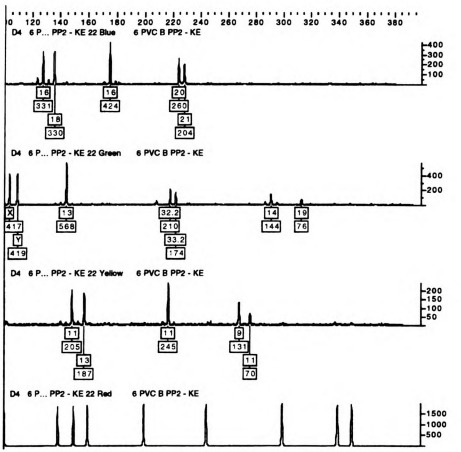
Post-deflagration, Second 310 Run



Pipe Bomb 6 PVC B

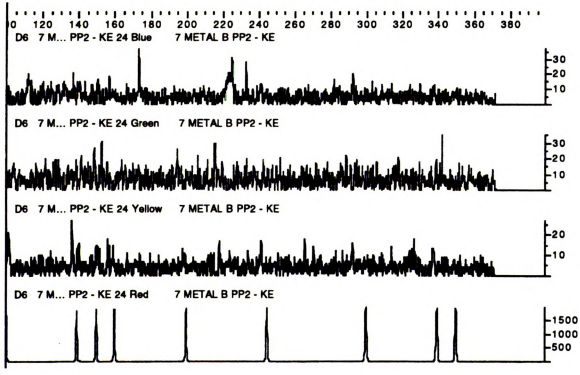
Post-deflagration, Second 310 Run





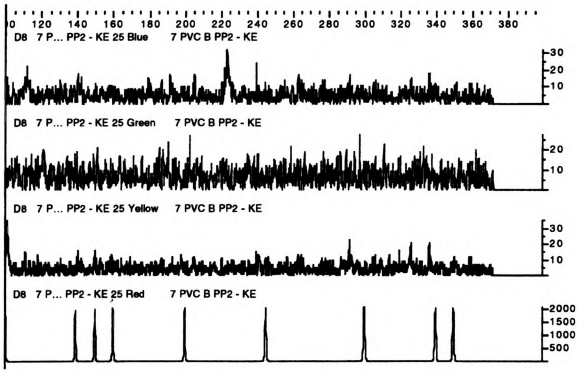
Pipe Bomb 7 Metal B

Post-deflagration, Second 310 Run



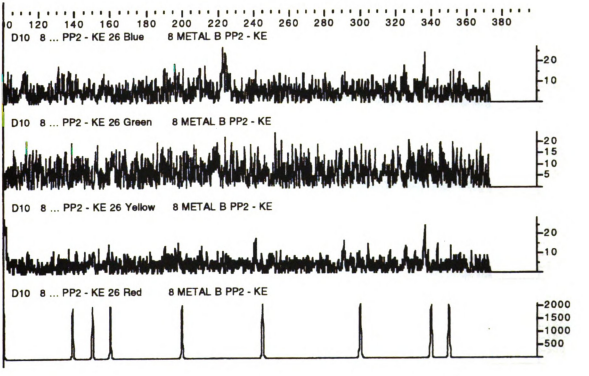
Pipe Bomb 7 PVC B

Post-deflagration, Second 310 Run



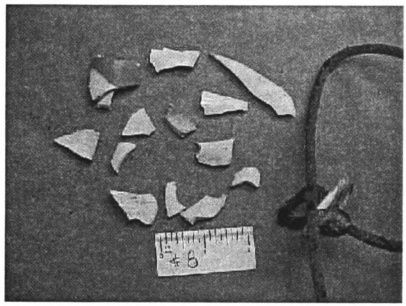
Pipe Bomb 8 Metal B

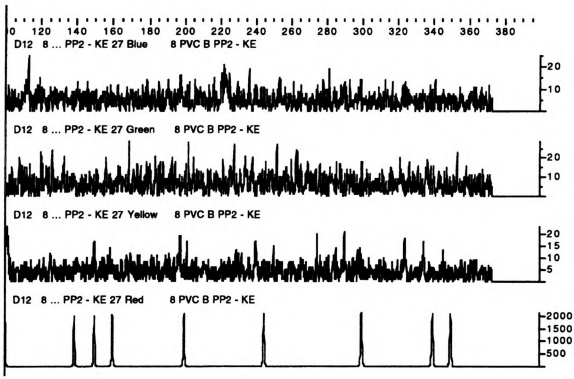
Post-deflagration, Second 310 Run



Pipe Bomb 8 PVC B

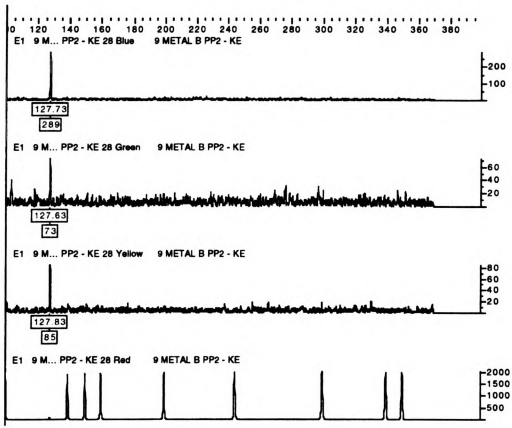
Post-deflagration, Second 310 Run





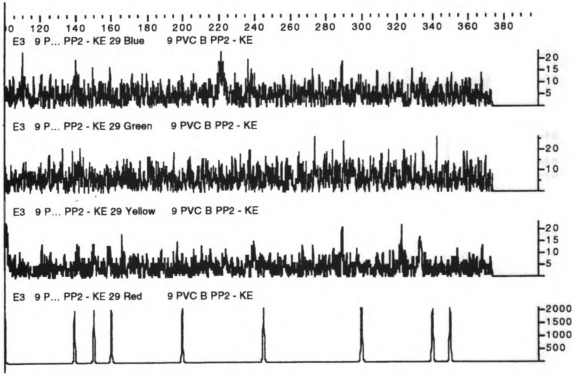
Pipe Bomb 9 Metal B

Post-deflagration, Second 310 Run



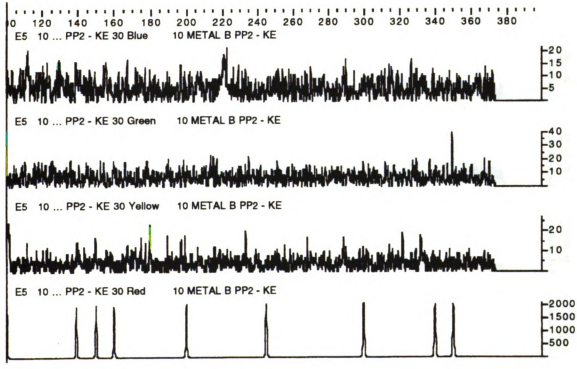
Pipe Bomb 9 PVC B

Post-deflagration, Second 310 Run



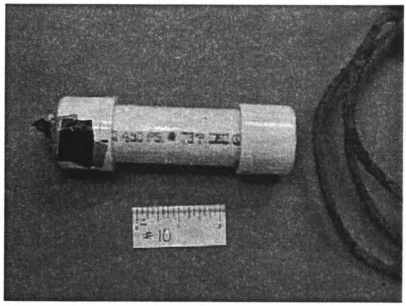
Pipe Bomb 10 Metal B

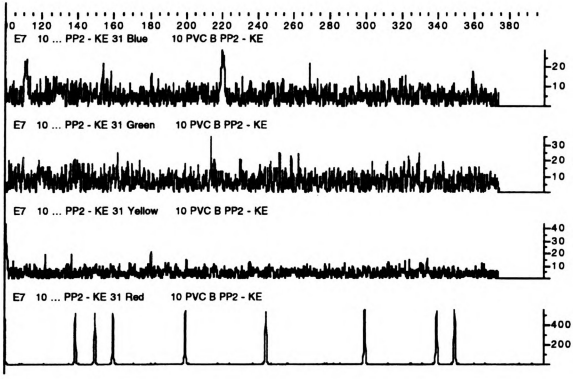
Post-deflagration, Second 310 Run



Pipe Bomb 10 PVC B

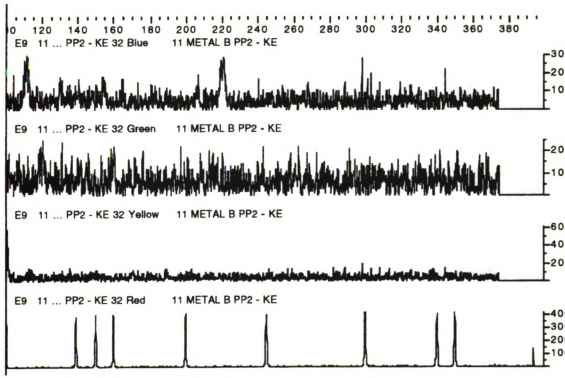
Post-deflagration, Second 310 Run





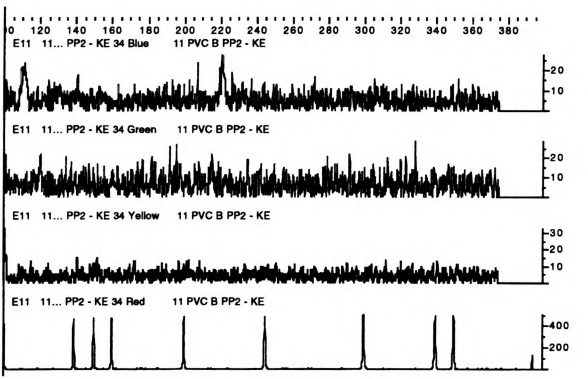
Pipe Bomb 11 Metal B

Post-deflagration, Second 310 Run



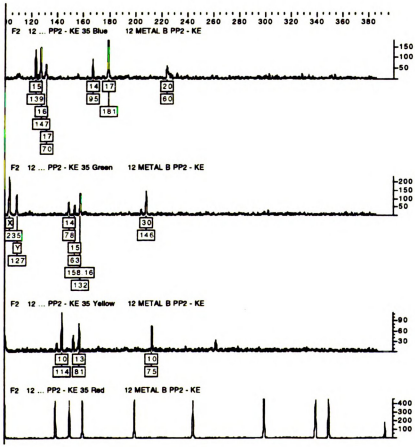
Pipe Bomb 11 PVC B

Post-deflagration, Second 310 Run

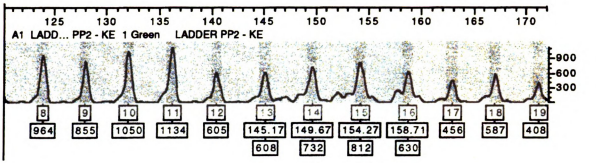


Pipe Bomb 12 Metal B

Post-deflagration, Second 310 Run

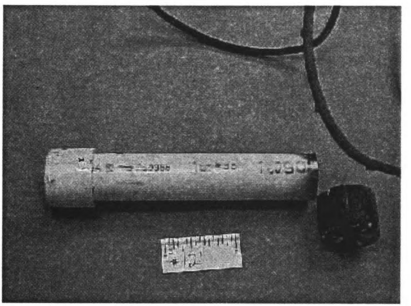


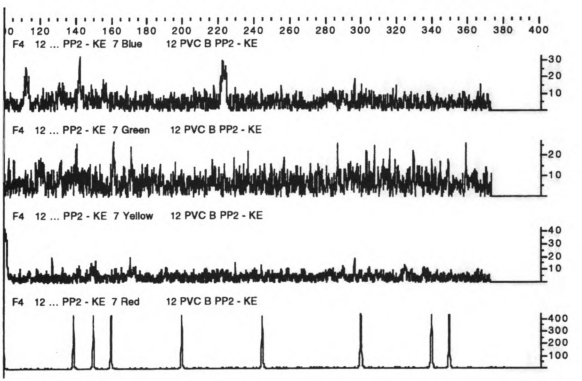
D8 LADDER


125
130
135
140
145
150
155
160
165
170
A1 LADD... PP2 - KE 1 Green
LADDER PP2 - KE
900
600
300
8
964
9
855
10
1050
11
1134
12
605
13
145.17
608
14
149.67
732
15
154.27
812
16
158.71
630
17
456
18
587
19
408

Pipe Bomb 12 PVC B

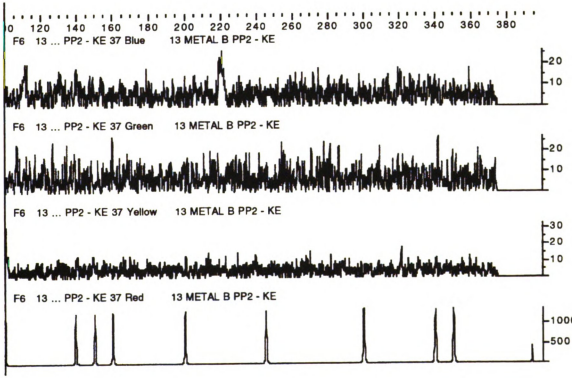
Post-deflagration, Second 310 Run





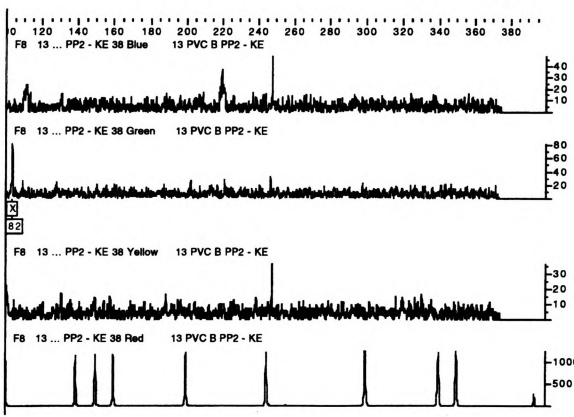
Pipe Bomb 13 Metal B

Post-deflagration, Second 310 Run



Pipe Bomb 13 PVC B

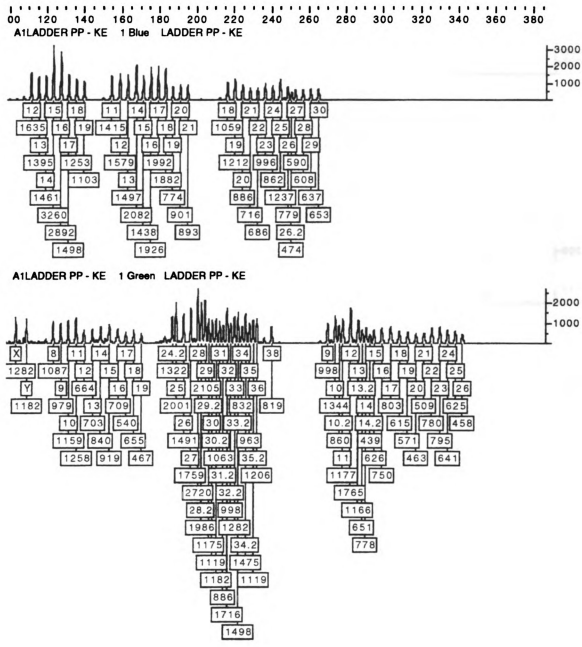
Post-deflagration, Second 310 Run



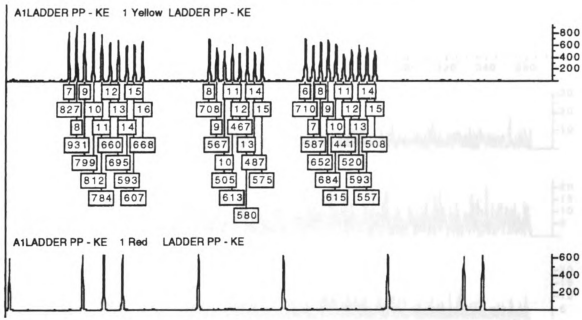
APPENDIX C

Appendix C contains electropherograms generated from Ladders and Control samples from the first and second 310 runs.

LADDER – First 310 Run

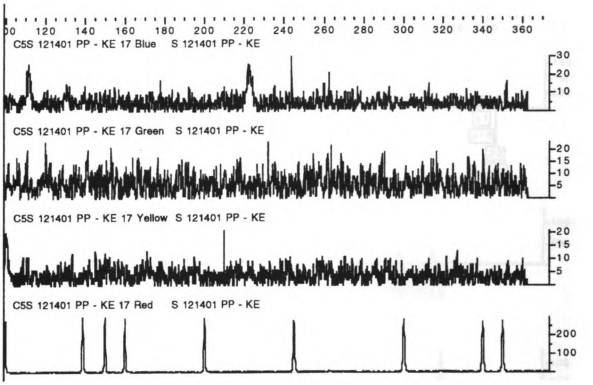


LADDER – First 310 Run (continued)



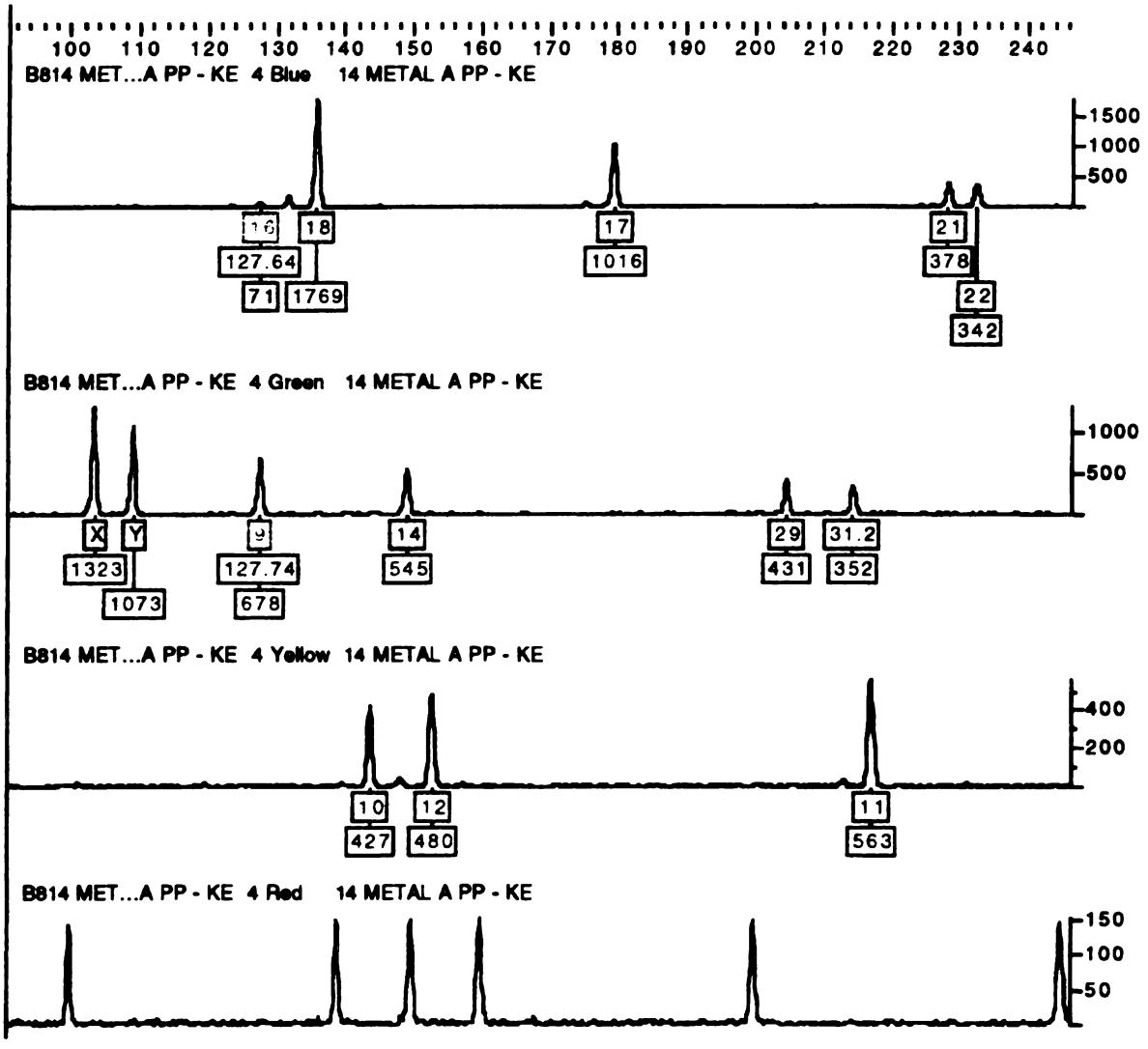
Stain Blank from Unconcentrated DNA Extract

First 310 Run

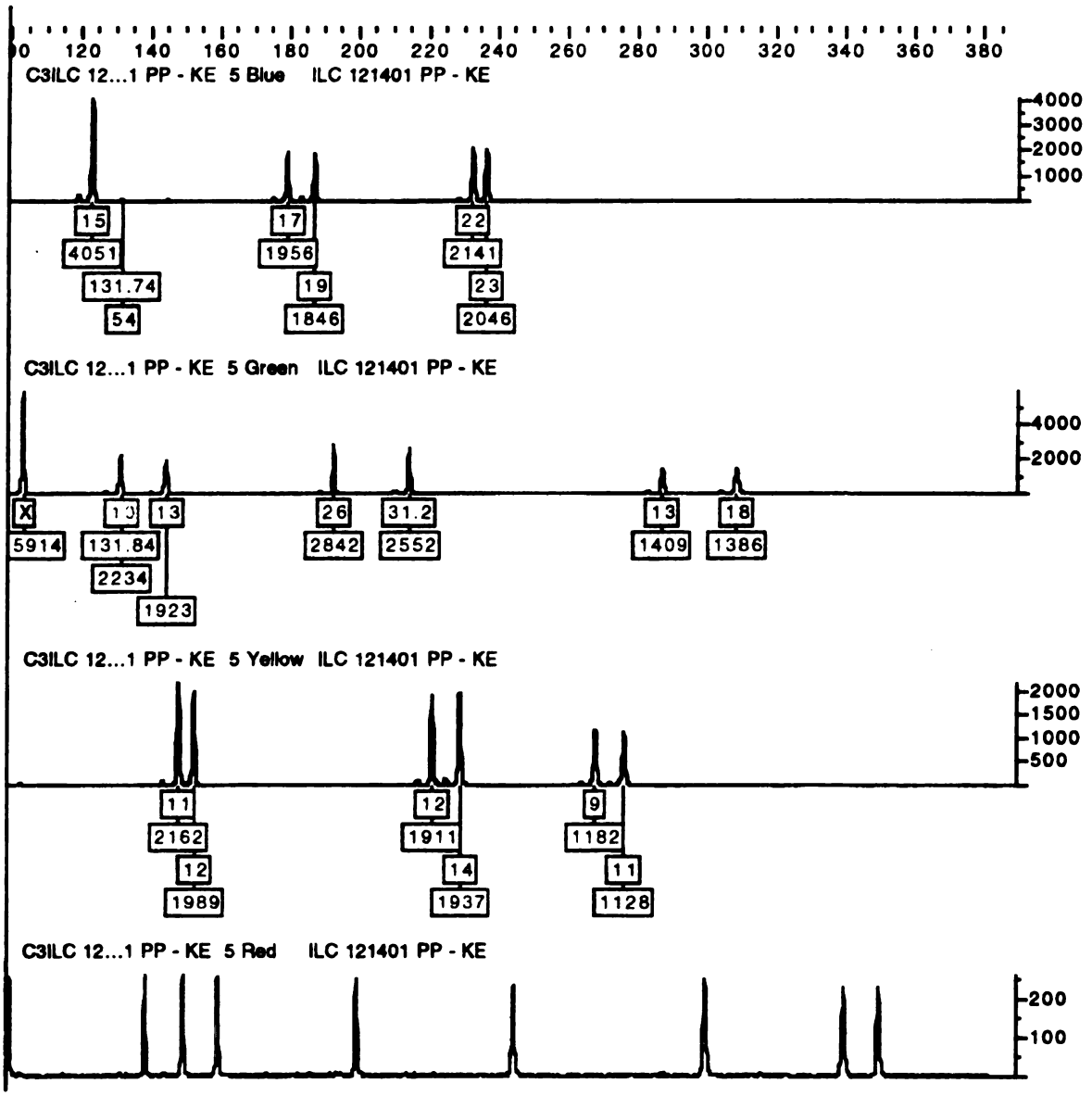


Metal Pipe Positive Control – Subject #14

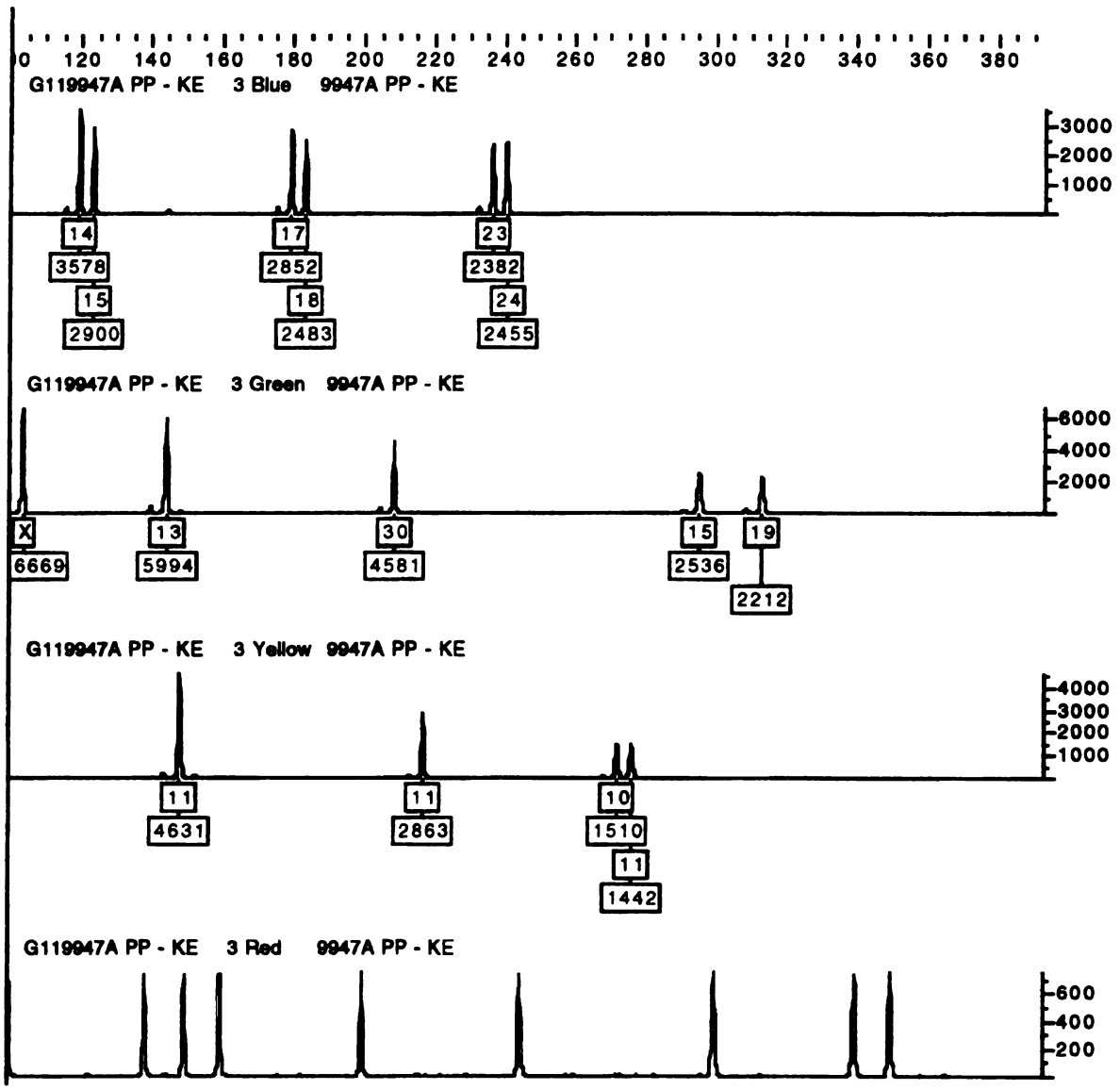
Pre-deflagration



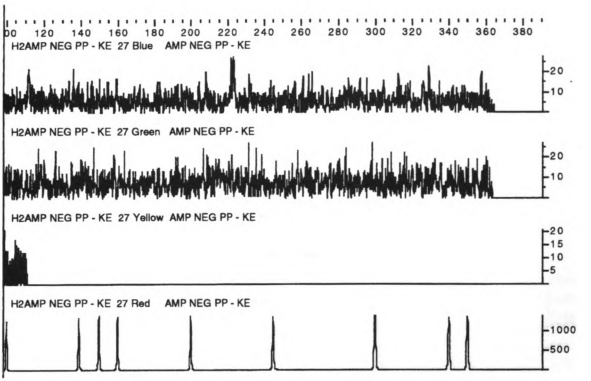
Internal Laboratory Control – First 310 Run



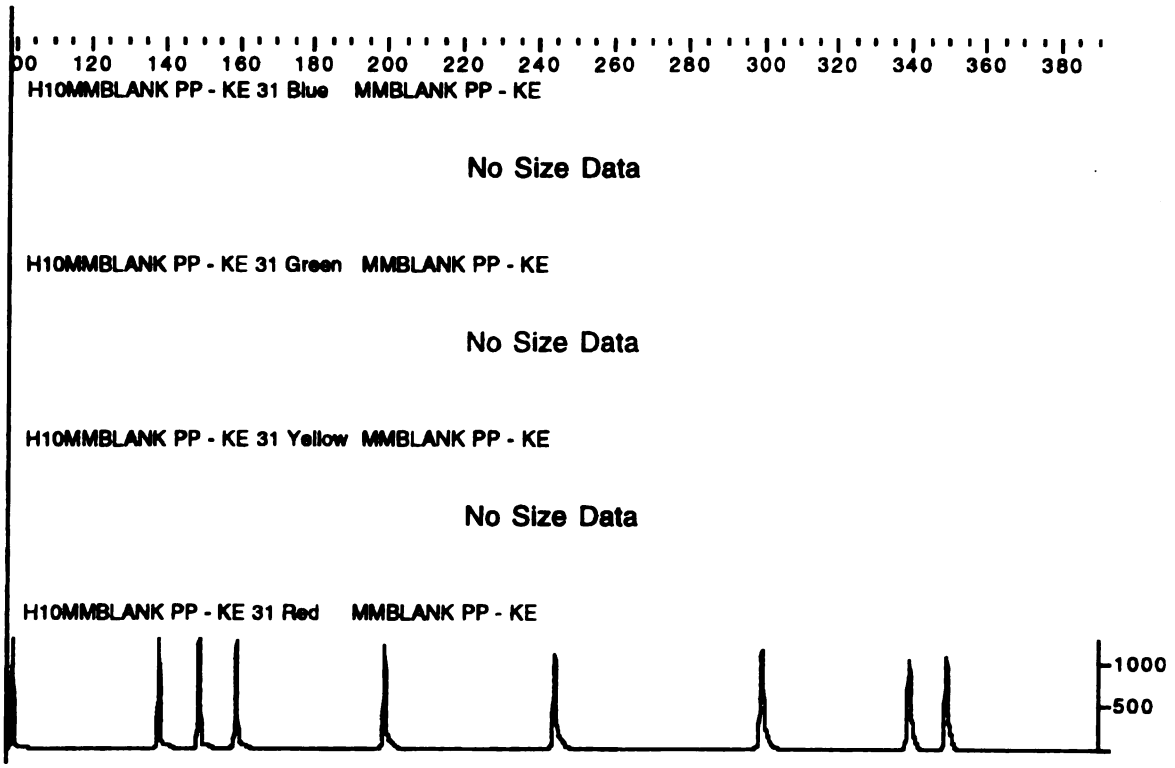
Amplification Positive Control – First 310 Run



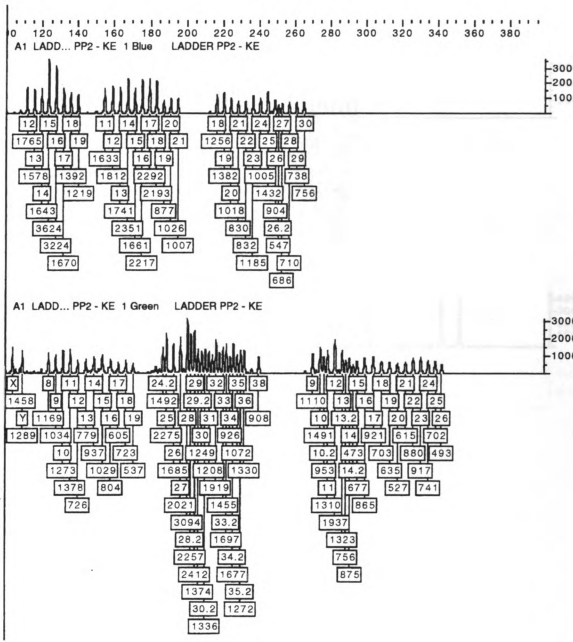
Amplification Negative Control – First 310 Run

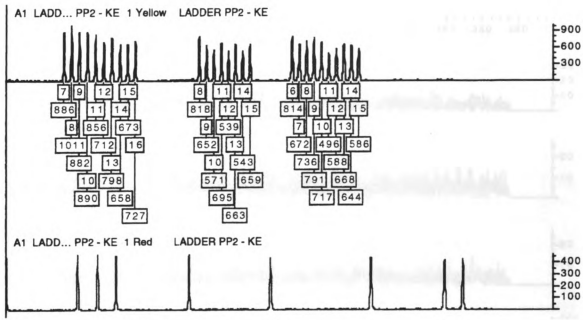


Master Mix Blank – First 310 Run



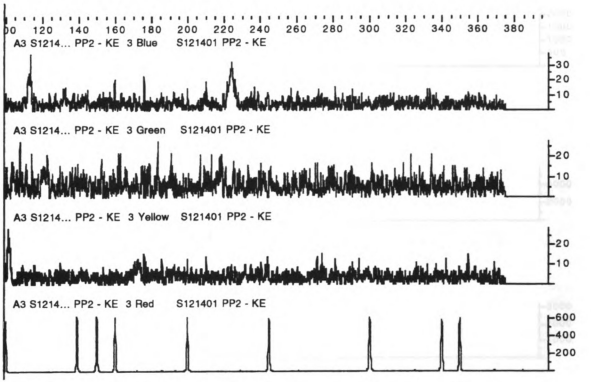
LADDER – Second 310 Run



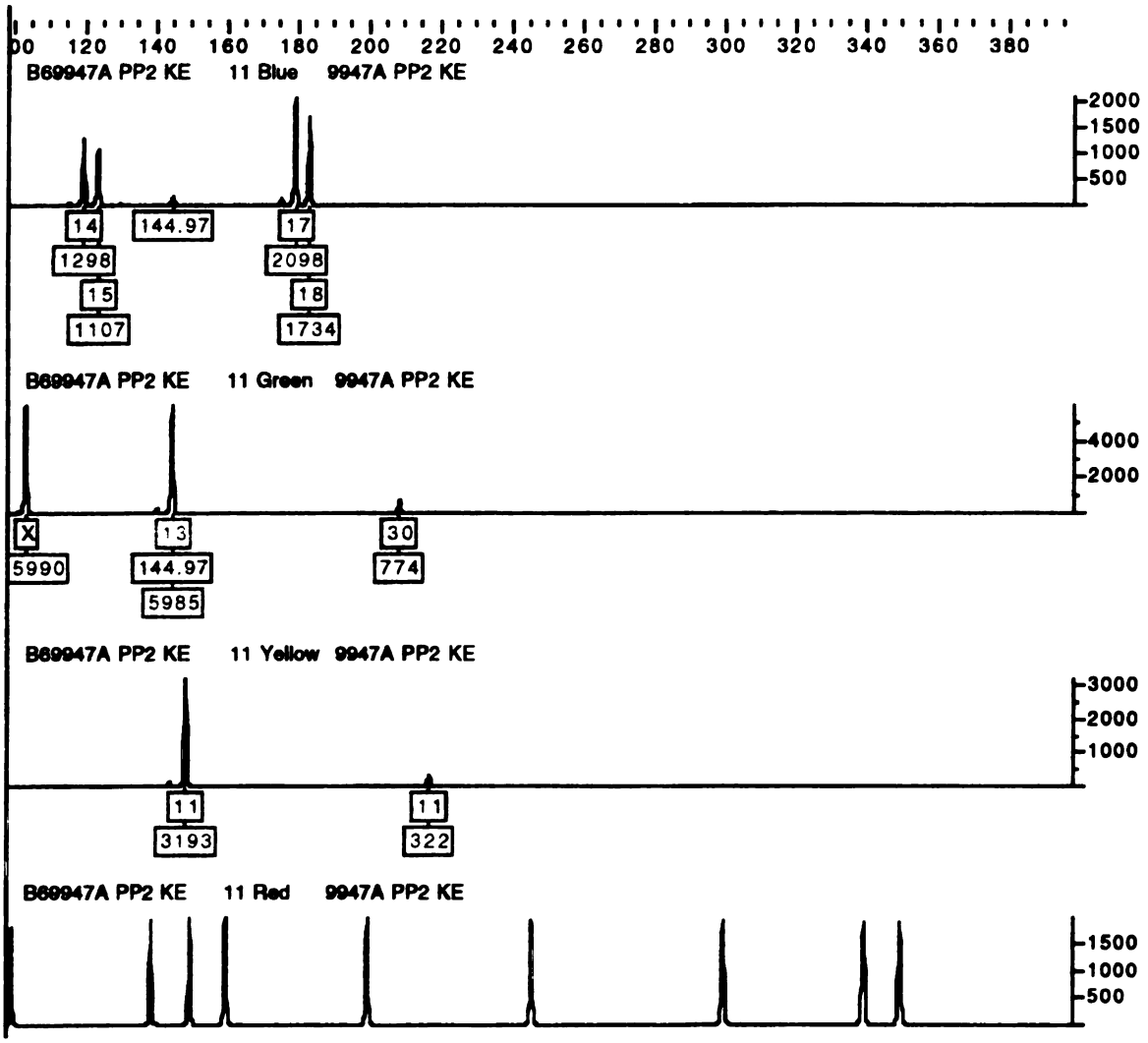

A1 LADD... PP2 - KE 1 Yellow LADDER PP2 - KE
900
600
300
7 9 12 15
886 11 14
8 856 673
1011 712 16
882 13
10 798
890 658
727
8 11 14
818 12 15
9 539
652 13
10 543
571 659
695
663
6 8 11 14
814 9 12 15
7 10 13
672 496 586
736 588
791 668
717 644
A1 LADD... PP2 - KE 1 Red LADDER PP2 - KE
400
300
200
100

Stain Blank from Concentrated DNA Extract

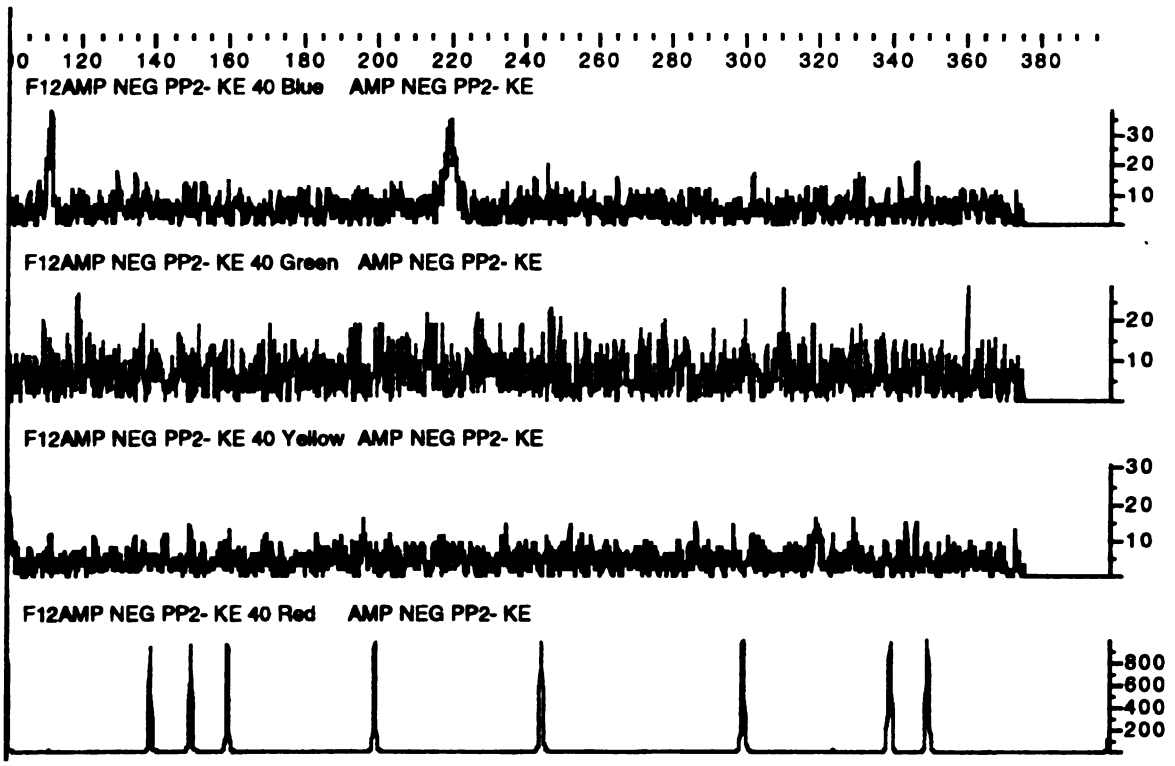
Second 310 Run



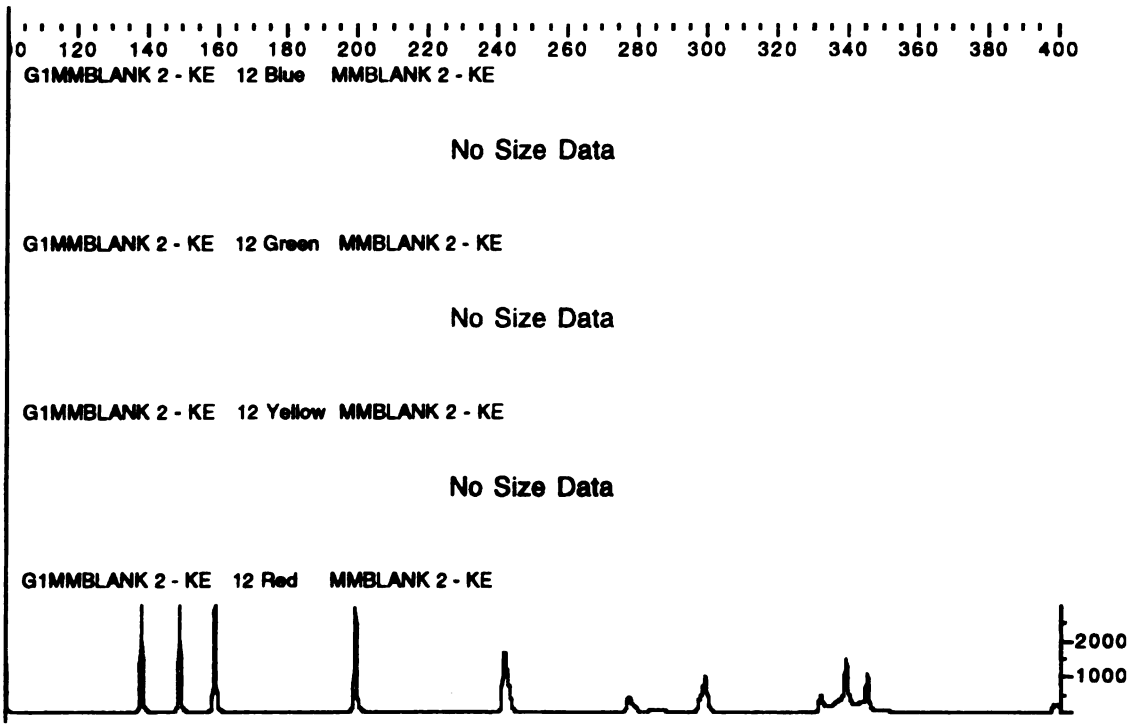
Amplification Positive Control – Second 310 Run



Amplification Negative Control – Second 310 Run



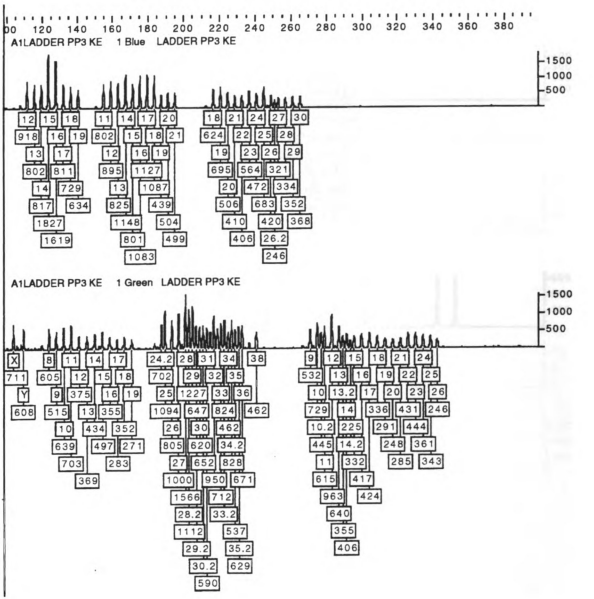
Master Mix Blank – Second 310 Run

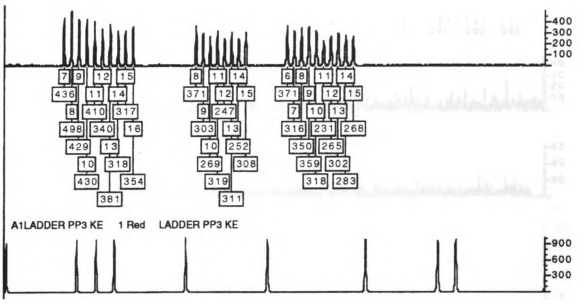


APPENDIX D

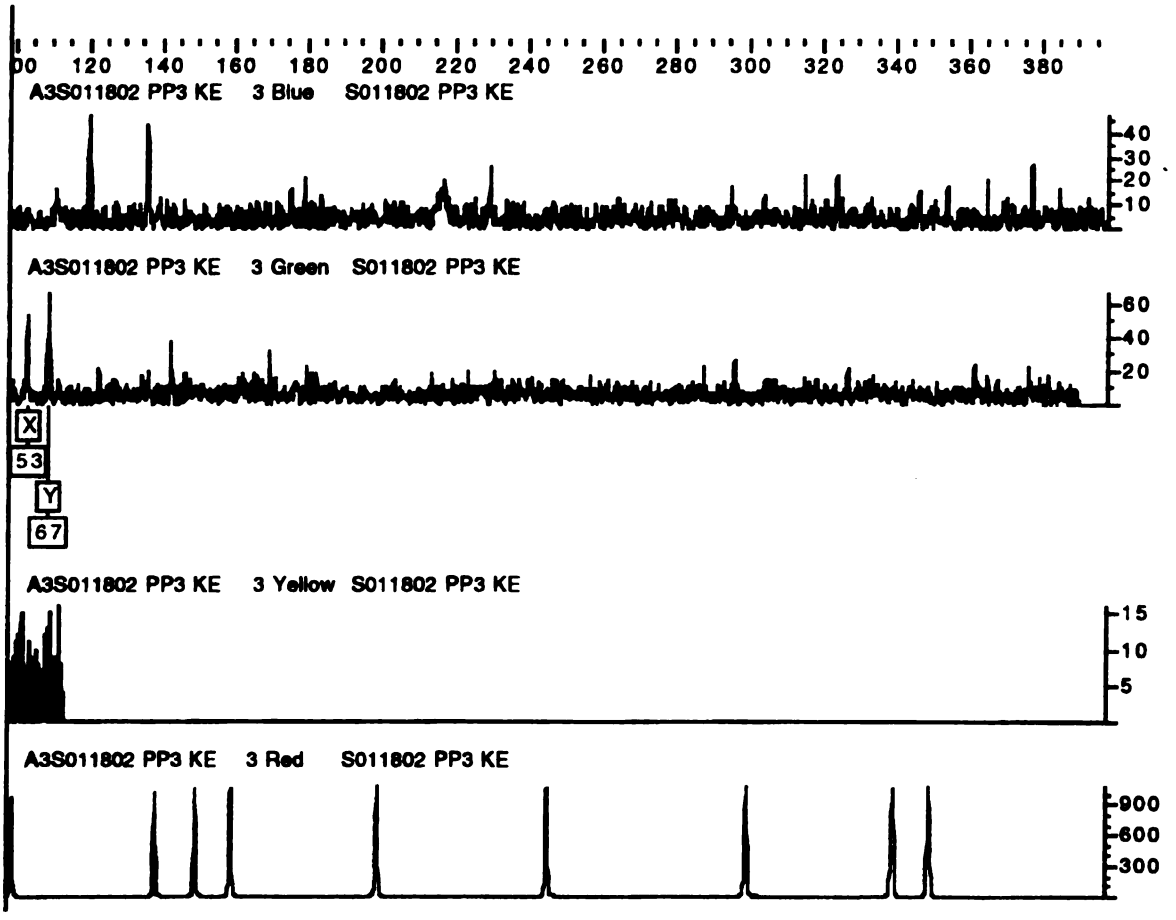
Appendix D contains electropherograms generated from the contamination study, including ladder, and positive and negative controls.

LADDER – Contamination Study

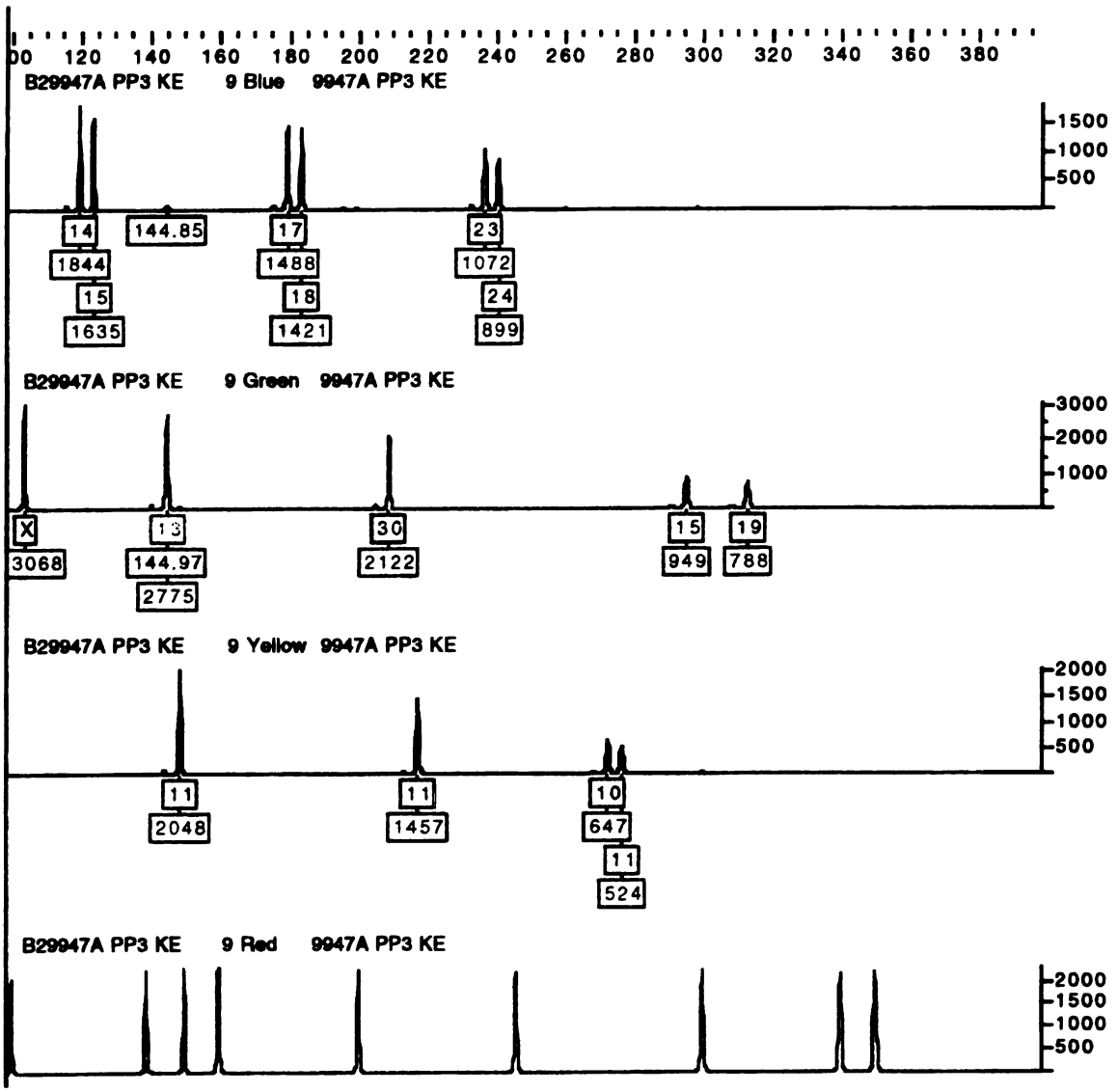



400
300
200
100
7 9 12 15
436 11 14
8 410 317
498 340 16
429 13
10 318
430 354
381
8 11 14
371 12 15
9 247
303 13
10 252
269 308
319
311
6 8 11 14
371 9 12 15
7 10 13
316 231 268
350 265
359 302
318 283
A1LADDER PP3 KE 1 Red LADDER PP3 KE
900
600
300

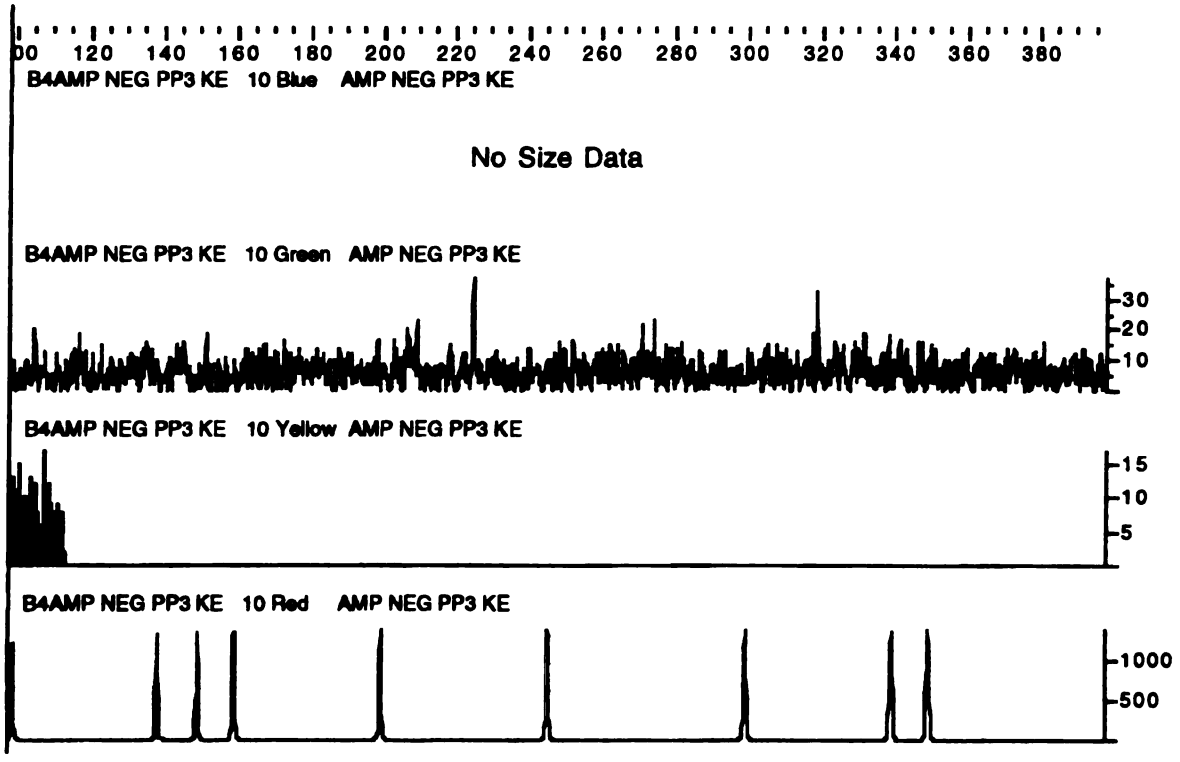
Stain Blank – Contamination Study



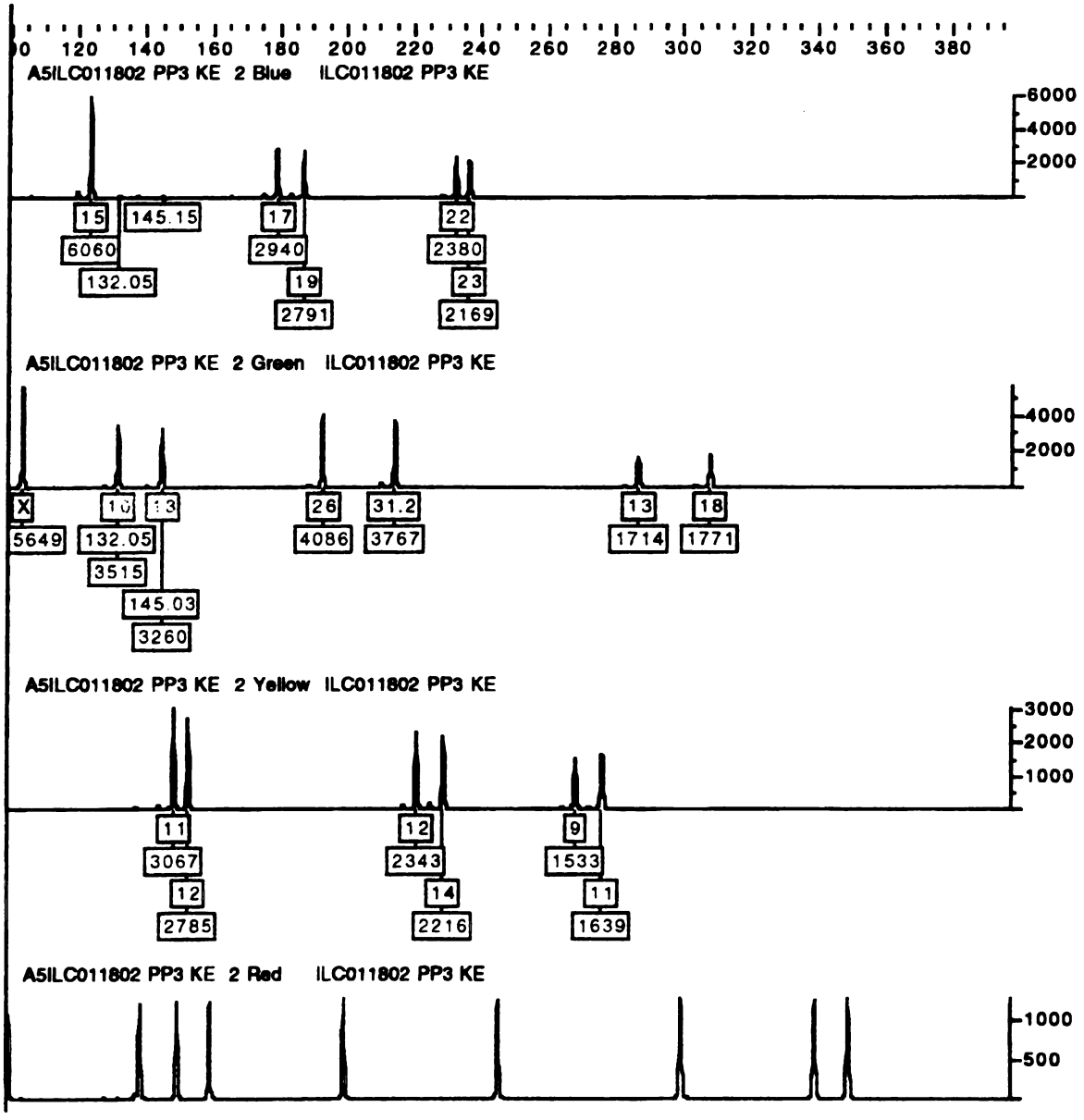
Amplification Positive Control – Contamination Study



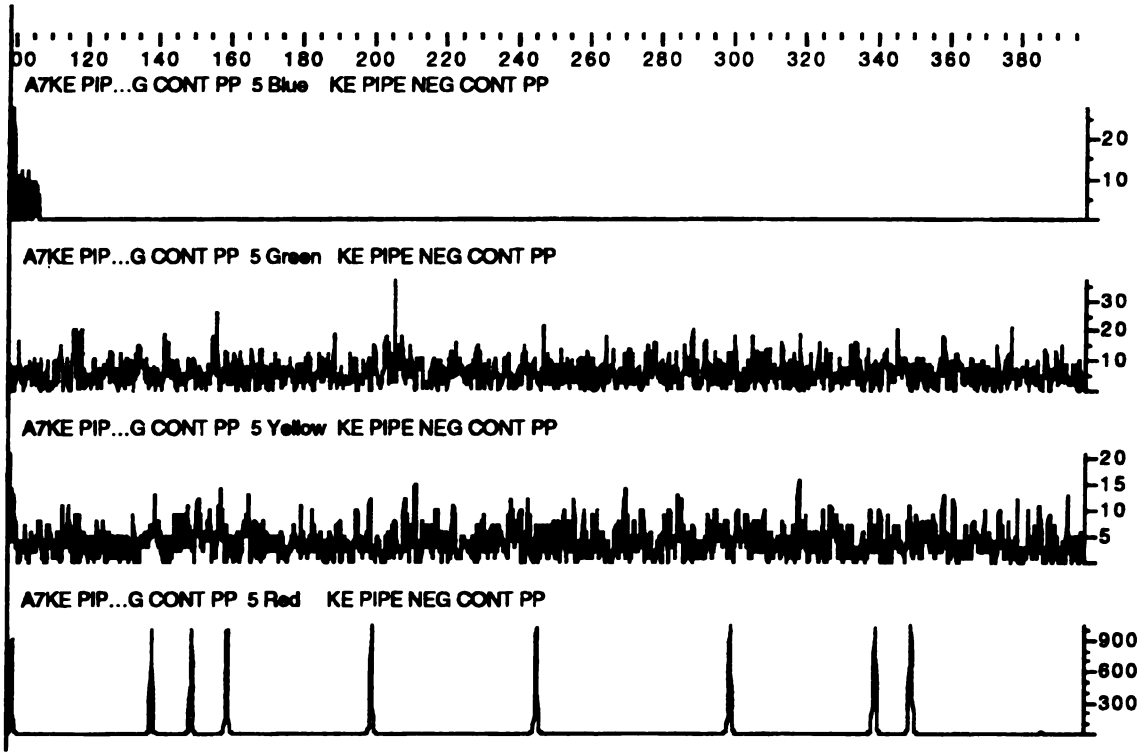
Amplification Negative Control



Internal Laboratory Control – Contamination Study

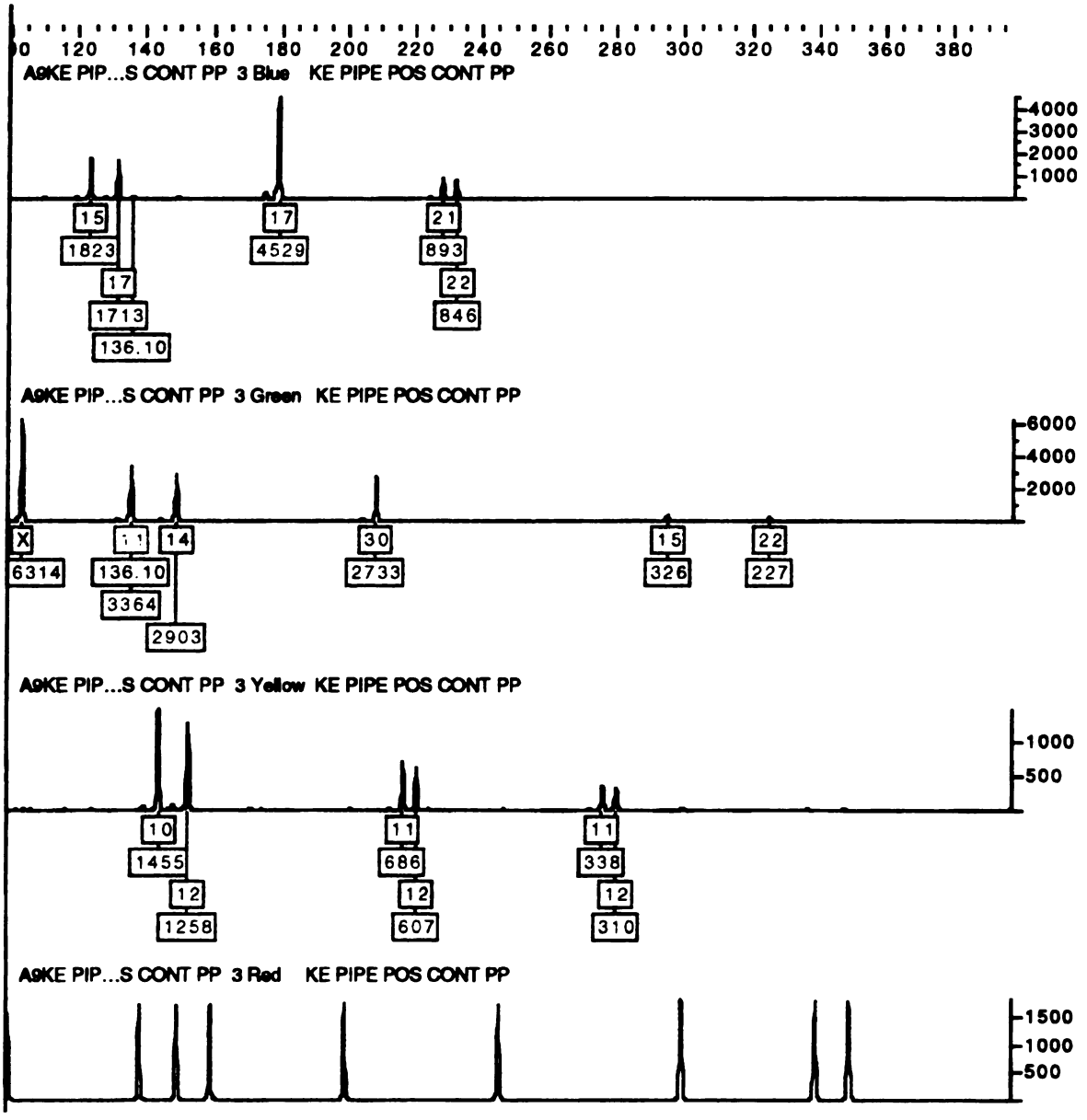


KE Pipe Negative Control – Contamination Study

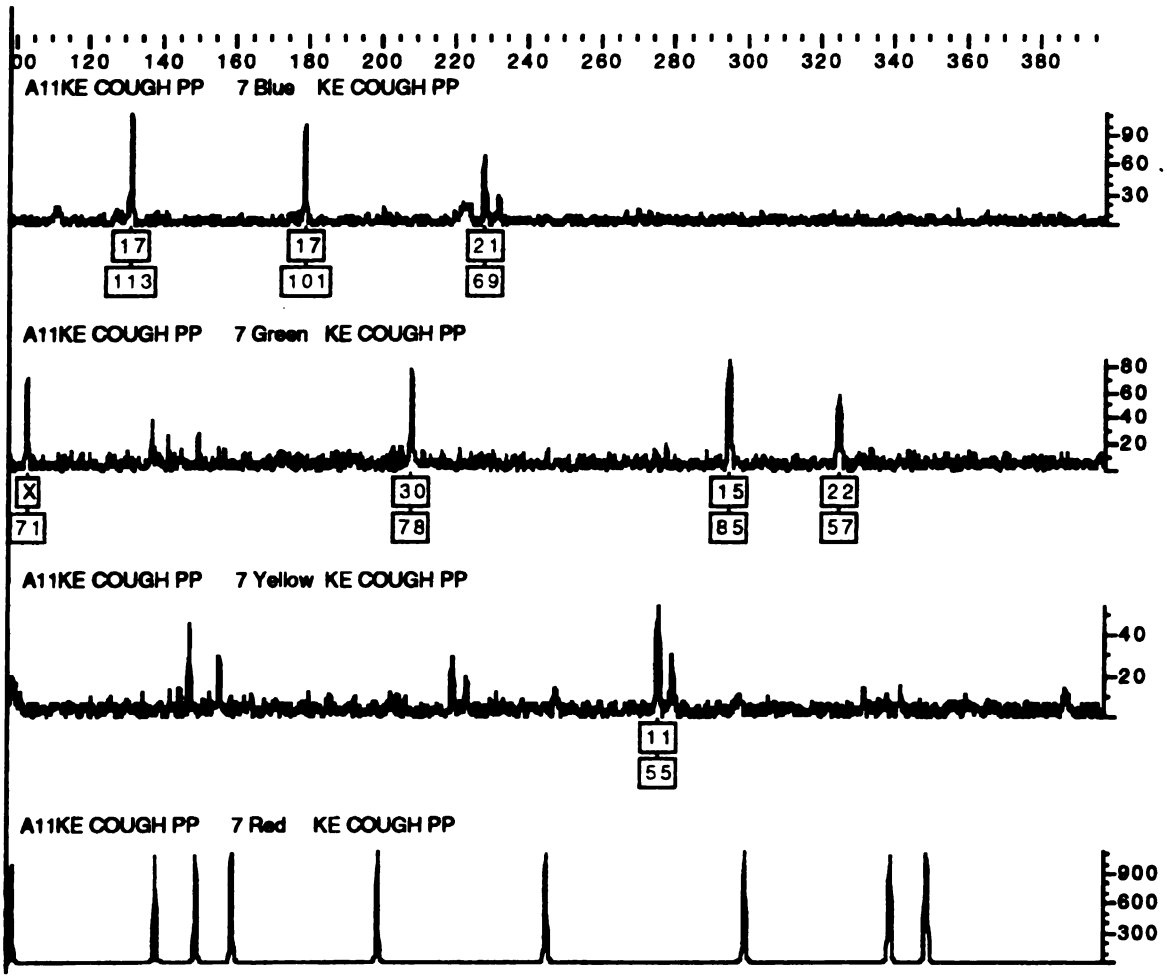


KE Pipe Positive Control – Contamination Study

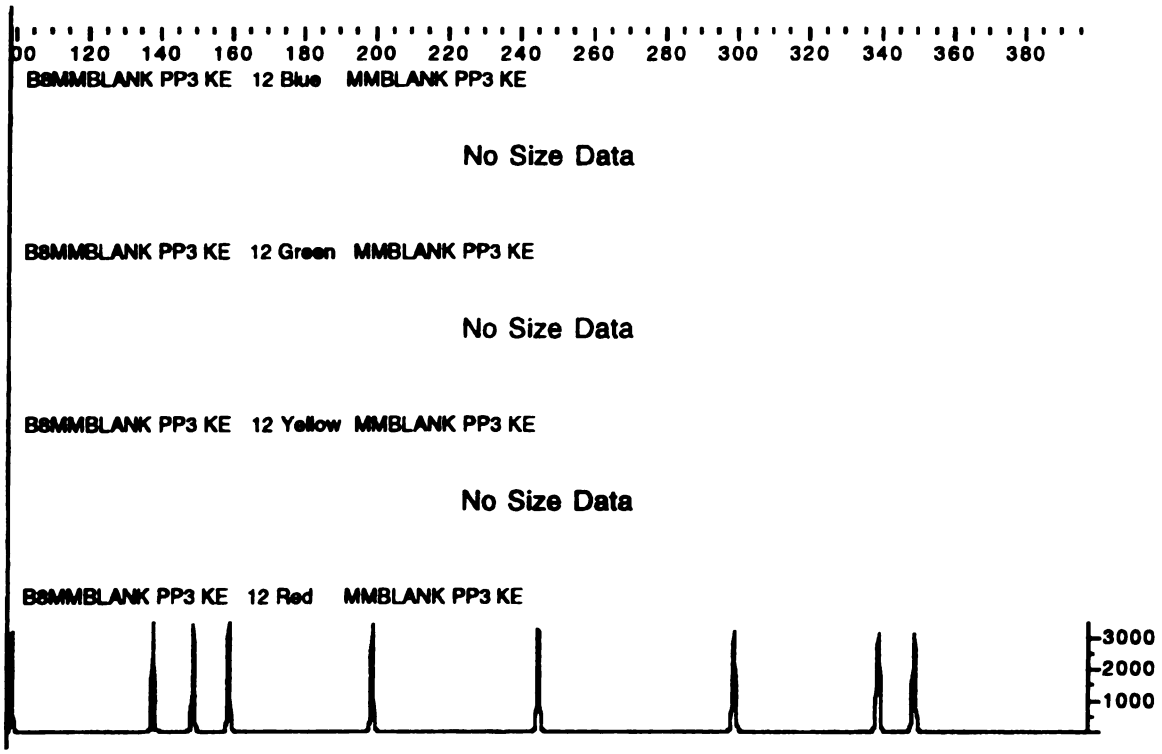
4 Second Injection



KE Cough – Contamination Study



Master Mix Blank – Contamination Study



APPENDIX E

Appendix E contains calculations using population statistics to demonstrate how often a particular genetic profile would expect to be observed in the general Caucasian population.

6 Metal B

	D3S1358		vWA		FGA		D8S1179		D21S11		D18S51		D5S818		D13S317		D7S820	
	16	18	16	16	20	21	13	13	32.2	33.2	14	19	11	13	11	11	9	11
US Cau Pop	0.232	0.163	0.202	0.202	0.145	0.173	0.339	0.339	0.112	0.031	0.173	0.036	0.410	0.146	0.319	0.319	0.148	0.202
Combin. Cau	0.075275		0.04544181		0.050448545		1.22E-01		6.87E-03		0.012389227		0.119918998		0.1082		5.97E-02	

Overall Cau	1.386398657E-12 OR 1 in 7.213 E +12 OR 1 in 7.213 Billion

6 PVC B

	D3S1358		vWA		FGA		D8S1179		D21S11		D18S51		D5S818		D13S317		D7S820	
	16	18	16	16	20	21	13	13	32.2	33.2			11	13	11	11		
US Cau Pop	0.232	0.163	0.202	0.202	0.145	0.173	0.339	0.339	0.112	0.031			0.410	0.146	0.319	0.319		
Combin. Cau	0.075275		0.04544181		0.050448545		1.22E-01		6.87E-03				0.119918998		0.1082			

Overall Cau	1.874612018 E - 9 OR 1 in 533,443,716 OR 1 in 5.334 Million

13 PVC B

	D3S1358		vWA		FGA		D8S1179		D21S11		D18S51		D5S818		D13S317		D7S820	
	18	18	17	17	21	22	9	14					10	12	11	11		
US Cau Pop	0.163	0.163	0.263	0.263	0.173	0.189	0.010	0.202					0.049	0.354	0.319	0.319		
Combin. Cau	0.03050978		0.074854		0.065495		4.11E-03						0.034479		0.1082			

Overall Cau	2.29E-9 OR 1 in 435,899,078 OR 1 in 4.359 Million

3 Metal B

	D3S1358		vWA		FGA		D8S1179		D21S11		D18S51		D5S818		D13S317		D7S820	
			16	17			11	13					11	11				
US Cau Pop			0.202	0.263			0.059	0.339					0.410	0.410				
Combin. Cau			0.105908				0.039812						0.168313					

Overall Cau	7.968 E -3 Or 1 in 126

Frequencies taken from the FBI

Formulas used in calculations are
2pq for heterozygotes

p^2 + p(1-p)(Theta) for homozygotes

THETA = 0.03

APPENDIX F

The following protocol is from a dated general reference for a Rendering Safe Procedure (RSP) reprinted from Robert R. Lenz's Explosive and Bomb Disposal Guide, copyright 1965 (16).

Rendering Safe Procedure (RSP)

Once access has been gained to any suspect package by remote means there are certain procedures which will take a natural course in the rendering safe procedures of various devices. These procedures will vary due to the unlimited amount of devices encountered, but generally speaking, they are:

1. **GAG Techniques:** Applied to movable plungers, clocks, etc. and consist of any measure taken to prevent movement of a mechanical device. One method is by use of plaster of Paris and water. Others are sugar, water solutions, syrup, or thick oil to stop clocks.
2. **Separation Techniques:** Separation of chemicals, detonators from main charges, and electrical separation are considered to be separation techniques. All electric circuits should be thoroughly traced before cutting any wire, due to the possibility of a collapsing circuit. When performing an electrical safing procedure (ESP), always cut and tape only one wire at a time and beware of a double strand contained in what may appear to be a single strand insulator. Separation of detonators from main charges should be done remotely due to internal booby traps in manufactured sabotage items.
3. **Replacing safety devices or pins:**

4. **Freezing procedures:** The use of CO_2 plus alcohol, liquid nitrogen, or other freezing materials may be applied or injected into certain devices to lower the firing potential of certain batteries in an electric circuit. One should bear in mind that, once freezing is started, the batteries must be kept frozen. Otherwise, the firing potential will rise as the temperature rises.
5. **Submerging techniques:** Consists of puncturing the package and submerging in oil to stop clocks or to saturate various explosive and chemical mixtures.
6. **Trepanning:** The use of strong nitric acids to corrosively eat a small hole into metallic containers by means of a fine acid spray directed against the container.
7. **Steaming:** Once access is gained on certain high explosive devices, steam directed at the explosive will melt the explosive into a water like mass. This technique should always be done remotely and once steaming is started, do not stop until it is completed. Never stop steaming and return later to commence re-steaming as detonation is likely to occur.
8. **Transportation phase:** This phase may have taken place during the contact phase if certain equipment (bomb trucks, special carriers, etc.) were available. In any case, there are certain rules to follow while transporting any live items. These rules will depend on the item before or after the rendering safe procedure, and the general size of the item. Certain general rules are:

    a. Provide escort if necessary.

    b. Use placards (explosive signs).

    c. Equip truck with sand bags, loose sand, and dunnage

d. Keep listening – if devices are transported intact. Clockwork could be activated.

e. Have police search area for additional devices.

f. Communications enroute to disposal area.

g. Shunt electrical blasting caps prior to transporting.

h. Take routes that are least conjected, based upon map of city.

i. Freezing enroute may be desired in some cases.

9. **All clear phase**

10. **Disposal area phase**

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