RAPID, MICROWAVE-ACCELERATED DNA EXTRACTION FROM SALIVA, SEMEN AND HAIR FOR DOWNSTREAM PCR TYPING

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Interest in rapid biometric identification capabilities for law enforcement, defense and intelligence applications have generated the development of automated rapid DNA systems. Currently available instruments can perform DNA typing from extraction through genotyping in less than 90 minutes[1]. Although these systems can provide highly probative information, they are too slow to permit identity of a suspect DNA in real time and they are not always successful on challenging samples.

Aboud et al. published a strategy using direct rapid PCR multiplexing, by-passing extraction and successfully generated a STR multiplex profile in under 25 minutes[2]. Others have published direct PCR on challenging samples such as those subjected to presumptive testing[3] and fingerprint enhancement[4], however, not all samples were properly amplified. Implementing a rapid, efficient DNA extraction method may enhance results on these types of challenging forensic samples.

The use of microwaves as a pretreatment to assist in the extraction of forensic biological samples, to the best of our knowledge, was first reported in our laboratory[5]. Use of a microwave can facilitate rapid cell lysis (in less than 1 minute), using intermittent microwave pulses[6]. The rapid lysis of cells using the microwave may greatly assist in the development of rapid, direct PCR methods and has been used on paraffin embedded tissues[7], environmental samples such as sludge[8], cyanobacteria[9], spores[10], in the rapid extraction and detection of *Neisseria gonorrhoeae*[11], in the decalcification and extraction from bone[12], serum [13], and blood[14] resulting in DNA suitable for direct PCR, real-time PCR analysis and fragmentation in library preparation for MPS applications[15]

In the present study, a standard commercial 800W microwave pre-treatment was evaluated for the ability to rapidly lyse cells and thereby replace the commonly utilized overnight, 24 hour 56° C incubation for extraction of saliva, semen and hair. Replicate samples of 15 ul saliva, semen and single hairs were either treated using microwave intermittent pulses of 4 seconds resulting in a total of 8 seconds, 16 seconds and 24 seconds or into a standard 56° C water bath incubation. Samples were placed in the same positions in the microwave based on preliminary 'calibration' of microwave energy performed by monitoring temperature to identify positions that received similar amounts of energy to avoid 'hot and cold spots'. Preliminary results of qPCR quantification (using Quantifiler Duo® Applied Biosystems and/or Plexor® Promega quantification kits) of microwave extracted 15ul saliva were variable ranging from 1.2-6.5ng/ul in a 20ul total) but were comparable to yields from 15ul saliva samples incubated at 56° C (0.9-2.0ng/ul in a 20ul total) demonstrating the ability to shorten the time of extraction by replacing the 24 hour 56° C overnight water bath with 8-24 seconds of microwaving. Additional results from replicate semen, hair and samples stored over 3 months, evaluation of the microwave for differential extraction of semen/saliva mixtures, direct multiplex PCR results from microwave treated samples and the evaluation of strategies to address differences in microwave energy imparted to samples will be presented.

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References

1. Butler JM. 2015. The future of forensic DNA analysis. Phil. Trans. R. Soc. B 370: 20140252.
2. Aboud M, Oh HH, McCord, B. 2013. Rapid direct PCR for forensic genotyping in under 25 min.
Electrophoresis. 2013 Jun;34(11):1539-47. doi: 10.1002/elps.201200570.
3. Thanakiatkrai P, Raham K, Pradutkanchana J, Sotthibandhu S, Kitpipit. 2017. Direct-STR typing from presumptively-tested and untreated body fluids. Forensic Sci Int Genet. 2017 Jun 6;30:1-9. doi: 10.1016/j.fsigen.2017.06.001.
4. Templeton JEL, Taylor D, Handt O, Linacre A. 2017. Typing DNA profiles from previously enhanced fingerprints using direct PCR. Forensic Sci Int Genet. Jul;29:276-282. doi: 10.1016/j.fsigen.2017.05.006.
5. Goodwin D.C., and S.B. Lee. 1993. Rapid microwave mini-prep of total genomic DNA from fungi, plants and protists for PCR. Biotechniques. 15: 438-444.
6. Giberson, R.T., R.S. Demaree, S.B. Lee and M. Lee. 1995. Days can be converted to hours, minutes or even seconds when using microwave technology in the Lab. Microscopy Today. 95:14-15.
7. Morgan, K, L Lam, N Kalsheker. 1996. Microwave-based DNA extraction from paraffin-embedded tissue for PCR amplification. Clin Mol Pathol. 1996 Jun; 49(3): M179–M180.
8. Wu S1, Qian X, Yu X, Sheng H, Lü B. 2012. Microwave heating of long-term formalin-fixed surgical pathology specimens improves quality of extracted DNA.
Appl Immunohistochem Mol Morphol. 2012 Oct;20(5):512-7. doi: 10.1097/PAI.0b013e3182434174.
9. M. Orsini and V. Romano-Spica. 2001. A microwave-based method for nucleic acid isolation from environmental samples. Letters in Applied Microbiology 2001, 33, 17±20
10. Rasmussen JP, Barbez PH, Burgoyne LA, Saint CP. Rapid preparation of cyanobacterial DNA for real-time PCR analysis. Lett Appl Microbiol. 2008; 46:14–19.
Aslan K, Previte MJR, Zhang Y, Gallagher T, Baillie L, Geddes CD. Extraction and Detection of DNA from Bacillus anthracis Spores and the Vegetative Cells within 1 minute. Anal Chem. 2008; 80:4125–4132.
11. Melendez JH, Santaus TM, Brinsley G, Kiang D, Mali B, Hardick J, Gaydos CA, Geddes CD. 2016. Microwave-accelerated method for ultra-rapid extraction of Neisseria gonorrhoeae DNA for downstream detection.Anal Biochem. 2016 Oct 1;510:33-40. doi: 10.1016/j.ab.2016.06.017. Epub 2016 Jun 17.
12. Imaizumi, K, K Taniguchi, Y Ogawa. 2013. An evaluation of the effect of microwave irradiation on bone decalcification aimed to DNA extraction. Legal Medicine 15 (2013) 272–277
13. Sandford AJ1, Paré PD. 1997. Direct PCR of Small Genomic DNA Fragments from Serum was also facilitated using a microwave extraction. Biotechniques. 1997 Nov;23(5):890-2.
14. Jadaon MM, Dashti AA, Lewis HL, Habeeb FM. Whole-blood polymerase chain reaction and restriction fragment length polymorphism: a simplified method by microwave irradiation. Med Princ Pract. 2009; 18:280–283. [PubMed: 19494534]
15. Yang Y, Hang J. Fragmentation of genomic DNA using microwave irradiation. J Biomol Tech. 2013; 24:98–103. [PubMed: 23814501]