Introduction

Identification of specific individuals through biological samples has many important forensic uses, such as the identification of a victim's remains at a crime scene, a perpetrator's blood at a crime scene, the paternity of an individual, and use of products produced from an endangered species. Prior to the advent of biotechnology in the mid-1970s, identification was mainly performed by fingerprint matching (dactylography) (Hazarika 2012), an individual's specific body measurements (biometrics) (Saini 2016), blood type analysis (Harbison 2016), and hair analysis (Deedrick 2004). Deoxyribonucleic acid, commonly known as DNA, is a double stranded right helix composed of two anti-parallel strands held together by hydrogen bonds between the bases. DNA contained in every cell in the human body is unique to each person, where DNA codes a sequence pertaining to a specific individual. DNA technologies advanced identification through the use of restriction fragment length polymorphism in the 1980s to the use of polymerase chain reaction (PCR), which is currently in use (Cormier et al 2005). Restriction fragment length polymorphism is an older bioanalytical technique that utilizes enzymes that cut DNA at specific sequences of DNA. These sequences vary on an individual basis, thus can be used in forensic genetics analysis (Bernatzky 1989). Furthermore, the incorporation of DNA as a forensic analytical tool completely revolutionized forensic science (Roewer 2013).

PCR proves to be beneficial in its ability to amplify specific segments of small amounts of DNA, thus targeting variable regions to be used for DNA fingerprinting (HHMI). Crime labs nationwide use PCR-based techniques targeted to autosomal DNA,

Y chromosome DNA, and mitochondrial DNA. Drawbacks to PCR, however, include the long length of time needed to process the sample, the hands-on tedious involvement leading to high cost in labor, the large quantity of sample needed, the high DNA integrity required, and the lack of statistics, as well as the opportunities for contamination (Wassenneger 2001).

For human identification, the Federal Bureau of Investigation utilizes short tandem repeat regions (STRs) of DNA at thirteen different autosomal loci (Federal, 2016). Short tandem repeats are repeated one to six base pair DNA sequences throughout the human genome. These segments are used for forensic identification because of the variation that is unique to each individual (Fan and Chu 2007). The DNA collected from individuals involved in violent crimes is collected and stored in the international database the Combined DNA Index System (CODIS), housing DNA information from over 90 labs in over 50 countries (FBI 2016). It is crucial in identifying victims and suspects to have as much genetic information as possible, so all thirteen loci are attempted for each sample. Further, allele frequency databases are then used to calculate the probability of a match by chance to the exact genotype identified in the sample. Therefore, the larger number of loci are preferred by both the forensic scientist and innocent individuals accused of committing a crime. In addition, investigators must also investigate motive and opportunity to commit the crime in question in order to procure a strong case against the defendant.

Criminals necessarily desire to dispose of the evidence of the crime and their linkage to it. For example, on July 9, 2003, Tim Schuster was murdered by his wife, a

biochemist at a research lab in California. Mr. Shuster's body was found half decomposed in a vat of acid (Morrison and Koch 2013). With this case, a partial body was recovered. Currently in media, entertainment sources such as the popular television shows *Breaking Bad* (Gilligan and Bernstein 2008) and *The Blacklist* (Bokenkamp and Mislano 2013), show criminals disposing of deceased bodies using drain cleaner to cover up homicides. This phenomenon in entertainment leads to an interesting proposition: is it possible for real criminals to decompose their victims in drain cleaner to the point where a DNA-based identification cannot be made? There were no forensic studies which answer this question when a literature search was conducted. Thus, there is a need to determine whether this method, which degrades tissue and therefore circumvents visual identification, can be used to degrade DNA to the point that no identification is possible.

A variety of drain cleaners can be found at local department stores, such as Walmart. Acidic and basic drain cleaners have near equal strength to that of the hydrochloric acid used by Larissa Schuster, the chemist who killed her husband. These drain cleaners chemically break down organic compounds into their individual components and change the organic molecules. Enzymatic drain cleaners work similarly, by initiating catabolic reactions within the organic matter (Gertzman, 2008). DNA is stable within the nucleus of a cell in part because the DNA is complexed with proteins and also because the cell maintains a constant pH 7. Disturbing the pH balance by either increasing alkalinity or acidity can result in decreasing the stability and eventually altering the structure of the DNA strands, thus denaturing the DNA and making it more likely to be degraded. A similar process was noted Anderson et al (1990), where in their

experiment they studied the effects of pH on the denaturing of proteins by increasing ion concentrations. Thus, in either acidic or basic conditions, the alteration of pH will likely decrease the integrity of the DNA in a time-dependent manner.

Since it is not feasible to perform these studies with human tissues in a first trial, a model organism must be used. *Sus scrofa*, commonly known as domestic pig, is a mammalian species that is often used in genetic research for studying genetic diseases relevant to humans and has tissue similar, such that the pigs are being investigated to grow human organs for organ transplant (Cozzi and White 1995). Utilizing *S. scrofa* will give a good approximation of the effects of drain cleaner decomposition on the DNA in *Homo sapiens*. The intent of this study was to determine whether any of the available drain cleaners could completely degrade DNA and at what time was the DNA degraded beyond use for identification purposes using the standard PCR-based methods used in forensics today. It was expected that DNA from tissue exposed to drain cleaner would degrade to unidentifiable at a faster rate than the pH-neutral solution.

Materials and Methods

Treatment of Pork Ribs and Isolation of DNA

Ribs from *S. scrofa* purchased at the local Walmart were used for this experiment. Approximately 22 grams of ribs were treated with 500 mL of one of the three drain cleaner solutions (acidic, basic and enzymatic) or a pH-neutral solution over the course of fourteen days. The drain cleaners were purchased from Walmart. The solutions were kept in two quart mason jars, also purchased at Walmart. Each of the four treatment methods had three replicates. The treatment was performed at room temperature, $25^{o}C$. For the first three days, approximately 25 mg were collected from each of the twelve jars. For the remaining days of the fourteen day treatment period, 25 µL were collected for the acid and base samples while 25 mg were collected for the enzymatic and water samples. The samples were then isolated by the DNeasy protocol (Qiagen) as directed by the manufacturer and stored at $-20^{o}C$.

Table 1. pH Measurements for Treatment Solutions. The pH of each solution was measured prior to addition of the pork rib tip using a pH strip, measuring from 0 (most acidic) to 14 (most basic). A pH of 7 is neutral (typical physiological pH).

Solution	**pH**
Acidic Drain Cleaner	0
Basic Drain Cleaner	14
Enzymatic Drain Cleaner	7
Water	7

Quantitation and PCR

DNA concentrations were measured using the Nanodrop and dilutions were made to 1.45 µg/mL using deionized water as a diluent. Primers for *Sus scrofa* Ss_STR11A and Ss_STR01A loci (Table 2) were purchased from Fischer Scientific. The primer sequences used were previously utilized with success by Lin et al (2014). An initial baseline was established to determine if the primer sequences given would work with the available pork rib samples.

Table 2. Sequence Names and Primer Sequences for the Primers Used for the *Sus scrofa*. The primers were selected from primers used by Lin et al (2014) based on success in PCR with control tissue sample for this experiment.

Sequence Name	**Forward Primer Sequence**	**Reverse Primer Sequence**	**Expected Size (bp)**
Ss_STR11A	CACGTGATCC TTTGCAA	GCAGGTGCATGC CTAAAAAG	200
Ss_STR01A	TGGTGTTGGTT TGATCCTCA	AAATCGGATTCTT TTCCCACCTA	250

For PCR, 64 µL 5x Phusion buffer green (ThermoFischer), 200mM DTT, 100% DMSO , forward Ss_STR11A primer, reverse Ss_STR11A primer, forward Ss_STR01A primer, reverse Ss_STR01A primer (all primers from Eurofins) 10mM dNTP mix

(ThermoFisher), and Phusion DNA Polymerase (ThermoFischer) were added to the diluted DNA. PCR was performed with the following parameters: 98^0C for 1 minute, 98^0C for 15 seconds, 55^0C for 30 seconds, 72^0C for 50 seconds. Steps two through four were repeated 35 times, then samples were kept at 72^0C for eight minutes and then held at 4^0C until samples were collected from the thermocycler. Amplified fragments were fractionated on 1.5% gel containing 0.04 mg/mL ethidium bromide at 120 volts for 50 minutes and visualized under UV light.

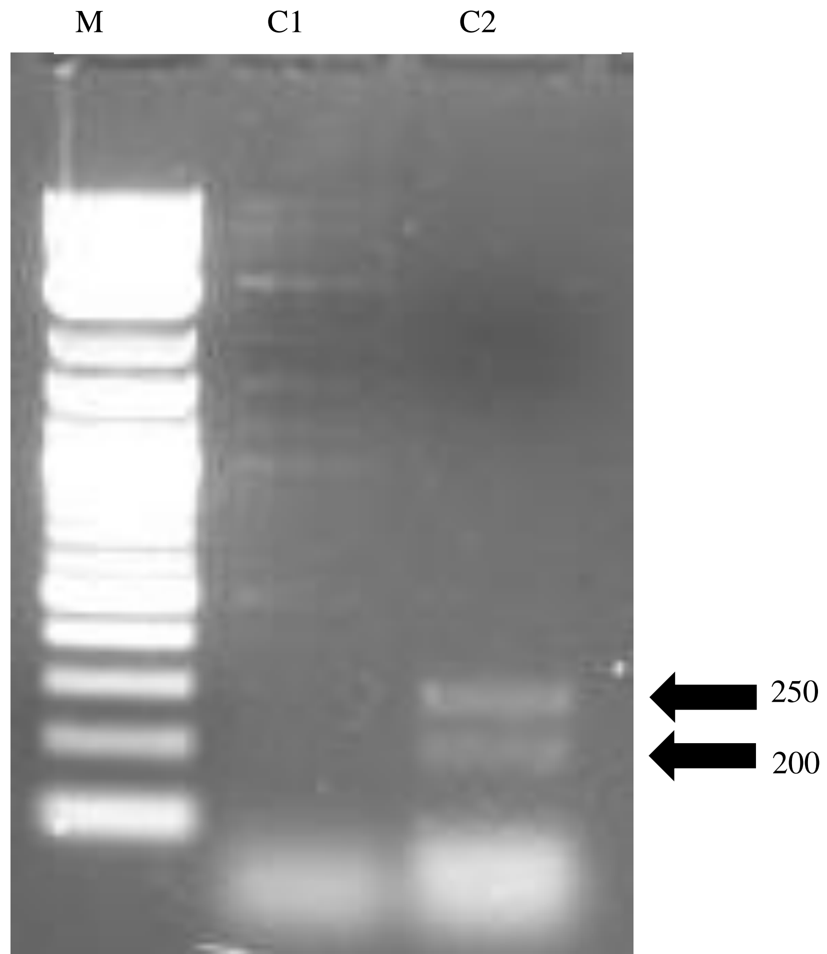

Figure 1. PCR Analysis of Untreated Pork DNA. In lane three, control bands can be seen at 200 and 250 base pairs for the primers Ss_STR11A and Ss_STR01A. M= marker (NEBlog2), C1= DNA from untreated pork sample 1, C2= DNA from untreated pork sample 2.

Results

To test the hypothesis that drain cleaner could be used to degrade DNA beyond its use as a genetic identification tool, pork ribs were incubated in acidic, basic, enzymatic drain cleaner or water. DNA was isolated each day of the fourteen days, quantified, and used as template for a forensic DNA-based analysis. First, to ensure that pork DNA could be detected using previously successful primers in our lab, DNA was isolated and used in gradient PCR to determine an optimal annealing temperature (data not shown). Both fragments were detectible using control DNA and an annealing temperature of 55^0C (Figure 1). With this information, the full experiment was began.

On day zero of the treatment, the ribs can be seen intact for all of the solutions. The acid and base solutions appeared denser than the enzymatic and water solutions, because the rib pieces can be seen floating on top of the solutions (Figure 2). By day three of the treatment period, the acid no longer contained any solid rib material and the base only contained the bone of the rib. The acid replicates contained a dark brown, gelatinous layer on the top surface of the liquid and the base replicates presented a foam on the top surface of the liquid. The enzymatic drain cleaner and water solutions became cloudy with the decomposed tissue. At the half-way point, day seven, the acid and base remained the same, where the top layers were thicker for all of these replicates. The enzymatic and water solutions displayed further decomposition of the rib tissue along with tissue swelling. At day ten, the top layers for acid and base replicates had grown even thicker. The enzymatic and water solution replicates became even cloudier. By the last day of the treatment, day fourteen, the acid replicates were nearly completely

gelatinous. The base replicate contained a solid, soapy foam layer on the top of the clear solution. The enzymatic and water solution replicates contained very cloudy solutions with swollen, decomposing rib.

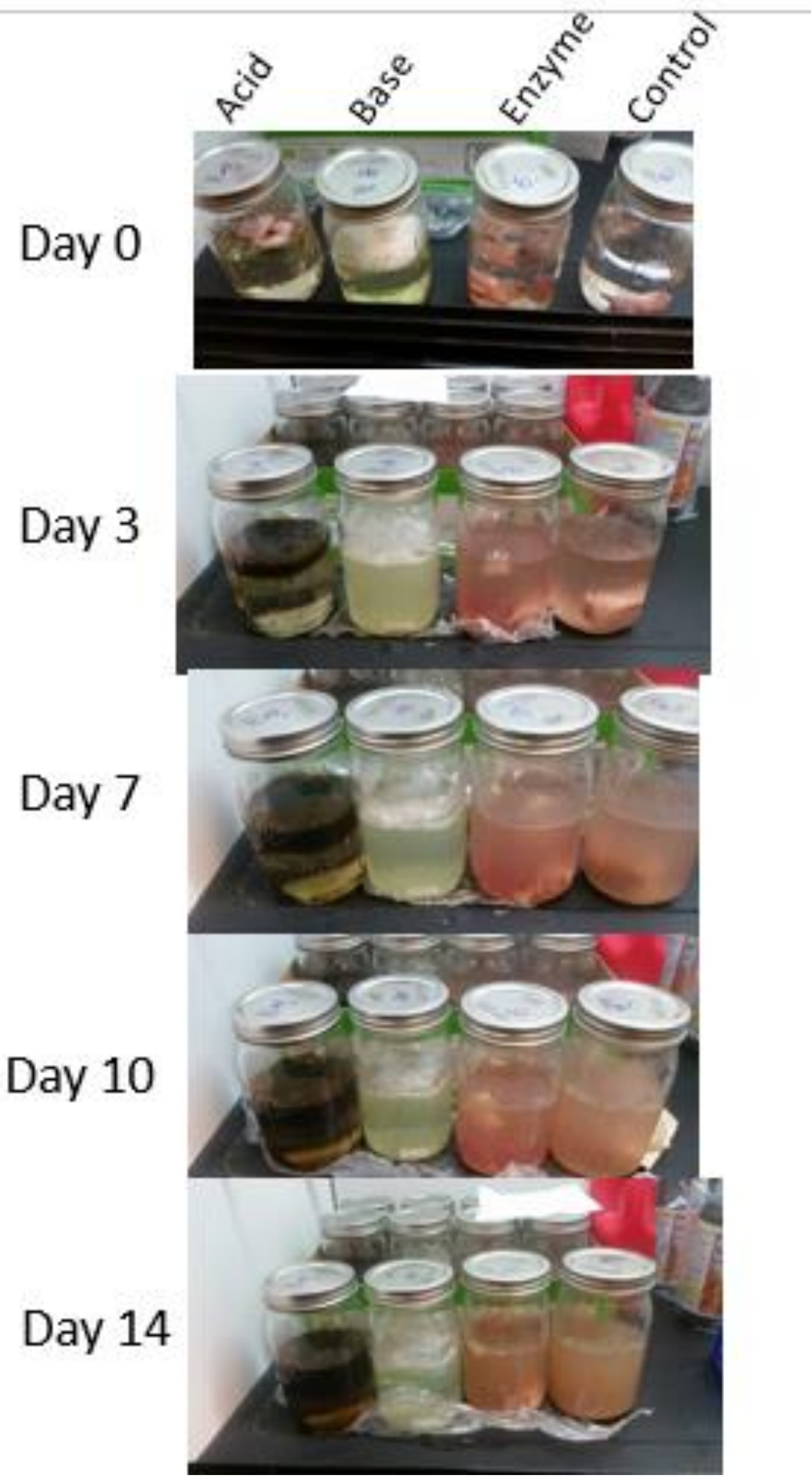

Figure 2. Visual Appearance of Treated Ribs from Day Zero to Day Fourteen.

DNA was extracted from each sample in triplicate. Each extraction was somewhat variable in yield, but DNA was able to be isolated and quantified throughout the entire fourteen day experiment for all treatments (Table 2). This measurement method utilizes absorbance values at a wavelength of 260 nanometers to detect the presence of DNA. However, it measures the integrity of the ring structure of the nitrogenous bases, not the integrity of the DNA helix. To test this, the extracted DNAs were used as templates in a PCR-based analysis similar to the forensic tests commonly used in forensic identification today.

Table 3. Median Nanodrop Concentrations Isolated from Treated Pork. Sample concentrations were read at 260nm.

Day	Acid Median µg/mL	Base Median µg/mL	Enzyme Median µg/mL	Water Median µg/mL
1	2.7 +/- 1.4	6.8 +/- 6.8	36.6 +/-25.0	12.9 +/- 9.0
2	3.7 +/- 0.9	8. 0 +/- 0.3	14.8 +/- 10.0	18.9 +/- 7.7
3	4.1+/- 1.8	3.5 +/- 1.4	7.6 +/- 6.5	13.3 +/- 9.9
4	2.8 +/- 0.8	6.5 +/- 9.8	12.1 +/- 4.2	8.5 +/- 10.4
5	-1.2 +/- 2.2	0.6 +/- 0.9	10.8+/- 4.5	7.2+/- 3.0
6	7.4 +/- 3.1	3.3 +/- 4.2	10.0 +/- 1.5	3.1 +/- 1.9
7	6.2 +/-1.2	1.2 +/- 0.7	9.1 +/- 31.6	25.4 +/- 12.6
8	18.4 +/- 15.5	0.4 +/- 0.5	14.8 +/- 15.8	16.0 +/- 6.0
9	1.9 +/- 0.6	1.9 +/- 1.0	35.2 +/- 11.0	7.1 +/- 35.9
10	8.3 +/- 9.2	1.9 +/- 8.2	21.6 +/- 15.2	20.9 +/- 5.9
11	10.1 +/- 8.0	2.9+/- 0.6	17.3 +/- 9.9	20.5 +/- 8.8
12	7.1 +/- 9.8	1.4 +/- 0.8	27.3 +/- 11.4	20.3 +/- 8.0
13	1.6 +/- 0.6	2.4 +/- 1.8	7.3 +/- 15.36	30.4 +/- 0.3
14	5.1 +/- 1.8	4.1 +/- 6.7	13.3 +/- 4.6	29.6 +/- 10.0

PCR was performed using primers to two loci in the pig genome. These primers were previously tested as identification tools (Lin, 2014). Two primer sets were chosen to generate different size fragments to possibly enable multiplexing of downstream analysis. Fragments were fractionated using standard agarose electrophoresis.

To observe the results from PCR using and primers, agarose gel electrophoresis was conducted. The lane labeled control on the gels was DNA from pork ribs that was isolated and amplified but received no treatment. Thus, the control for the agarose gel electrophoresis demonstrates the effectiveness of the PCR itself. First, all three replicates showed different results, but two of three replicates showed DNA fragment amplification in early samples, (Figure 3), but no amplification in late samples. The controls shows a band at 250 base pairs.

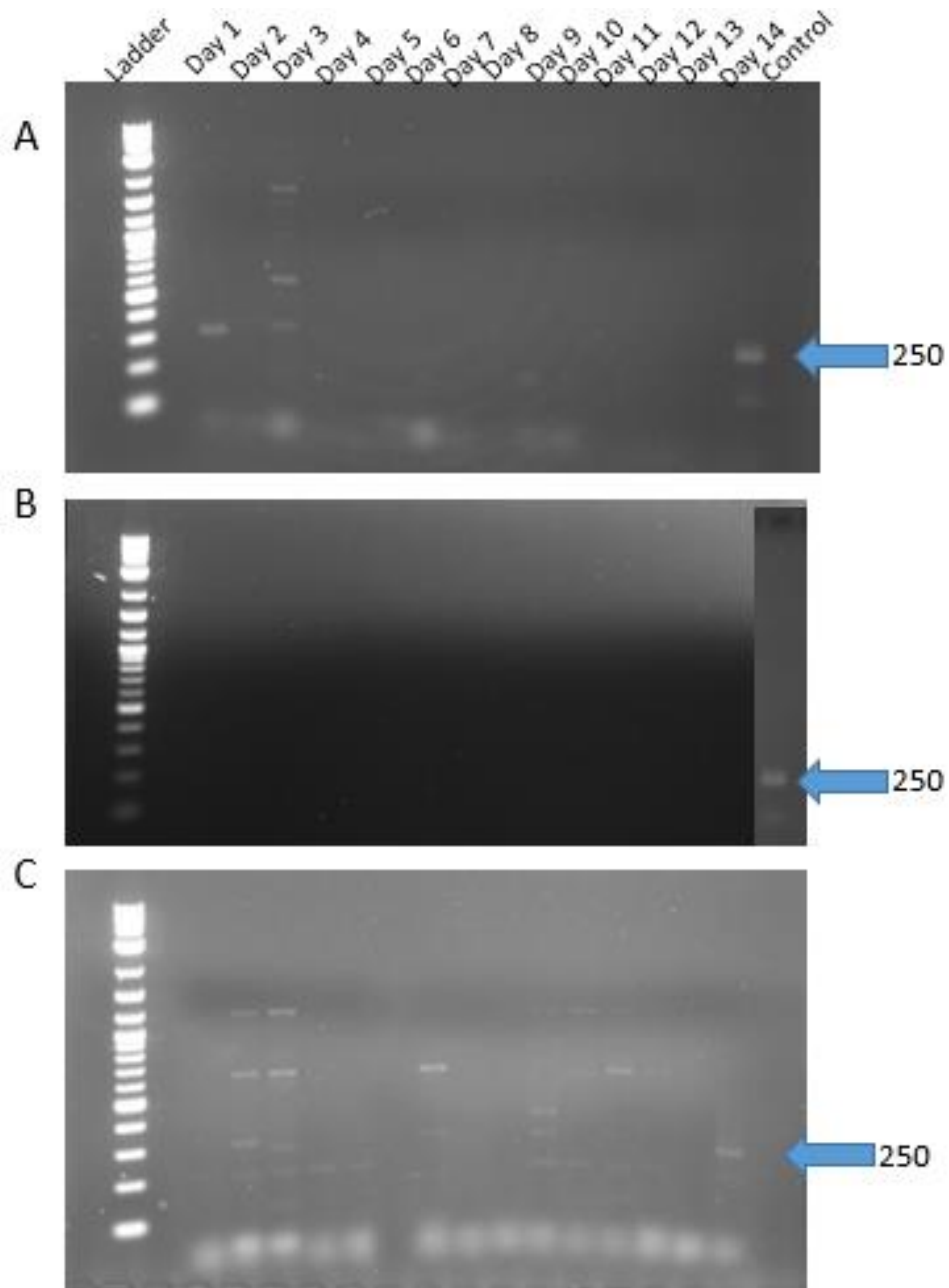

Figure 3. Forensic DNA Analysis of Acid Drain Cleaner Treated Samples (replicates A, B, and C). Gel electrophoresis of PCR with Ss_STR11A and Ss_STR01A loci primers. Control DNA with no treatment. Ladder = NEBlog2

The PCR results for the base set of replicates is displayed in figure 4. Next, DNA from base treated tissue was amplified throughout the treatment for one of three replicates (Figure 4B).

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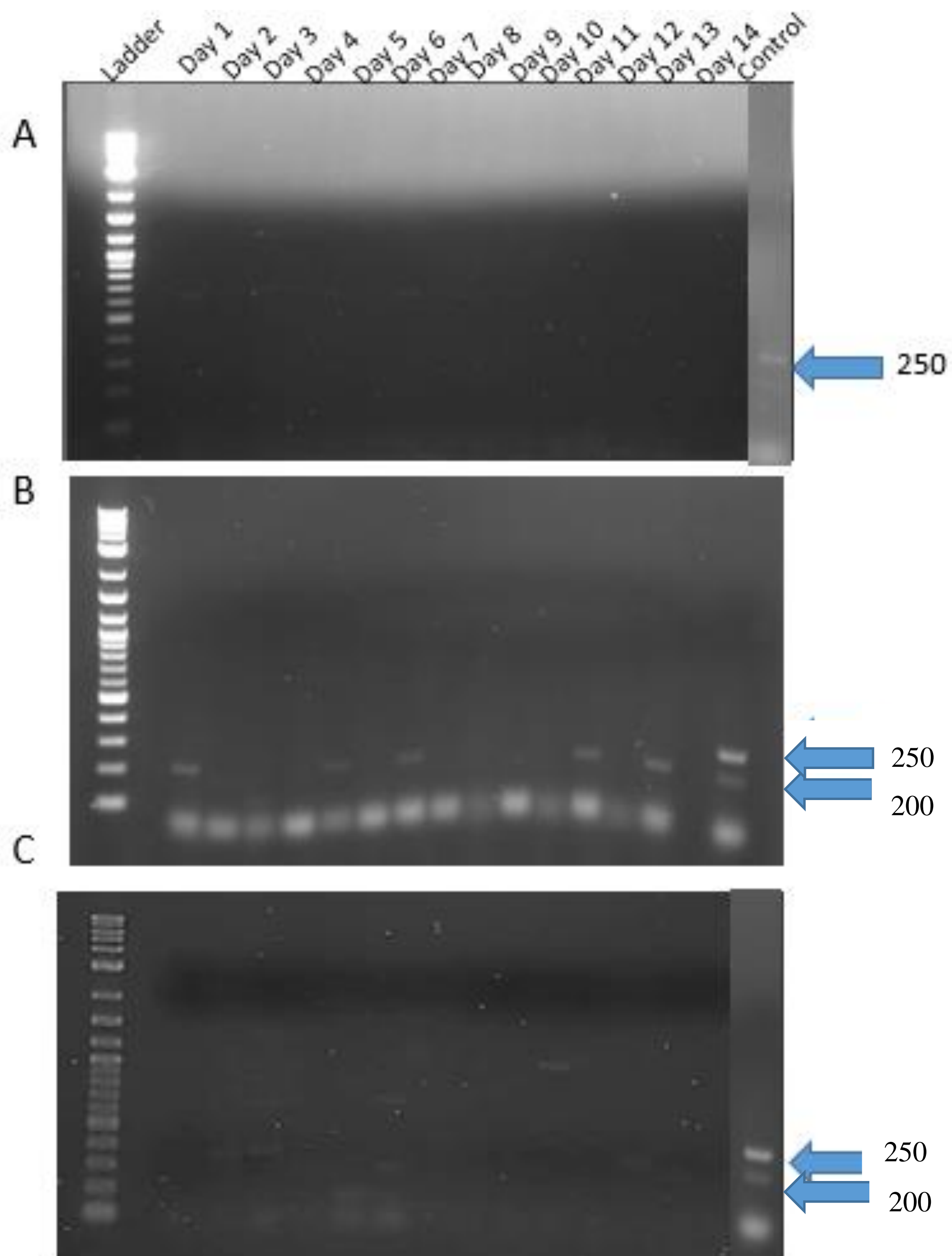

Figure 4. Forensic DNA Analysis of Base Drain Cleaner Treated Samples. Replicates A, B, and C. Gel electrophoresis of PCR for with Ss_STR11A and Ss_STR01A loci primers. Control DNA with no treatment. Ladder NEBlog2

Thirdly, DNA from enzymatic drain cleaner-treated tissue was amplified throughout the treatment for two of three replicates (Figure 5A and 5C).

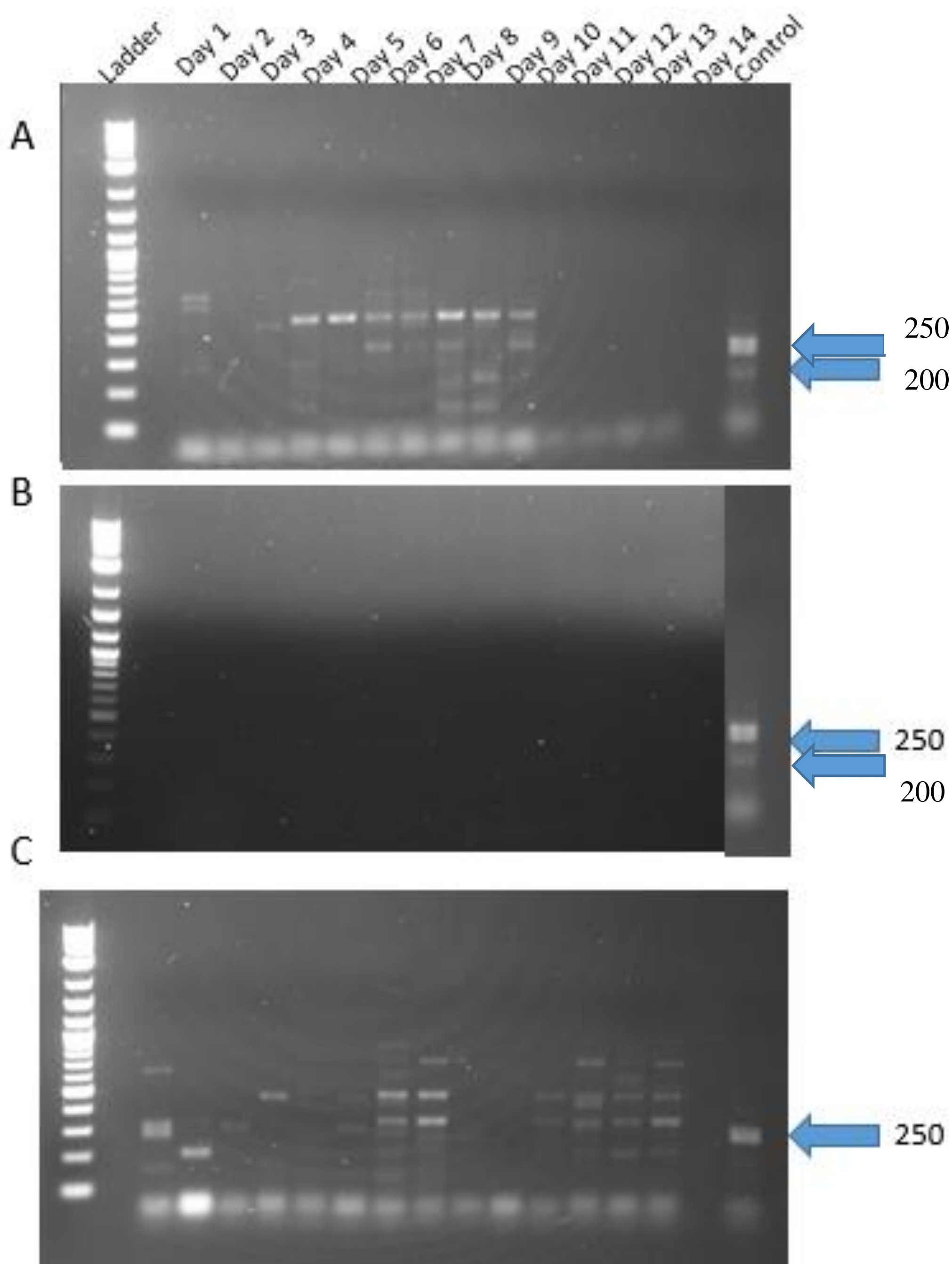

Figure 5. Forensic DNA Analysis of Enzymatic Drain Cleaner Treated Samples.

Replicates A, B, and C. Gel electrophoresis of PCR with Ss_STR11A and Ss_STR01A loci primers. Control DNA with no treatment. Ladder NEBlog2

Finally, as a control, DNA isolated from water treated tissue was amplified in one of three replicates (Figure 6).

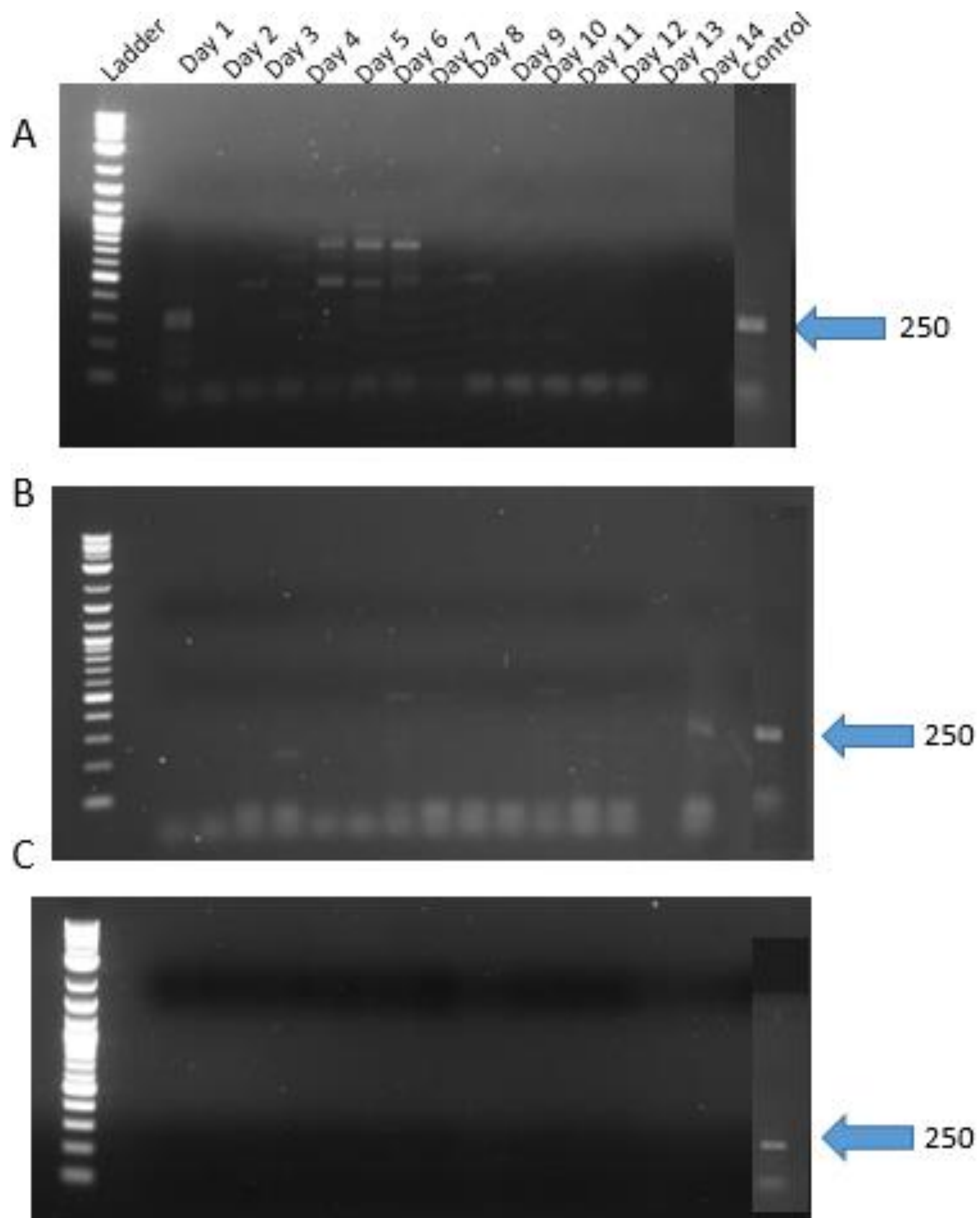

Figure 6. Forensic DNA Analysis of Water Treated Samples. Replicates A, B, and C. Gel electrophoresis of PCR with Ss_STR11A and Ss_STR01A loci primers. Control DNA with no treatment.

Discussion

DNA-based identification requires small amounts of DNA, but that DNA must be intact at least in the regions used for identification. While the popular media has suggested that drain cleaner can degrade both tissue and DNA, scientific analyses have not been conducted to determine whether this is possible. To begin investigating this hypothesis, pork ribs were treated with acid, base, or enzymatic drain cleaner or water over fourteen days. DNA was successfully extracted from samples each day for all treatments. Further analysis of the DNA using PCR based techniques revealed inconsistent results across the replicates and treatments. However, some conclusions can be drawn from the amplification results. First, some amplification was observed in at least one replicate's DNA for all treatments. Comparison of the most successful replicate of each treatment condition (acid C, basic B, enzymatic C, water A) revealed that enzymatic treatment was least effective in degrading DNA (day fourteen), even less effective than water. Basic drain cleaner treatment was the next least effective at DNA degradation (day thirteen). Water and acid treatments were most effective (day eight) at DNA degradation, based on these results.

These conclusions are preliminary, so the experiment should be replicated to provide additional evidence. Additionally, this method of analysis, while more sensitive than past techniques, is not as sensitive a visualization method as what is used by the FBI currently. Using this advanced visualization method involves detection of fluorescent PCR products using capillary electrophoresis. Combining additional experiments with a more sensitive detection method should allow determination of whether drain cleaner can

conclusively be used as a DNA degrading agent, as has been purported in the popular media.

Similar research using bovine (cow) bones was performed, testing pH variation of aquatic solutions on the integrity of cow bone. This experiment was tested over a year, finding that bone was best preserved in basic condition (sodium hydroxide, pH 14 and least conserved in the strong acid solution (nitric oxide, pH 1) (Christensen et al 2011). Comparably, research done by Eichmiller et al (2016) studied the effects of aquatic temperature on the integrity of eDNA, where eDNA (environmental DNA) is the DNA left behind by an organism and used to study biodiversity in ecosystems. From a forensic standpoint, DNA evidence collected from a crime scene is also a form of eDNA. The study by Eichmiller et al concluded that DNA decay increased exponentially with increase in temperature. In a forensics setting, temperature of the water as well as pH of the water at a potential crime scene should be taken into account when collecting a DNA sample as evidence. Another media emphasized aspect of forensics is cleaning a crime scene with bleach. Research has shown that using bleach, a strong compound also known as sodium hypochlorite, resulted in DNA degradation and denaturation (Passi et al 2012). Thus, while drain cleaner may not be the most effective means of decomposing a body, as supported by television views, bleach does have adverse effects on DNA analysis.

While much progress has been made within the last few decades in making forensic science a true science, seen especially with the advancements in the sequencing of the human genome (Venter et al 2001), more research is still necessary in order to understand the best way to preserve the DNA in the evidence collection process to hold

the fidelity of the DNA analysis process. With this, there is a need for studying the effects of the environment on maintaining the integrity of DNA, such as temperature of storage as well as temperature of location collected. Once current methods of DNA collection are understood, then scientists can begin to develop more specialized ways to examine different DNA samples, such as potentially modifying DNA isolation and purification techniques if the sample was collected from an acidic, basic, or enzymatic drain cleaner.

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