## B47 Accelerating DNA Extractions Using a Microwave Toward Increasing the Speed and Success of Rapid DNA Analysis

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**Learning Overview:** After attending this presentation, attendees will better understand how a microwave can be used to help extract DNA for Rapid DNA analyses.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees how the use of a microwave to extract DNA can greatly increase the speed of extractions and has the potential to also inactivate downstream Polymerase Chain Reaction (PCR) inhibitors.

The goal of this project is to develop a quick microwave-based extraction technique as a front end for rapid DNA analysis. The recent development of rapid and microfluidic technology has made it possible to perform field-able analysis of saliva samples from suspects.[1] However, when confronted with forensic samples such as blood, semen, and touch DNA, these same instruments often perform less well. Currently available instruments can perform DNA typing from extraction through genotyping in fewer than 90 minutes and this newly developed ultra-rapid DNA system can yield genotypes in fewer than 15 minutes.[2,3]

The problem is that the processes these instruments use to isolate DNA are slow and inefficient when utilized with samples other than saliva and may not be fast enough nor effective enough for certain sample types and critical time-sensitive intelligence applications. Results from studies involving crime scene samples indicate the need to mitigate issues with sensitivity and PCR inhibition and to improve the speed of rapid DNA systems. Furthermore, processing difficult samples in remote locations is important for rapid on-site intelligence applications. Thus, there is a need to develop a rapid and efficient front end for processing difficult samples in remote locations. This project will develop microwave DNA extraction methods as a front end to a variety of commercial- and laboratory-based rapid Short Tandem Repeat (STR) genotyping systems to improve the speed and quality of results for rapid genotyping of challenging forensic samples.

The importance of effective forensic DNA extraction methods was reviewed by Lee and Shewale, and while there are many effective procedures, several are time-consuming, use hazardous chemicals, unstable chemistries, and/or are generally not amenable to field deployment.[4]

Microwave DNA extraction methods were first published for gram positive bacteria in 1991 and for eukaryotic organisms in 1993.[5,6] Microwave extraction has been applied to paraffin-embedded tissues, sludge, cyanobacteria, spores human disease vectors, bone, serum, and blood resulting in DNA suitable for both Polymerase Chain Reaction (PCR) and Massively Parallel Sequencing (MPS) applications.[7-16] In a study comparing chemical, enzymatic, and microwave extractions, results showed that the microwave method outperformed the other two methods in both the quality and quantity of recovered DNA.[16] This study concluded that the microwave-based procedure is easy, rapid and cost-effective for high-yield isolation of analytical-quality DNA.[17]

Three different microwaves have been successfully used for DNA extraction in the laboratory. In the first study, a dramatic increase in DNA isolation speed was demonstrated using a conventional 400W microwave oven on eukaryotes, including plants, fungi, snake and protists.[6] In a second study, a microwave DNA isolation method was successfully applied to human forensic samples: blood, saliva, semen, fingernails, and hair, in collaboration with Ted Pella Inc using a computer-driven microwave: Ted Pella Inc., Model 3440 at 800W.[18] Two eight-second pulses at full power with an intermittent rest of 20 seconds could be used to isolate DNA from 16 samples in 400µl of extraction buffer simultaneously while only slightly raising the temperature (4–6ºC) in less than one minute, resulting in comparable quality and quantity to conventional methods.[18]

A third study tested the compatibility of microwave-extracted DNA with direct rapid PCR. Replicate 15µl saliva samples were microwave extracted, then amplified directly with a rapid two-step PCR protocol for amplification of a seven locus multiplex. Direct STR amplification and detection were achieved in 13 minutes from microwave-extracted saliva samples.

Microwave DNA extraction can increase the speed of extraction and DNA yield, resulting in high-quality DNA suitable for direct rapid PCR protocols. The system has been applied to a variety of single-source human saliva, blood, and epithelial samples. The protocols will be tested on samples deposited on different substrates, samples spiked with inhibitors, and mixtures. Furthermore, a set of mini STRs has been developed for rapid direct PCR of the microwave extracts with amplification in less than nine minutes.

The inclusion of the microwave digestion at the front end of the analytical stream may help to mitigate PCR inhibition, improve the lysing of cells, and increase the overall yield of input DNA for rapid DNA analysis. These positive effects should greatly improve overall speed and success rates using a rapid DNA processing stream for both laboratory and commercial systems.



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**Microwave, DNA, RAPID**



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