

# Comparison Of Quantity And Quality Of DNA Recovered From Burn Samples In Which Burn Temperatures And Conditions Were Varied<sup>1</sup>

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## Citation

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## Abstract

The use of DNA to identify human remains after an accidental fire, arson fire, or a mass disaster has become a standard technique in the forensic community. The objective of this study is to determine systematically the relative quantity, quality, and amplifiability of DNA extracted from pig muscle tissue and bone marrow subjected to controlled burns in an open flame or convection oven. Burn times range from twenty to forty minutes. Our results indicate that the length and type of burn, and location of the extracted muscle tissue/bone marrow affect DNA recovery from the sample. Agarose gel electrophoresis of burned pig muscle tissue resulted in the recovery of mostly low molecular weight, degraded DNA with undetectable yields from the top tissue after the forty minute burn time. Recovery yields were higher for the bone marrow samples and proved the most amplifiable as evidenced by real-time PCR.

## INTRODUCTION

The use of DNA to identify human remains after an accidental fire, arson fire, or even a mass disaster has become a standard technique in the forensic community. Documented mass fatality events such as the Kaprun cable car fire disaster with 155 victims (1) as well as the 9/11/2001 attack on the World Trade Center in New York City (2) highlight the need for systematic methods to evaluate human samples for potential DNA typing using autosomal DNA.

Previous research in the field of arson has been focused primarily on evaluating the accelerant components used in acts of arson. Arson accelerants have been identified by electron ionization Fourier transform ion cyclotron resonance high-resolution mass spectrometry (3) and proton transfer reaction time-of-flight mass spectrometry (4). Gas chromatography ion-trap mass spectrometry has been used as a post-mortem test for low-boiling arson residues (5). Ignitable liquid residues have been extracted from human skin using SPME and were analyzed using GC (6). Pyrolysis GC-MS has been used to evaluate the thermal degradation of amino acids in fingerprint residue to develop new latent fingerprint developing reagents (7). However, a recent human DNA recovery study involving a house fire in which a clothed pig carcass with a blood stain on the shirt and other

blood and body fluid stains placed in a house to simulate a dead body showed that the substrate and accelerant types were not found to affect the quality of the DNA recovered. Fire temperature was found to be an important factor given that only 25% of the DNA recovered from rooms in which the fires were started yielded interpretable results (8). Additionally, De Haan has emphasized that burns are not always hotter in the presence of an accelerant such as gasoline (9).

A method employing the organic DNA extraction prior to real-time PCR was recently published (10). Extracting DNA from tissue preserved in buffered formalin and alcohol-based fixative (11), old bone in mass graves (12), skeletal remains from a World War II mass grave (13), and remains from an explosion in a military fort (14) are important to the community and have used organic extraction to extract ancient, degraded, or mass disaster DNA to obtain a high yield. Crime labs have historically employed this method for difficult samples and degraded DNA. Additionally, the phenol-chloroform method has been shown to recover DNA after the use of Hemastix strips when a more modern method failed (15). The recovery of DNA from ancient and burned bone has been very well studied (9). Although the recovery of DNA from blood after a simulated arson event and STR

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analyses were recently investigated (8, 16), the literature contains no report of a systematic analysis of the effects of temperature and burn time to determine at what times and temperatures DNA becomes unrecoverable from burned tissue. In recent studies, DNA from blood and body fluids was recovered by swabbing and scraping after simulating arson events in the presence and absence of inhibitors (8) and systematic burns were used to evaluate the recovery of DNA from tooth pulp (17).

The objective of this study was to vary the temperature and burn conditions in the absence of accelerants to evaluate the recovery threshold for DNA detection and amplification from burned tissue and bone marrow using pig (*Sus scrofa*) ribs with muscle to provide systematic results of what happens when exposed muscle and bone are exposed directly to heat and flame. The present investigation is the first to compare the effects of simulated open air (open flame) and closed room or vehicle (convection-oven) burns at varying times and temperatures on DNA extracted from flesh and bone marrow. Pig (*Sus scrofa*) muscle (18, 19) and bone marrow (in the form of pork spare ribs) were utilized in the experiments because they best parallel human flesh, have been well accepted in the scientific community as a substitute for human tissue in experiments, and are widely available for study.

Agarose gel electrophoresis was used as a preliminary assay to determine the quality and relative quantity of post-burn, pre-amplification, recovered DNA using an organic extraction. Real-time PCR was used to evaluate the concentration, amplifiability, and extent of degradation of the DNA (10). Real-time PCR was used as a comparative or qualitative method to evaluate concentration and amplifiability as no pig quantitation standards were commercially available. The determination of which samples provide amplifiable DNA and which yield no DNA or unamplifiable DNA will aid the crime scene investigator in deciding which samples to collect and package for further DNA processing and which are less likely to produce results for autosomal DNA typing.

## **METHODS**

### **OVERVIEW**

A rack of pig ribs (King Soopers) was uniformly cut into approximately ten gram pieces with a hobby knife and hack saw (The Home Depot), massed using an analytical balance, repackaged in plastic bags, labeled, and placed in a

refrigerator (4 °C). Using different samples, burns were conducted with an open flame using a Bunsen burner or cooked in a convection-oven to evaluate different conditions (e.g. open burn vs. closed room) (9). The burn times ranged from twenty to forty minutes.

### **THE BURNS**

The convection oven (Maytag Gemini Double Oven MER6755aab) provided a constant maximum temperature of 550 °F (287 °C). Four samples (10.2730 g, 10.4432 g, 10.6033 g and 10.6101 g) (approximate dimensions 1.5x1.8x1.5 cm and 1.8 cm bone width and 1.0 cm bone height) were placed in separate disposable aluminum mini-loaf pans and cooked at the maximum oven temperature of 287 °C for 20, 30, 35 and 40 minutes, respectively. Samples were frozen after burning (left in pan and bagged individually) and thawed prior to extraction using the phenol-chloroform-isoamyl alcohol (PCIA) organic method (10).

The open flame burn was performed using a Bunsen burner, and the temperature was measured with a Traceable Workhorse Thermometer with Type-K probe (Fisher Scientific). The sample was supported over the Bunsen burner using a ring stand with ring holder with a piece of wire mesh placed on the ring holder. The meat/rib sample was placed on the wire mesh with the bone down and meat up. The measured distance between the top of the Bunsen burner itself to the bottom of the ring holder was 5 inches. Whereas the best estimation of temperature with an open flame, in which the temperature fluctuates, is an average temperature (9), a temperature range was determined by monitoring the flame with the thermometer at set time intervals to determine the average. The sample (11.5974 g, bone placed down/meat up) was burned for twenty-five minutes with the heat of the flame being monitored two minutes at a time, five different times (3, 8, 13, 18 and 23 minutes, respectively). The temperature was lowest at 888 °F (475 °C) and highest at 1072 °F (577 °C) with an average temperature of 980 °F (526 °C). DNA from the open flame burn was extracted using the PCIA method (10) immediately after the sample was burned and cooled to room temperature.

Control samples were taken from unburned pork spare ribs (samples, 10.1538 g, 11.6946 g and 11.4340 g) on the surface (top) of the tissue and in the bone marrow.

### **EXTRACTION**

Upon thawing the oven sample and immediately after

completing the open flame burn, tissue samples from the top, middle, next to the bone, and bone marrow were taken with a hobby knife, in triplicate, from each cooked sample, and DNA was extracted using the PCIA organic extraction method favorable for degraded DNA as documented in the literature (10-15) and as communicated to us by the Colorado Bureau of Investigation. In addition to the burn samples, a set of controls were evaluated: reagent negative controls (no tissue added to extraction, reagents only) and unburned positive controls (unburned tissue and bone marrow) also using the PCIA extraction method. The knife was cleaned with soap and water between each sample taken. Specifically, the DNA extractions were conducted using 300  $\mu$ L of organic stain extraction buffer (0.20 M NaCl, 0.020 M Tris (pH 8.0), 0.020 M EDTA, 5 % SDS), 12  $\mu$ L 1M dithiothreitol, and 4  $\mu$ L of 10 mg/mL proteinase K and were added to 1.5 mL microcentrifuge tubes containing the samples. The mixture was briefly vortexed and spun in a microcentrifuge for 2 to 5 seconds to force the sample into the extraction fluid prior to an overnight incubation at 56°C. After the incubation, the samples were spun in a microcentrifuge for 2 to 5 seconds to force the condensation into the bottom of the tube with the sample and 300  $\mu$ L of phenol-chloroform-isoamyl alcohol (pH 7.8-8.2) (Acros Organics, Geel, Belgium) was added and subsequently vortexed for 2-5 seconds until a milky emulsion was obtained. The aqueous layer was separated from the phenol layer after centrifuging for three minutes and the former was added to a Microcon YM-100 filter equilibrated with 100  $\mu$ L of 10 mM Tris- 0.100 M EDTA (TE) buffer (pH 8.0) for concentrating and filtering the sample according to the manufacturer's instructions. The samples were washed with TE buffer, eluted into 100  $\mu$ L of TE buffer and subsequently frozen at -20 °C after preliminary quantification by agarose gel electrophoresis.

### **GEL ELECTROPHORESIS**

A 1% agarose gel loaded with the DNA logic molecular weight ladder (Lambda Biotech, St. Louis, MO) was used to determine DNA size and quantity. The DNA was visualized using SYBR Green intercalating dye. A 10  $\mu$ L sample of each extracted DNA solution was aliquoted into separate, sterile, 1.5 mL microcentrifuge tubes with 4  $\mu$ L of bromophenol blue loading dye (6x bromophenol blue/xylene cyanol/ficoll loading buffer containing 15% (w/v) Ficoll 400, 0.25% bromophenol blue, and 0.25% xylene cyanol) with SYBR Green (2:1 ratio, respectively). Ten microliters of the DNA ladder (sizes: 100-1000 bp in increments of 100

bp and 1500 bp and labeled on the gels) with 1  $\mu$ L of the bromophenol blue/SYBR Green mix served as the sizing and quantitation standard, the unburned extracted DNA served as the positive control and loading buffer plus sterile water alone (no DNA) served as the negative control. Agarose gels were prepared using a 50 mL 1x TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) buffer to a final percentage of 1% (15). After heating to dissolve the agarose, the gel material was cooled to < 60 °C and a 14-well comb was inserted into the poured gel. The samples were completely pipetted into the wells using micropipettes and sterile tips. Electrophoresis of the gels was conducted in 1x TAE buffer at 148 volts for 30 to 60 minutes. For the real-time PCR reactions (25  $\mu$ L), only 1  $\mu$ L of bromophenol blue loading dye (to visualize the sample during electrophoresis and sediment the sample in the well) was added, since the mixtures already contained SYBR Green dye and only 10  $\mu$ L of that mixture was added to each well in the agarose gel. Upon completion, digital photographs were recorded of each gel upon illumination under UV light and a SYBR filter.

### **PCR PRIMERS**

PCR primers were designed to amplify a 261 base pair amplicon (41 % GC content) of the *Sus scrofa* cocaine- and amphetamine-regulated transcript protein (CART) gene intron on chromosome 16 (NCBI Accession number: GI:147907868) from bases numbered 980-1240 (20). The 5'-primer sequence is TCC TTT CCC TCC AAT TTT CTT CCT and has a melting temperature of 56.3 °C. The 3'-primer sequence is CTG GCT CAT TTA GTT TCT CAT CCA and has a melting temperature of 54.9 °C (IDT, Coralville, IA). The expected melting temperature of the amplicon is 82 °C as calculated using the biotools software from Northwestern University (21).

### **REAL-TIME PCR**

Real-time PCR was conducted by the authors on a BioRad iQ5 instrument (BioRad, Hercules, CA) using the iQ5 software (v. 2.0) and SYBR Green dye on-site at Metropolitan State College of Denver. Pig-specific primers (20) were used to amplify the recovered samples and gauge the DNA quality by determining if the DNA produced the expected 261 base pair amplicon. An optimization study was conducted using a single stock of extracted, previously frozen (-20 °C), unburned, positive control DNA (number 14 in our collection, originally 11.6946 g, Q = 2.17) for a serial dilution and temperature gradient, to help identify the optimal annealing temperature of the primers (for a total of

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56 samples) and a 96-well plate. The samples included a negative (no template) control, non-diluted, 10x dilution, 100x dilution, 1,000x dilution, 10,000x dilution and 100,000x dilution. The gradient consisted of eight different temperatures, 64.0 °C, 63.5 °C, 62.3 °C, 60.4 °C, 57.9 °C, 56.1 °C, 54.8 °C and 54.0 °C. The optimal amplification temperature was determined to be 60.4 °C and used in subsequent reactions.

Subsequent plates were loaded with DNA from the open flame and convection oven burns and with unburned samples (same as for optimization, inter-plate control) and a negative control to evaluate the quality of the recovered DNA from each burn. No other calibrator standards were used. In each well, 12.5 µL of 2x iQ SYBR Green Supermix (BioRad, Hercules, CA), 1 µL of each of the 5 µM primers (CART 3' and 5'), 8.5 µL of nuclease free water, and 1 µL of undiluted DNA template were pipetted, mixed and amplified using the BioRad iQ5 instrument according to the following protocol: an initial 3 minute denaturation at 95 °C and 40 cycles of denaturation at 95 °C for 15 seconds and annealing/extension

at 60.4 °C for 60 seconds. A melting curve was produced by conducting 91 cycles of 30 seconds of melting every 0.5 °C from 50 °C to 95 °C.

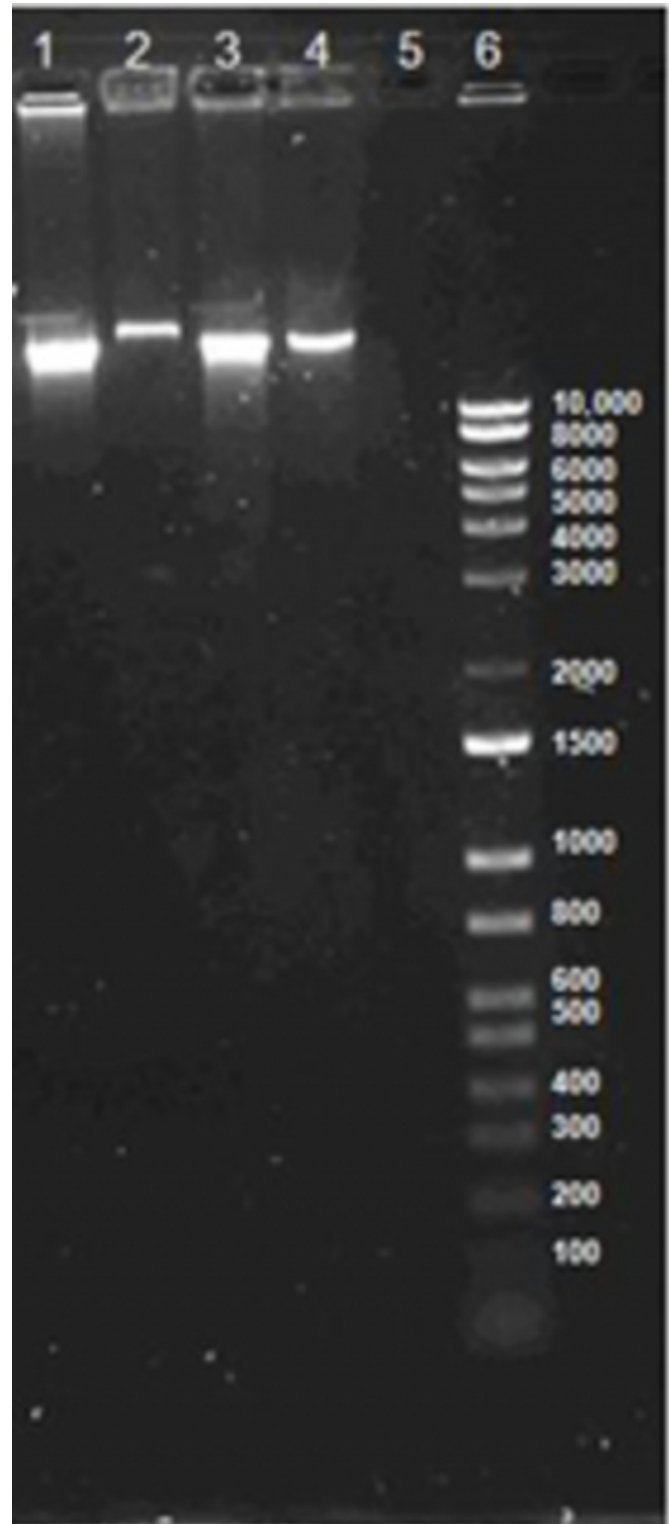
For all of the real-time PCR experiments, the maximum value from the first derivative of the melting curve for each sample was recorded. Graphs were produced by plotting this point versus the burn time in minutes at temperatures 73 °C and 82 °C. Error bars were included on the graphs representing the standard deviation between the three replicates of each like sample.

### **RESULTS**

All recovered DNA samples were initially analyzed for relative quality and quantity using agarose gel electrophoresis and RT-PCR. Figure 1 contains results from the DNA agarose gel electrophoresis on the unburned control tissue and bone marrow samples. The recovered DNA was of high quality as indicated by the tight bands (lanes 1-4) and of high molecular weight as indicated by comparison to the 10 kb to 100 bp ladder (lane 6).

**Figure 1**

Agarose gel of extracted DNA from control unburned tissue and bone marrow from two different samples. Lanes 1 is extracted bone marrow from one sample, lane 2 is tissue from the same sample as in lane1, lane 3 is bone marrow from a second sample, lane 4 is tissue from the same sample as lane 3, lane 5 is empty, and lane 6 contains the DNA Logic ladder (sizes indicated).



Interestingly, the yield from the bone marrow was significantly higher than that of the tissue (both were greater than the 100 ng standard). The results of the negative controls on the pre-amplification agarose gels, in the RT-PCR experiments, and on the post-amplification agarose gels demonstrated no DNA present in those controls (data not

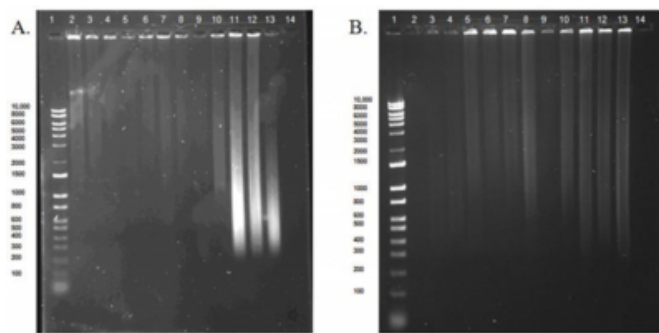
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shown).

Figure 2 shows agarose gel electrophoresis results from two representative burns: open flame for 25 minutes (Figure 2A) and 20 minute oven burn (Figure 2B).

**Figure 2**

Agarose gel of extracted DNA from open flame burn (25 minutes, 11.5974 g, average temperature for the burn was 526 °C) (Figure 2A). Lane 1 is the DNA Logic ladder (sizes indicated), lanes 2, 3 and 4 are replicates of tissue from the top, lanes 5, 6 and 7 are replicates of tissue from the middle, lanes 8, 9 and 10 are replicates of tissue from next to the bone, lanes 11, 12 and 13 are replicates of tissue from the bone marrow. Agarose gel of extracted DNA from oven burn 1 (20 minutes, 10.2730 g, temperature for the burn was 287 °C) (Figure 2B). Lane 1 is the DNA Logic ladder (sizes indicated), lanes 2, 3 and 4 are replicates of tissue from the top, lanes 5, 6 and 7 are replicates of tissue from the middle, lanes 8, 9 and 10 are replicates of tissue from next to the bone, lanes 11, 12 and 13 are replicates of tissue from the bone marrow.



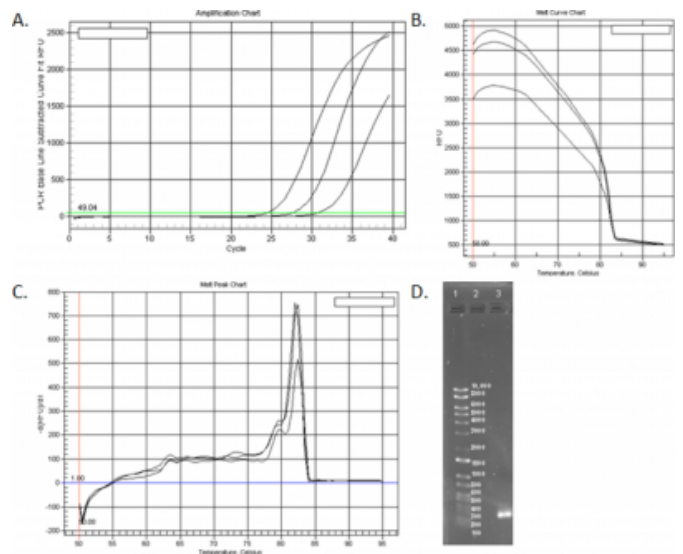
In both gels, lane 1 contains the DNA Logic ladder, lanes 2, 3 and 4 are the three replicates of the top samples; lanes 5, 6 and 7 are the three replicates of the middle samples; lanes 8, 9 and 10 are the three replicates of the next to bone samples; lanes 11, 12 and 13 are the three replicates of the bone marrow samples and lane 14 is empty. The open flame burn at an average temperature of 526 °C (Figure 2A) showed that the DNA was very degraded and of little quantity in the top, middle and next to the bone samples of DNA extracted from the tissue. The bone marrow samples still showed a significant quantity of DNA but also with degradation as indicated by the smear in the gel. The 20 minute oven burn at 287 °C (Figure 2B) showed significant degradation and limited quantity but fairly similar results throughout the replicate samples taken from all of the top, middle, next to the bone and bone marrow regions. The gels for the last three oven burns (30, 35 and 40 minutes, respectively) were devoid of any visible DNA on these pre-amplification gels except for a faint smear for one of the 30 minute middle

replicate samples (data not shown).

An optimization experiment (as previously described in the Methods) was used to evaluate the effects of varying the annealing temperature and the quantity of DNA using the pig-specific primers (20). Figure 3 shows the results of optimization study amplification curve (Figure 3A), melting curve (Figure 3B), and first derivative of the melting curve (Figure 3C) that was performed on an unburned positive control sample and the associated agarose gel results (Figure 3D).

**Figure 3**

Figure 3A shows the results of a real-time PCR with a positive control sample of serially diluted 1:1, 1:10 and 1:100, respectively (left to right traces), for the 64 °C annealing temperature. The 1:1000, 1:10,000 and 1:100,000 and the negative control samples are indistinguishable from the baseline as none amplified. The threshold cycle (C) was 24.35, 27.56, and 30.73 for the 1:1, 1:10, and 1:100 dilutions, respectively. Figure 3B shows the melt curve post-amplification for the same three samples shown in Figure 3A. Figure 3C shows the first derivative of the RFU units of the melt curve versus temperature. All dilutions exhibit the 82 °C melting temperature. Figure 3D shows the results of an agarose gel of extracted DNA from the real-time PCR serial dilution and gradient experiment on a control unburned sample. Lanes 1 is the DNA Logic ladder (sizes indicated), lanes 2 is empty, and Lanes 3 is the amplicon result for the 64.0 °C annealing temperature from the gradient. The 63.5 °C, 62.3 °C, 60.4 °C, 57.9 °C, 56.1 °C, 54.8 °C and 54.0 °C annealing temperatures produced equal quantity of product.



Shown in Figure 3A are the results of the real-time PCR experiment with the temperature gradient and the serial dilutions for an unburned control for the 1:1, 1:10, and 1:100

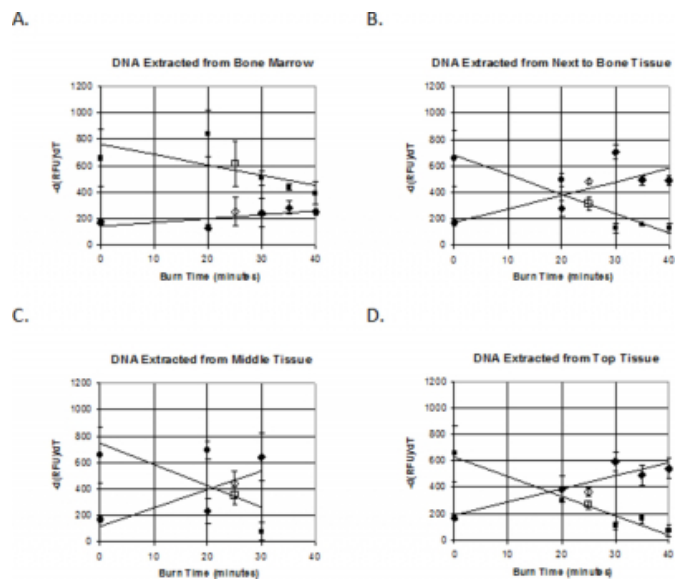
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dilutions (left to right traces, respectively) for the 64 °C temperature. The 1:1000, 1:10,000 and 1:100,000 and the negative control samples were indistinguishable from the baseline as none amplified. The pig-specific primers proved successful at amplifying the amplicon in an unburned positive control sample based on the expected 82 °C melting temperature (as predicted by Biotools, 21). The threshold cycle ( $C_T$ ) was 24.35, 27.56, and 30.73 for the 1:1 (left), 1:10 (middle), and 1:100 (right) traces, respectively, demonstrating congruence with the 3.33 cycles expected for a serial dilution (Figure 3A). Figure 3B shows the melt curve post-amplification for the same three samples shown in Figure 3A. Figure 3C shows the first derivative of the RFU units of the melt curve versus temperature. All dilutions exhibit the 82 °C melting temperature for the amplicon. Figure 3D shows the associated agarose gel results of the serial dilution and gradient from the real-time PCR on the positive control unburned sample with lane 1 containing the DNA Logic ladder, an empty lane 2 and lane 3 showing the expected amplicon produced (for representative annealing temperature 64 °C as all annealing temperatures produced equal quantity of product). Careful measurement and graphical analysis of the ladder and amplicon bands indicated that the expected amplicon of 261 base pairs was produced. The annealing temperature chosen for the real-time PCR (60.4 °C) was based on the melting temperature of CARTF ( $T_m$  56.3 °C) and CARTR ( $T_m$  54.9 °C) and the results of this experiment.

Figure 4 shows the results of the melting curve experiments conducted after the real-time PCR on the same instrument performed using DNA extracted from the bone marrow, next to the bone, middle of the muscle, and top of the muscle for the oven and open flame burns. The data from the triplicate samples have been averaged and the standard deviation is shown for each averaged data point. For interpretation purposes, a real-time PCR negative control series of three replicates each of nuclease-free water, primers and water, SYBR Green mix with nuclease-free water, and SYBR Green mix with nuclease-free water and the forward and reverse primers was conducted. The amplifiability of the negative reagent control from the PCIA extractions was also evaluated.

### Figure 4

Results of the average first derivative of the melting curve RFU maxima from the RT-PCR experiments for the triplicate samples of each type are plotted against the length of burn in minutes (e.g. 20, 25, 30, 25, or 40 minutes, respectively) for two temperature points: 73 °C (closed diamonds for the oven burns and open diamonds for the open flame burn) and 82 °C (closed squares for the oven burns and open diamonds for the open flame burn) for the DNA samples extracted from bone marrow (Figure 4A), next to the bone (Figure 4B), middle tissue (Figure 4C), and top tissue (Figure 4D). The standard deviation is shown for each averaged data point using error bars.



In Figure 4A, the average first derivative of the melting curve RFU maxima are plotted against the length of burn in minutes for two temperature points: 73 °C and 82 °C for the DNA samples extracted from bone marrow. As the burn time increases, the amplifiability (as indicated by the averaged intensity of the first derivative of the RFUs) of the DNA extracted from bone marrow decreases (82 °C melting temperature peak for the expected amplicon) as the remaining primers increases (73 °C melting temperature peak of the primers). Interestingly, the 25 minute open flame burn yield was directly in between the 20 and 30 minute oven burns. Figures 4B, 4C, and 4D are the same plots for DNA recovered from the next to bone samples, middle of the muscle samples, and top of the flesh samples, respectively. The highest DNA yield after the forty real-time PCR cycles was observed with the bone marrow samples, followed by the next to the bone samples, middle of the muscle samples, and top of the flesh samples with all of the samples. For all of the locations, as the burn time increases, the average amplifiability of the DNA extracted decreases as

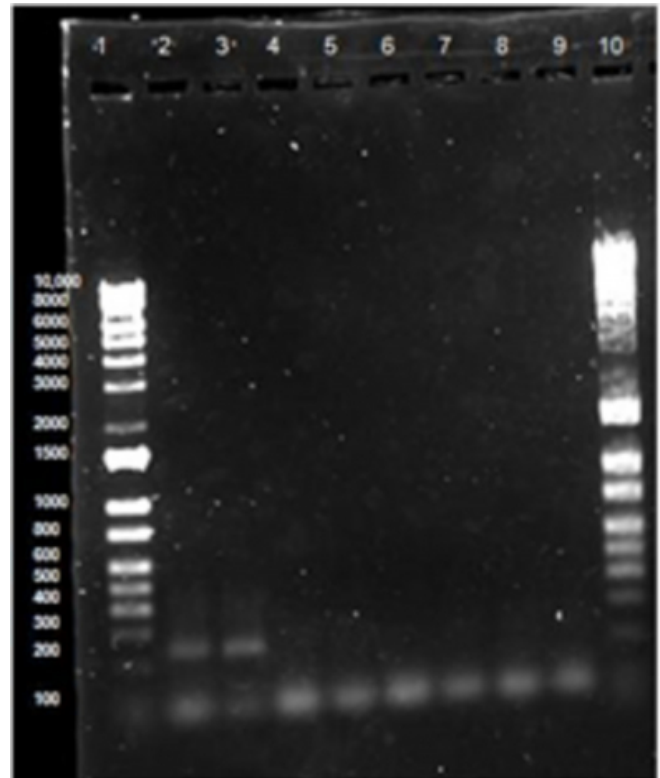
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the remaining primer peak increases but the plots flattened for samples extracted from the inner portion (bone marrow) of the sample to the top of the sample as the top samples were least amplifiable, as expected. The wells consisting of nuclease-free water, primers and nuclease-free water and SYBR Green mix with nuclease-free water showed no RFU, as expected (data not shown). The SYBR Green mix with nuclease-free water and the forward and reverse primers and the same reaction components with the negative PCIA control sample showed a prominent peak at 73 °C (24 bp) with a smaller peak at 77 °C (134 bp), and a very minor bump at 81 °C (261 bp) (data not shown).

Figure 5 shows post-PCR agarose gel results from the real-time PCR of all next to the bone samples from the twenty, thirty, thirty-five and forty minute oven burns. Samples are in duplicate, instead of triplicate, due to evaporation of the first replicate following real-time PCR.

### Figure 5

Agarose gel of extracted DNA from the real-time PCR experiments of all next to bone samples from the oven burn experiments. Samples are only presented in duplicate due to evaporation of first replicate during real-time PCR. Lanes 1 and 10 are the DNA Logic ladder (sizes indicated), lanes 2 and 3 are next to bone taken from oven burn 1 (20 minutes), lanes 4 and 5 are next to bone taken from oven burn 2 (30 minutes), lanes 6 and 7 are next to bone taken from oven burn 3 (35 minutes), lanes 8 and 9 are next to bone taken from oven burn 4 (40 minutes).



Lanes 1 and 10 contain the DNA ladder, lanes 2 and 3 are next to bone from the twenty minute oven burn, lanes 4 and 5 are next to the bone from the thirty minute oven burn, lanes 6 and 7 are next to the bone from the thirty-five minute oven burn and lanes 8 and 9 are next to the bone from the forty minute oven burn. Lanes 2 and 3 (20 minute burn) show faint primer bands as well as faint band above (the primer bands) which is the 261 base pair amplicon indicating amplifiability of these samples. The remaining lanes (4-9) are from the longer oven burn times (30, 35 and 40 minutes, respectively) and show no amplicon bands but all have slightly more intense primer bands due to the greater proportion of unused primers.

### DISCUSSION

Crime labs are most interested in yields of amplifiable DNA.



When comparing the gel results, it was clear that the convection-oven degraded the DNA more evenly than the open flame, even at half the temperature of the open flame (Figure 2A versus Figure 2B). The quality of the extracted DNA after the burns was degraded as indicated by the further migrating fragments at the longest burn time indicating smaller and more degraded DNA. Agarose gel electrophoresis results showed that an open flame burn for twenty-five minutes at an average temperature of 980 °F (526 °C) affected quality and quantity of the top and middle tissue more severely than that of the bone marrow, as expected. By contrast, the convection-oven burn for twenty minutes at a constant 550 °F (287 °C) degraded the tissue and bone marrow evenly. The agarose gel from the thirty minute convection-oven burn (287 °C) revealed only faint traces of extracted DNA from the bone marrow and none from the other sample locations and the thirty-five and forty minute increased times in the convection-oven at 287 °C revealed no DNA on the agarose gels (gels not shown). The forty minute oven burn yielded “green” bone that easily cracked and demonstrated full penetration of the burn (1). The longest burn times yielded no detectable post-extraction, pre-PCR DNA. As expected, the surface DNA was most degraded and had the lowest recovery, which is observed both on agarose gels and in the real-time PCR experiments.

Overall, as expected, the surface DNA and longest burn times exhibited the most degraded and least amplifiable of all of the burn samples as demonstrated by the real-time PCR experiments (Figure 4). Conversely, real-time PCR indicates that the best amplifiability was with the bone marrow samples from the twenty minute oven burn (Figure 4) but that for all of the locations sampled, the 73 °C peak increases as the 82 °C peak decreases in the melt experiments. The pig muscle samples had significant remaining tissue after the short burns times (e.g. 20-25 minutes) than what might be found in a human sample since burn times can extend significantly longer than the forty minutes examined in this study and the small pieces of bone and meat in this study do not mimic the a human body in terms of size and coverings. Whereas the skin was removed from the pig muscle and small ten gram samples were used, the short burn times were sufficient to burn the meat and bone completely in the forty minute oven burn. After forty minutes in the oven, only flake material was left on the bone in our simulated arson samples, and the bone itself crumbled. As the burn time increases, the bone marrow becomes the DNA source of choice for amplification,

whereas the top of the muscle decreases in recoverable DNA. Agarose gel electrophoresis was used in lieu of capillary electrophoresis fragment analysis (which would be expected to produce one peak) to verify the amplicon produced in the real-time PCR experiments in accordance with published real-time PCR guidelines (22). Figure 5 contains a post-PCR gel for the real-time PCR experiment of next to bone samples and supports the data in Figure 4.

The present study could be extended in the future to include other common extraction methods that are automatable including the Chelex® method (23) or the use of commercially available tissue extraction kits such as the Qiagen DNeasy blood tissue kit® or Promega DNA IQ® kit. The concentration of the extracted DNA sampled here was sufficiently large for amplification (>> 1 ng) and potentially for STR analysis, especially analysis of short STRs. Fragmented DNA may pose a problem for longer amplicons as observed in a recent study of controlled burns with teeth (17). In conclusion, the threshold of DNA degradation for use in DNA typing such as single nucleotide polymorphism (SNP) or short tandem repeat (STR) analysis appears to have been met even after the short forty minute burn on the top, middle and next to the bone samples, but the bone marrow DNA proved amplifiable, as expected.

## ACKNOWLEDGEMENTS

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