B48 Increasing the Speed and Efficiency of 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 understand how a microwave can be used to rapidly extract DNA for improving Rapid DNA analyses.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by informing attendees how a microwave can be used to increase the speed and efficiency of extractions for rapid direct Polymerase Chain Reaction (PCR) analysis.

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 fieldable 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 less than 90 minutes and a newly developed ultra-rapid DNA system can yield genotypes in under 15 minutes.[2,3]

The problem is that the processes these instruments use to isolate DNA are slow and inefficient and may not be fast enough nor effective enough for certain sample types and critical time-sensitive intelligence applications.[4] Additionally, results from studies involving crime scene samples indicate the need to mitigate issues with sensitivity and PCR inhibition as well as improving the speed of rapid DNA systems. Processing difficult samples in remote locations is important for rapid on-site intelligence applications. Thus, there is a need to develop a more efficient front end for processing difficult samples in remote locations.

In this study, microwave DNA extraction using both conventional and computer-driven microwaves have been tested for rapid direct PCR. Previous research results have demonstrated successful microwave extraction from both eukaryotes and prokaryotes, including human bodily fluids and tissues.[5-20] Single-source human saliva, blood, and semen samples were prepared on replicate swabs at different total cellular amounts between 25–500 cells. Protocols using different microwave energy levels at no microwave energy, 300, 400, 500, 600, and 700W of energy at 40 seconds were tested first on replicate saliva swabs containing varying cellular loads. Improved DNA yield for samples microwaved at 300W for 40 seconds was observed. Further experiments with no microwave controls, and 100, 150, 200, and 300W microwave treatments at 40 seconds were carried out. Improved yields were again observed at 300W; however, some loss and variation was observed across replicates due at least in part to saliva sample heterogeneity. Short Tandem Repeat (STR) RapidHIT™ testing of replicate 1% saliva dilutions demonstrated increased allele detection and peak heights for the microwave-treated saliva samples on the Rapid HIT™ system. On average, microwaved replicates of 1% saliva swabs resulted in correct allele detection of 92% (34 of 37), whereas unmicrowaved replicates resulted in only 78% correct allele detection (29 of 37). Microwaved sample peak heights increased an average of 215 Relative Fluorescence Units (RFU) versus non-microwaved samples (with a range of 31–594 RFU for 26 alleles). For seven alleles, microwaved peak heights decreased an average of -198 RFU (with a range of -24 to -341 RFU).

All alleles were correctly detected at 10% saliva for both microwaved and non-microwaved samples.

In addition to testing the commercial RapidHIT™ systems, a rapid direct STR multiplex has been developed. The multiplex consists of the following loci: D10S1248, FGA, D8S1179, D7S820, AMELOGENIN, D2S441, D18S51, D2S1338, D21S11, TH01, and vWA. Results of optimization experiments under different combinations of primer concentration, cycling temperature, cycle number, and template has resulted in successful rapid 15 minute co-amplification of nine STRs with no extraction step other than microwave processing. Microwaved 1% saliva samples were directly amplified with no additional extraction resulting in increased peak heights over non-microwaved samples.

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, resulting in improved recovery of low template samples for rapid DNA analysis. These positive effects should greatly improve overall speed and success of rapid DNA processing for both laboratory and commercial systems.

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