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Development of a microwave-based extraction for forensic biological samples

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ABSTRACT

In this study, a quick microwave-based treatment was developed as a front end for DNA analysis of forensic samples. The effect of microwave treatment is to cause cell disruption which can improve the release of DNA during direct PCR as well as with extraction methods. Exposure to microwave preprocessing improved the quality of rapid genotyping, particularly when used with low level samples. Optimal results were obtained when samples were microwaved at 300W for 40 s, resulting in improved allele detection. Overall, the addition of this simple preprocessing step improves sensitivity and allele recovery for low level DNA samples when combined with expedited DNA analysis workflows. Its main advantages include speed, low cost, compatibility with downstream DNA methods and application to a wide variety of samples.

1. Introduction

Forensic human biological evidence consists of biological fluids and tissues, such as blood, semen, saliva, epithelial cells, hair, bone, teeth, fingernails, and putrefied tissues. Collected evidence may contain mixtures of tissues or dried stains, as well as polymerase chain reaction (PCR) inhibitors from the crime scene. To generate the most complete DNA profiles, the collected samples are subjected to nucleic acid extraction and purification methods. Some of the common extraction methods involve the use of automated DNA extraction systems and/or phenol chloroform based separations, as well as silica based extractions and others collated in a recent review [1]. Although these conventional methods are utilized in forensic DNA laboratories, they can be labor intensive, relatively slow, costly, and inefficient [2]. Furthermore, because complex extraction methods require multiple steps and tube transfers, (e.g., cell lysis, protein digestion, DNA isolation to purification) they can result in sample loss resulting in reduced peak heights and allele calls [1,2].

Recently, direct DNA amplification systems have been implemented for forensic applications to streamline the processing of reference samples [3,4]. In these systems, samples undergo a crude digest without purification, skip quantification entirely by relying on the laboratory's validation of the process, and are amplified directly prior to analysis by capillary electrophoresis [4]. Sample processing can be further sped up by the use of fast thermal cyclers which use specialized PCR tubes with increased surface area so that heat may be transferred more efficiently [5]. Although the operation time is shorter than traditional procedures, direct PCR DNA systems are currently designed for use with buccal swabs, samples which generally contain abundant quantities of recoverable DNA. Such systems are generally less effective when confronted with crime scene samples, particularly those involving low quality (degraded and inhibited samples) or quantity (touch DNA) [6]. To improve the recovery from these challenging samples, a possible solution is to add a brief sample pre-treatment step to improve extraction efficiency. The goal of this study was to develop a microwave irradiation treatment to improve the recovery of forensic samples (saliva, blood, and semen) when used with rapid and direct DNA amplification methods. The rapid and direct amplification system used was an in-house developed 9 miniSTR multiplex. This newly developed system was derived from a previously developed multiplex [7].

The first published microwave extraction research from Bollet et al., in 1991 used an ordinary microwave oven to isolate chromosomal DNA

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from Gram positive or acid-fast bacteria [8]. Later, microwave irradiation was applied to extract DNA from eukaryotes including fungi, plants, protists and animals [9]. Computer driven microwaves have been evaluated for their impact on DNA yield using various power settings and durations, on different amounts and types of biological samples [10]. Studies found a setting of two 8 s microwave pulses at 800W with an intermittent rest of 20 s, effectively isolated DNA in under 1 min with 400 µL lysis buffer and resulted in only a slight increase in temperature [10].

Microwave extraction methods have been reported to result in higher DNA yield and purity than chemical and enzymatic extractions [11]. Furthermore, microwave extracted DNA has been successfully used as template for downstream qPCR and PCR applications [12]. Microwaves are already well established as a method for heating samples to isolate analytes [13,14]. Cell disruption leading to DNA release has also been reported [15]. Therefore, microwave-based extraction may provide an alternative for rapid and effective isolation of DNA [16]. For forensic applications, this microwave-based technique can simplify on-site sample preparation by eliminating the use of additional chemical treatment, making it an efficient front-end for processing difficult samples [17]. Moreover, the use of a fast thermocycler and a rapid direct miniSTR PCR protocol can further speed up the process.

The workflow used for this study is shown in Fig. 1. This consists of 1) collection of samples, 2) pretreatment in a microwave (20–40 s) followed by 3) manual rapid direct PCR using the 9 miniSTR multiplex (13 min).

2. Materials and methods

2.1. Sample collection

Five biological samples (two saliva samples, two blood samples and one semen sample) were collected from three volunteer donors using the Institutional Review Board (IRB-19-0354) from Florida International University. The methods used for collection of the body fluids consisted of the following steps: Saliva samples were collected by having volunteers spit into 1.5 mL tubes. The samples were then diluted to 1% using deionized water, creating a stock solution of 200 µL final volume (2 µL saliva and 198 µL water). Blood samples (drops) were collected using a finger prick into 500 µL tubes. The samples were then diluted to 1% using water, (2 µL blood and 198 µL water) creating a stock solution of 200 µL final volume. Semen samples were collected in 50 mL tubes. The samples were then diluted to 0.5% using water, (2 µl semen and 398 µL water) creating a stock solution of 400 µL final volume. The samples were kept frozen until processing. Replicate samples from each stock were taken for each experiment as described below.

2.2. Commercial microwave calibration

Since one goal of the project was to develop a new, low cost extraction technique, we first employed an Amazon Basics Microwave (700W).

Microwave energy imparted to samples will differ depending on the sample volume, position, and load which includes diluent used, tube number and type and energy settings. Too little energy may result in inefficient DNA recovery and too much energy may result in DNA degradation. Thus, calibration of the microwave oven is necessary before implementation. Specifically, we targeted areas of the microwave that would impart medium level, continuous energy to the samples resulting in the highest DNA recovery.

Both neon bulbs and water condensation tests were used to identify the cold and hot spots in the chamber of the commercial microwave oven. The circular, rotating plate inside the microwave was removed and a rectangular tube rack was placed inside (Fig. 5). Neon bulbs (Uxcell 100Pcs F6 6 × 16mm Bright Red Light Neon Light Bulb Indicator Lamp) in 1.5 mL tubes were placed in each row of the rectangular tube rack, and exposed to continuous microwaves for 20 s at Power 10. During microwave operation, the excitation time of the bulbs and the strength of the emitted light were recorded. Afterwards, 100 µL of water in 1.5 mL tubes were placed in the same tube rack and exposed to the same microwave power and time. The temperature of the water, levels of condensation and the status of whether tubes were opened or closed due to excessive internal pressure were recorded (Fig. 2).

2.3. Computer driven microwave calibration

Commercial microwaves do not include the ability to directly adjust and monitor microwave wattage; therefore, additional tests on saliva samples were performed on a computer-driven microwave using adjustments to the microwave energy wattage to establish the most efficient time and power required for DNA recovery from the samples.

In the first studies, three 1% saliva replicates from the same donor were exposed at the highest energy power of 700W for different durations: no microwave, 10 s, 20 s, 30 s, 40 s and 50 s. They were later quantified using Real time PCR (refer to section 2.6 below).

In the second test, another set of 1% saliva sample replicates from the same donor were exposed to 40 s of microwaves for different ranges of power: no microwave, 300W, 400W, 500W, 600W and 700W. The goal of the last test was to check the yield of DNA, using the power and time settings of the Microwave oven determined in the first studies. Since the highest yield was observed for 300W, three replicates of 1% saliva samples from the same donor were exposed to 40 s of microwaves at the following lower energy levels: no microwave, 100W, 150W, 200W and 300W. The samples were quantified following the microwave treatment.

2.4. Conductivity test

Saliva samples were used to determine the effect of buffers and detergent on the efficiency of extraction. Therefore, dH_2O, Tris Ethylenediaminetetraacetic acid $^{-4}$ (TE^{-4}) 1X and Tween-20 solutions were utilized to dilute saliva samples and evaluate their effects. For this experiment, fresh saliva sample replicates were either used immediately after collection, were stored in a 4 °C fridge for a short time (less than 1 h), stored in a −20 or −80 °C freezer for longer durations (over 1 h- 24 h) or were collected with swabs and then completely air dried.

The saliva was diluted to concentrations of 75%, 25%, 10% and not

Fig. 1. Overview and speed of the microwave based rapid direct STR process.

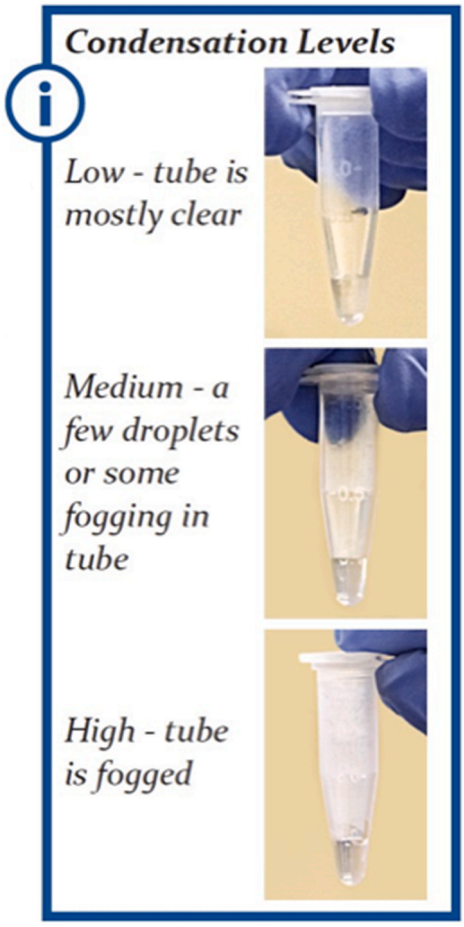

Fig. 2. Three different condensation levels with pictures from the condensation test.

diluted. Each buffer dilution was measured in triplicate using a Horiba B-173 Twin Cond Conductivity Meter according to the manufacturer's protocol [18].

2.5. Microscopy test

Three replicates of 200 μL of fresh saliva were pretreated with either 20 s of microwaving or non-microwaved. The liquid samples were transferred by cotton swabs onto glass slides, and subsequently dyed with 1% Methylene Blue and 1% Trypan Blue as previously reported [19]. The cellular content of microwaved versus non-microwaved saliva was observed using the Leica Laborlux 12 Pol S polarized microscope (Leica Microsystems, Wetzlar, Germany). Cells were counted manually using a grid.

2.6. Quantification test

All samples were quantified with a Qubit 4.0 (Thermo Fisher) and a qPCR method. The Qubit assay used the Qubit 1X dsDNA HS (High-Sensitivity) Assay Kit according to the manufacturer's protocol [20]. The qPCR method utilized a Rotor Gene 6000 from QIAGEN with Rotor-Gene SYBR Green PCR Kit for Real-Time PCR analysis according to the manufacturer's protocol [21]. The primer sequences were Alu-F (5′ GTCAGGAGATCGAGACCATCCC 3′) and Alu-R (5′ CCACTACGCCCGGCTAATTT 3′) and the PCR and thermal cycling protocol was performed as previously reported [22]. A series of saliva dilutions, 100%, 90%, 85%, 50%, 25%, 10%, and 0% with the buffers, d.i. H_2O, TE^{-4}, and Tween-20, were quantified in triplicate.

2.7. MiniSTR kit development

The blood, semen, and saliva samples were prepared according to their specific dilutions and amplified using an in-house 9 loci miniSTR direct amplification multiplex as described by Boelens et al. [7]. The use of these miniSTRs (ranging in size from 78 to 281 bp) can improve typing on highly degraded samples over conventional STR systems. This test kit includes D2S441, D18S51, D10S1248, FGA, AMEL, D8S1179, D7S820, D21S11, and vWA and was amplified using a Philisa® thermal cycler (Streck Biosciences) using a hot start of 94 °C for 90 s followed by 30 cycles of 94 °C for 2 s and 62 °C for 20 s based on the procedure used by Aboud et al. [5].

2.8. DNA yield test

DNA recovery was tested using eight 1% saliva samples, four 1% blood samples, and two 0.5% semen samples collected as described in section 2.1. Donor A contributed saliva and blood samples while Donors B and C contributed saliva and semen samples, respectively. Prior to amplification, half of total number of samples were microwaved for 40s at 300W in the commercial microwave while the other half remained non-microwaved. Samples were analyzed on the Applied Biosystems™ 3130XL Genetic Analyzer and average peak height at each locus was calculated by averaging relative fluorescence units (RFUs) for each heterozygous locus and dividing each homozygous allele in half. Allele recovery was determined by the percentage of expected alleles produced by each sample. Random match probabilities (RMP) were calculated using the allele frequencies from the NIST 1036 database [23] after removing those loci in which dropout was observed. Multi-way ANOVAs were performed to evaluate whether there was a significant difference in average peak heights and allele recovery based on microwave treatment status while accounting for multiple body fluids and loci.

3. Results and discussion

3.1. Buffer

Saliva samples were diluted with d.i. H_2O, 10% Tween 20, and TE^{-4} 1X, to examine the effect of detergents and buffers on extraction as well as to simulate low template samples commonly encountered in forensic casework. The main reason for altering the sample by dilution is that the salt concentration impacts the efficiency of the microwave since electromagnetic radiation from microwaves interacts with dipoles in water molecules as well as solvated ions. Dilution of samples is also a commonly used approach to decrease the impact of any substances that may be co-extracted [24]. For example, residues of compounds in cigarette smoke present in saliva are known to inhibit PCR [25]. Conductivity decreased linearly with the diluted concentrations of saliva samples (Fig. 3A). Additionally, due in part to the presence of bacterial nucleases in saliva [26] that may result in loss of DNA over time, high variability in recovery of DNA from stored saliva replicates may be observed. Therefore, saliva samples were preserved at very low temperatures or air dried completely if they could not be used immediately.

Saliva samples diluted with water, detergent or tris-EDTA were quantified to evaluate the impact on DNA yields. The results shown in Fig. 3B indicated that 90% and 75% dilutions with water and TE^{-4} solutions provided comparable results to 100% saliva. Moreover, water provided comparable yields to TE^{-4} while yields were reduced in Tween-20. Based on these results, d.i.H_2O was selected to dilute saliva samples for the remainder of the experiments. Water has additional advantages as it is, a very good microwave-absorbing solvent, due to its dipole moment [27].

3.2. Microscopy of microwaved vs non-microwaved saliva samples

Measurements of cell disruption were performed using polarized

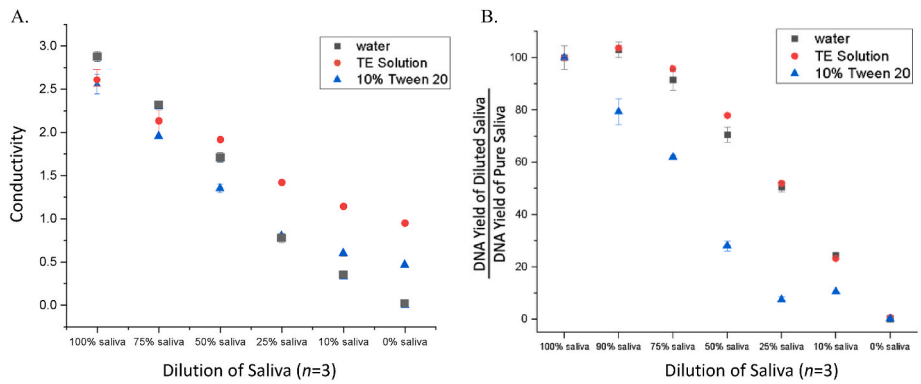

Fig. 3. Impact of different buffers on the (A) conductivity and (B) DNA yield from saliva dilutions.

light microscopy. Saliva samples were dyed with Methylene Blue, and cells were observed before and after 20 s of commercial microwave treatment (Fig. 4). For the microwaved samples, the number of stained intact cells decreased compared to the non-microwaved samples when replicates at the same volume when observed under the same field of view and magnification (Fig. 4A and B). It was also noted that treating a sample one time for 20 s (Fig. 4C), produced more cell disruption than a similar sample that was microwaved three times at 8 s each. Samples prepared with trypan blue, showed a similar result (Supplemental Information and Figures: Figure SI1).

These results indicate that the microwave process reduces the percentage of intact cells remaining in the sample. Based on these observations, it appears that microwave treatment disrupts cell and nuclear membranes as discussed in previous reports [28]. In general, electromagnetic radiation from microwaves interacts with dipoles in water molecules as well as solvated ions. It is likely that increased ionic strength within cells can cause localized heating within cells, resulting in the rupture of cell membranes and the release of the components inside [29]. In addition, localized disruption of cell membrane structure has also been seen, indicating that microwave radiation can specifically interact with cell membrane components resulting in poration of the cells and leakage of cellular components [30].

3.3. Computer-driven microwave calibration

The microwave power and time were optimized with a computer-driven microwave that permitted real time monitoring of energy and temperature in the oven. When diluted liquid saliva samples (1%) were microwaved for 40 s in the computer driven microwave at 0, 300, 400, 500, 600, and 700W, the microwave treatment of 300W resulted in the highest average DNA yield of 0.40 ng/μL (Table 1).

Since results using 40 s at the lowest energy tested (300W) provided the highest yield, further testing was performed at lower energies from 100 to 300W (Table 2). Microwave DNA yield results at 40 s with 300W

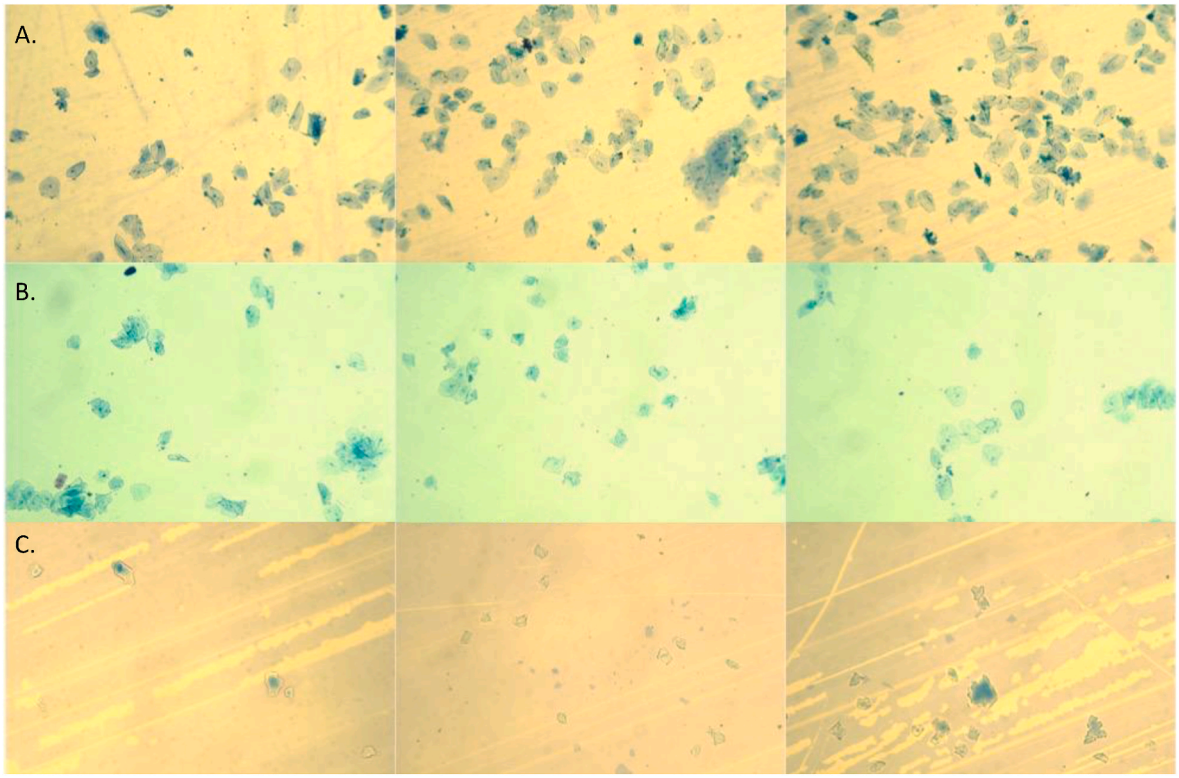

Fig. 4. Microscopy of swabs dipped into saliva and then smeared on the slide. Each column represents a replicate sample. A. Non-Microwave saliva sample B. Microwaved sample for 8 s three times. The number of cells decrease; however, some cell membranes and nuclei remain intact. C. Microwave sample for 30 s (For interpretation, the reader is referred to the web version of this article).

Table 1
qPCR DNA yield results (ng/μL) from replicate liquid saliva samples under varying microwave energy levels from 300 to 700W with 40 s microwave duration.

40 s tests	Sample 1	Sample 2	Average (ng/ul)	STD
no microwave	0.352	0.286	0.319	0.047
microwave 300W	0.381	0.417	**0.399**	0.025
microwave 400W	0.277	0.323	0.300	0.033
microwave 500W	0.29	0.307	0.299	0.012
microwave 600W	0.289	0.363	0.326	0.052
microwave 700W	0.321	0.276	0.299	0.032

Table 2
qPCR DNA yield results from replicate saliva samples under varying microwave energy levels from 100 to 300W with 40 s microwave duration. Note that sample 2 for the 300W treatment was lost due to a processing error.

	Sample 1	Sample 2	Sample 3	Average (ng/ul)	STD
microwave 100W	0.303	0.362	0.128	0.264	0.122
microwave 150W	0.289	0.314	0.216	0.273	0.051
microwave 200W	0.451	0.317	0.158	0.309	0.147
microwave 300W	0.454		0.307	**0.381**	0.104

provided the highest yields at 0.38 ng/μL (Table 2) versus the no microwave test shown in Table 1 (0.32 ng/μL).

Another test was performed using the same energy with different time durations to evaluate if shorter microwave treatments at higher energies would increase DNA yield. The diluted saliva samples were microwaved at 700W varying times as described in methods above, and qPCR DNA quantities were measured as shown in Table 3. During this microwave duration testing, additional measurements of total cell counts and cell viability as an indicator of cell disruption were performed using differential dye staining (see microscopy section).

The DNA yield obtained using 40 s of 700W energy (0.34 ng/μL) was lower than the results for the 300W, 40 s treatments shown above (0.40 and 0.38 ng/μL vs. no microwave 0.32 ng/μL). Overall, the microwave calibration data indicated that the highest yields are obtained at 40 s of irradiation using 300W energy.

During the microwave time test, microscopy was performed, and DNA yield measured. While a correlation of cellular disruption (intact cell decrease) was observed with increasing microwave time (Fig. 4 and Table 3), the level of cellular disruption was not always proportional to DNA yield (Table 3). As mentioned above, microwave irradiation may not always lyse the cells but instead act by causing damage to the membranes permitting the release of cellular components, potentially including nucleic acids [15,30]. It should also be noted that when an excess of nuclear DNA is present in a sample, there is little need for additional microwave treatment, as there is more than sufficient sample for a complete DNA profile, regardless of treatment. In addition, a high degree of variation in DNA yields from saliva sample replicates was observed during calibration. The use of known stabilization buffers and additional swab types including Copan flocked swabs were evaluated; however, they did not improve reproducibility. This variation may be due to saliva sample non-uniform collection efficiency, recovery, storage, stability, or differences in cell free DNA between replicates.

3.4. Commercial microwave calibration

To translate the optimized microwave energy power (300W, 40s) from the computer-driven, laboratory-based microwave to a commercial microwave, a series of calibration tests were performed. Neon bulb excitation, temperature measurement tests, and sample retention tests were employed to determine the energy intensity and distribution in the commercial microwave oven. Various sample positions were identified as the most suitable locations in the microwave oven to extract samples, based on mapping samples in a test tube rack located in the same fixed space in the microwave oven.

Using a neon bulb test, the light emitted from different positions was observed to vary depending on the energy imparted. The level of light generation observed was designated as none, weak, medium, or strong and these observations were also used to determine if the light emitted was continuous or discontinuous. To avoid the hot/cold spots in the microwave oven, only positions 1–4 in Row 5 at the front of this microwave (Fig. 5 and Supplementary Information and Figures: Figure SI2) were selected for further experiments because the bulbs at these positions emitted a continuous but medium level light. These four locations were utilized for further condensation tests, temperature tests, and low levels of condensation were observed from tubes in these positions. Interestingly, with the same microwave time and tube locations, when higher volumes of water were used in the tubes, samples were found to reach higher temperatures.

Following a series of empirical tests in locations 1–4, with varying power levels at different total times in the commercial microwave, results showed that 95 s at power level 5 with the commercial microwave is needed to deliver 40 s of actual microwave energy due to the cycling of the oven. Using 95 s at power level 5 in the 700W commercial microwave produced similar energy to 40 s at 300W in the computer driven laboratory-based microwave (Supplementary Information and Figures: Table SI1). The commercial microwave manufacturer manual states that power levels (scale of 1–10) translate to 10–100% of the highest wattage level.

Since commercial microwave ovens may impart different levels of energy depending on the uniformity of magnetron energy, sample position, buffer type and volume used, it is recommended that laboratories perform their own calibration. Therefore, a set of instructions was

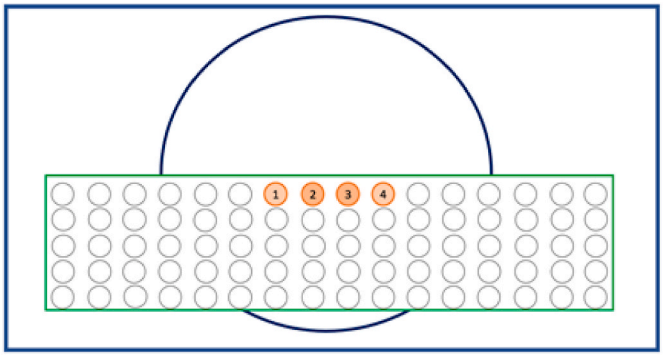

Fig. 5. Based on Neon Bulb Calibration of Microwave, energy positions 1–4 in Row 5 were selected for further experiments.

Table 3
qPCR DNA Yield and Cell counts for replicate saliva samples microwaved at 700W using 10, 20, 30, 40 and 50 s durations.

700W tests	Sample 1	Sample 2	Average -concentration (ng/ul)	STD	Viable cells	Non viable cells	Total Cells
microwave 10 s	0.31	0.276	0.293	0.024	95000	435000	5.3E+05
microwave 20 s	0.222	0.148	0.185	0.052	35000	455000	4.9E+05
microwave 30 s	0.257	0.341	0.299	0.059	75000	520000	6.0E+05
microwave 40 s	0.342	0.341	**0.342**	0.001	70000	540000	6.1E+05
microwave 50 s	0.27	0.313	0.292	0.030	20000	575000	6.0E+05

developed to permit users to utilize optimal parameters for sample processing. To do this, neon bulb tests are used to determine the hot spots inside the microwave and additional tests can be performed to identify areas with high condensation (Supplementary Information and Figures: Video SI1). Afterwards, selected rows should be tested at different power levels for 80, 90, 95 and 100 s to determine the optimal settings to mimic a 40 s microwave treatment at 300W (See Supplementary Information and Figures: Table SI1). During these tests, it was determined that many microwave ovens control energy level input by simply turning on and off at certain times, thus the energy input is not continuous (Table 4).

3.5. DNA yield comparison

To evaluate the effect of microwave treatment on rapid direct DNA analysis, eight replicates of 1% saliva, four (4) replicates of 1% blood, and two (2) replicates of 0.5% semen, were either not microwaved or microwaved for 40 s at 300W, directly amplified on a Philisa thermal cycler without any quantitative analysis, and analyzed on a 3130XL genetic analyzer according to the manufacturer's protocols. As the raw data was skewed, it was log transformed prior to calculating summary statistics. The geometric mean of the average peak heights for the microwaved samples was 74% ± 5% higher than that of their non-microwaved counterparts (Supplementary Information and Figures: Table SI2). Notably, 10 loci of 126 completely dropped out 2 loci partially dropped out for non-microwaved samples while only 4 loci dropped out for microwaved samples. This is also reflected by the percent allele recoveries where there was a 13% ± 45% increase in allele recovery for the microwaved samples over the non-microwaved samples. For reference, the random match probability (RMP) of a full profile for microwaved blood from Donor A = 1 in 3.01 billion while the modified RMP after removing D2S441 and D21S11 was 1 in 9.16 million for the non-microwaved blood sample. Similarly, the modified RMP for non-microwaved 0.5% semen sample after removing D8S1179, D7S820, D21S11, and vWA was 1 in 44.4 thousand while the microwaved sample only had dropout at D8S1179 and D21S11 resulting in a modified RMP was 1 in 5.91 million. In each instance, there is a difference in RMP of several orders of magnitude between microwaved and non-microwaved samples.

A multi-way ANOVA accounting for microwave treatment status, body fluid, and specific locus revealed a significant difference in the log transformed average peaks heights for microwave treatment status (F-statistic = 4.18, p-value = 0.044). Further analysis by a Tukey post-hoc test indicated that the significant difference in the log transformed average peak height by body fluid (F-statistic 24.2, p-value = $< 2.6x10^{-9}$) was based on the difference between saliva and blood (p adj = <0.001) and between saliva and semen (p adj = <0.001). The difference in average peak height between loci (F-statistic = 1.92, p-value = 0.065) was explored because the miniSTR multiplex used in these experiments was homemade and found to be insignificant.

A two-way ANOVA of the percent allele recovery for the three body fluids with microwave treatment status and body fluid as factors was statistically significant for body fluid (F-statistic = 5.04, p-value = 0.031) but not for microwave treatment status (F-statistic = 3.430, p-value = 0.094). Tukey post-hoc testing confirms that the body fluid difference depends saliva versus semen (p-adj = 0.031) which is likely due to the higher average peak heights in both microwaved and non-microwaved saliva samples of 1340 ± 850 RFU as compared to semen samples at 270 ± 120 RFU. However, it is noted that percent allele recovery is bounded by the total number of possible alleles and therefore the entire trend cannot be observed. All allele designations were concordant for microwaved and non-microwaved samples (See Supplementary Information and Figures: Table SI2 for data and peak height calculations).

Microwaved vs non microwaved blood sample results demonstrated increased peak heights on average of 522% (range 12–963 RFU- Fig. 6 and Supplementary Information and Figures: Table SI3). Note that the increase in amplification for the blood sample shown represents the highest increase observed.

Experiments examining semen samples using microwave treatment (40 s at 300W) vs non-microwave using 0.5% dilution of semen (10uL) demonstrated increased peak heights of an average of 100% as well as an increase in the number of alleles detected (Supplemental Information and Figures SI4). However, there was no significant difference observed in the peak heights for not microwaved versus microwaved 0.5% semen possibly due to increased dropout.

Table 4
Lightbulb activity observed from neon bulb calibration in row 5 position 4 at Power Level 5 for 95 s.

Observations	Seconds	Lightbulb Activity observed
Microwave starts	2.55	
Lightbulb on	5.11	2.56
Lightbulb off	16.38	**11.27**
Lightbulbs on	33.27	16.89
Lightbulb off	45.56	**12.29**
Lightbulb on	62.45	16.89
Lightbulb off	74.23	**11.78**
Lightbulbs on	91.64	17.41
Microwave stops	97.27	**5.63**
Total time lightbulbs on		**40.97**

4. Conclusions

In this study, a microwave pretreatment method was developed to improve the recovery of profiles from trace levels of DNA in forensic samples. The experiments performed demonstrate that microwave treatment can improve the yield of DNA from diluted saliva, blood and semen samples, based on increased STR allele recovery and peak heights using direct amplification. The results obtained indicate that the microwave treatment can provide a fast, safe, and inexpensive method for sample extraction compared to the common methodologies currently in use.

In summary, microwave treatment increased DNA recovery, STR peak heights, allele detection, and provided a more informative RMP across all sample types tested. Additional studies being prepared for publication include the evaluation of microwave treatment for increased sensitivity on mixtures, mock touch samples, and the impact on very low template sample results from the automated RapidHIT ID system (ThermoFisher, CA).

The microwave method developed herein, has broad application to clinical diagnostics, environmental and microbial forensics, biodefense and biothreat agent detection, on site field applications and others in which rapid, sensitive molecular detection is of paramount importance. The method has potential for wide adoption due to the potential increase in sensitivity, speed, simplicity, low cost, omnipresence of microwave ovens, portability and compatibility with downstream direct PCR applications. Finally, further studies are underway to evaluate the impact of our calibrated microwave treatment for inactivation of inhibitors as previously reported for serum samples [12], and recovery from additional challenging forensic sample types such as bone [31], formalin-fixed paraffin-embedded samples [32] and for differential extractions of sexual assault evidence.

Financial disclosure

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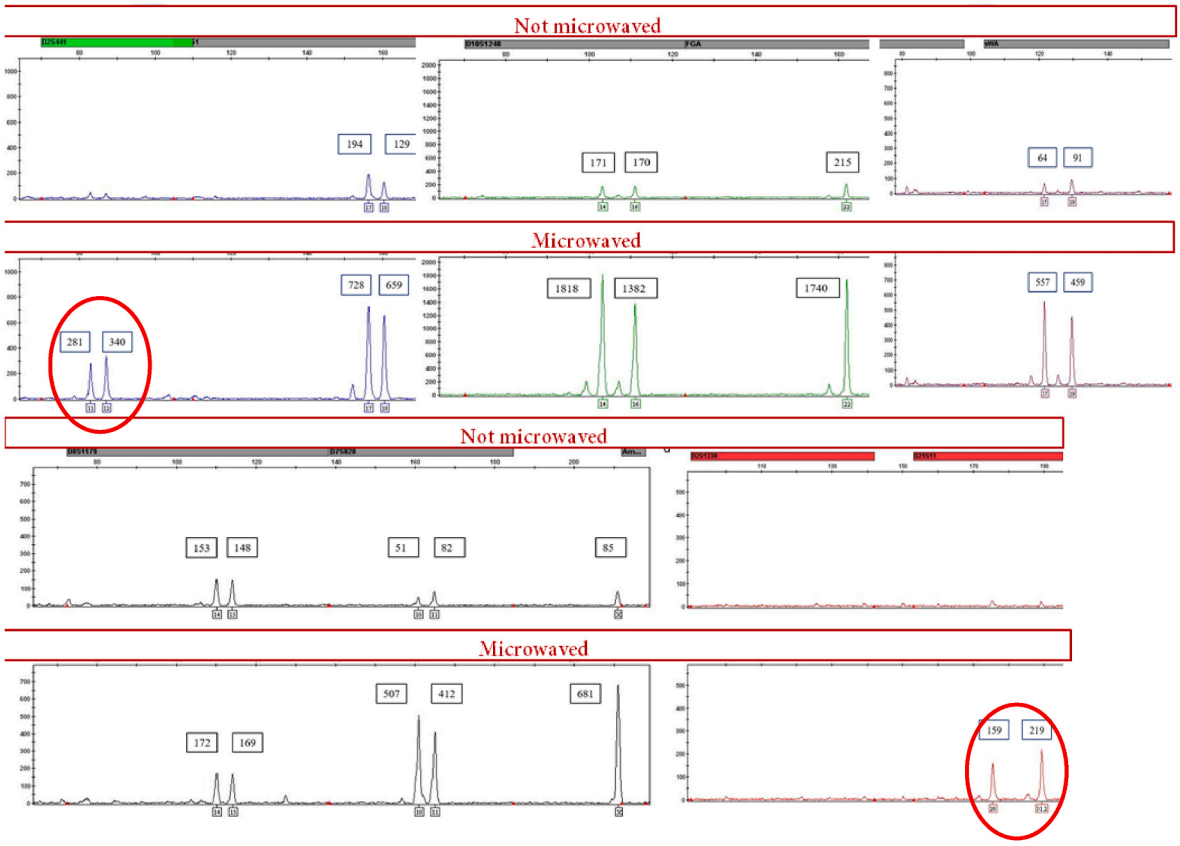

Fig. 6. Manual Direct STR Electropherogram result of female donor A4 blood sample diluted to 1%. Control replicates were non-microwaved. Treated replicates exposed to 40 s of microwave at 300W. Microwaved and non-microwaved DNA profiles are shown. The boxes above the peaks represents the Peak Height values expressed in RFU (Relative Fluorescence Units). An average increase in peak height of 522% and additional allele recovery shown in red circles were observed.

CRediT authorship contribution statement

Fabiana Taglia: Investigation, Validation, Formal analysis, Writing – review & editing, Visualization. **Ling Wang:** Formal analysis, Writing – review & editing, Visualization. **Casandra H. Setser:** Investigation, Validation, Formal analysis, Writing – review & editing, Visualization. **Nicole Fernández-Tejero:** Investigation, Validation, Formal analysis, Writing – review & editing, Visualization. **Bruce R. McCord:** Conceptualization, Methodology, Resources, Writing – review & editing, Funding acquisition. **Steven B. Lee:** Conceptualization, Methodology, Resources, Data curation, Formal analysis, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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