University of Technology Sydney
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EVALUATION OF NOVEL MITOCHONDRIAL DNA PANELS FOR FORENSIC USES

A thesis submitted in fulfillment of the requirements for a Master of Science (Research) degree

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October 2018

Certificate of Original Authorship

I, Ka Tak Wai declare that this thesis is submitted in fulfilment of the requirements for the award of the Master of Science (Research) degree, in the Faculty of Science at the University of Technology Sydney. This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

This research is supported by an Australian Government Research Training Program Scholarship.

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Acknowledgements

This project would not have been possible without the support of everyone involved in it. Associate Professor Peter Gunn (University of Technology Sydney) and Dr. Mark Barash (University of Technology Sydney) are thanked for their supervision and guidance throughout the project. The volunteers who kindly provided their DNA sample for the project are thanked. The project recognises the funding and support provided by UTS, Centre for Forensic Science. This includes the travel grants, which were provided in the Vice Chancellor's Postgraduate Research Student Conference Award and by Professor Claude Roux for attending the 27th Congress of the International Society for Forensic Genetics (Seoul, South Korea). Researchers from the UTS Forensic Biology Unit, Elisha Prasad, Samara Garrett-Rickman, Andrew Walton, Alicia Khuu, Liam Pullan and Alexandra Summerell are acknowledged for their support and collaboration throughout the project. Furthermore, the technical support provided by Sophie Winiarski and Connie Soilemezis (UTS Science), James Cooke (Bio-Rad Laboratories, Inc.) and Beatrice Goh and Lucy Dagostino (Thermo Fisher Scientific) is greatly appreciated.

Presentation of Thesis

This thesis is presented in six chapters and follows the process of mitochondrial DNA testing in forensics. Chapter 1 introduces the theory of mitochondrial DNA, its testing and forensic uses in cases. Sequencing technologies for mitochondrial DNA are reviewed as well as the limitations in DNA markers for quantification and sequencing, providing a strong justification for this research in developing and evaluating novel mitochondrial DNA markers for human identification. Chapters 2-5 listed below describe the methods and results, and discuss the two projects undertaken in this research. Note that Chapter 3 is prepared as a manuscript for publishing and Chapter 5 is accepted as a research article. As such, the references of Chapters 3 and 5 are independent to the thesis. Both of these manuscripts were written in collaboration with the candidate as the primary contributor.

Chapter 2 introduces the qualities of DNA that can be encountered in compromised forensic samples and the research methods, which were used to mimic these samples.

Chapter 3 describes the development of a quantification assay which was used to assess the mitochondrial DNA quantity. While it does appear prior, it should be noted that the assay in Chapter 3 was developed last and is discussed retrospectively.

Chapter 4 presents the nuclear typing of DNA samples as an indication of DNA quality.

Chapter 5: ‘Performance of the Early Access AmpliSeq™ Mitochondrial Panel with degraded DNA samples using the Ion Torrent™ platform’

Chapter 6 provides a final discussion of the projects and its conclusions as well as recommendations for the research field.

Research Output

Publication

Wai, K.T., Barash, M., and Gunn, P., *Performance of the Early Access AmpliSeq™ Mitochondrial Panel with degraded DNA samples using the Ion Torrent™ platform.* Accepted in Electrophoresis, 2017.

Wai, K.T., Barash, M., and Gunn, P., *Development of a real-time quantification of mitochondrial DNA copy number in degraded samples.* Manuscript in preparation.

Presentation

August, 2017 'Evaluation of the Early Access AmpliSeq™ Mitochondrial Panel utilising Massively Parallel Sequencing'.

Poster Presentation: 27th Congress of the International Society for Forensic Genetics, Seoul, South Korea

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List of Abbreviations

DVI	Disaster victim identification
DNA	Deoxyribonucleic acid
ATP	Adenosine triphosphate
BGA	Biogeographical ancestry
BP	Base pairs
CE	Capillary electrophoresis
DI	Degradation Index
HVR	Hypervariable region
INDEL	Insertion and Deletion
ISFG	International Society for Forensic Genetics
MPS	Massively parallel sequencing
mtDNA	Mitochondrial DNA
nDNA	Nuclear DNA
NUMT	Nuclear inserts of mtDNA
PCR	Polymerase chain reaction
PHR	Peak height ratio
qPCR	Quantitative PCR
RFU	Relative fluorescence unit
S.E.M	Standard error of mean
SD	Standard deviation
SNP	Single nucleotide polymorphism
STD	Standard
STR	Short tandem repeat

Abstract

Mitochondrial DNA testing is expanding the use of DNA as a forensic tool for human identification. The testing of mitochondrial DNA is a common practice for biological samples, which are compromised, degraded and contain limited STR information. In these cases, the amplification and sequencing of mitochondrial DNA becomes a valuable tool in determining the source of DNA samples. A review of mitochondrial DNA testing in forensic science reveals a number of improvements which can be made to this process. The project therefore aimed to improve the process of mitochondrial DNA testing. In particularly, it focuses on the developing and testing of mitochondrial DNA markers involved in the quantification and amplification of samples.

The quantification of mitochondrial DNA is important to optimising the amplification of DNA samples. Methods for quantification of mitochondrial DNA commonly involve estimating mitochondrial DNA quantities from nuclear DNA. This is usually unreliable as a high variability of mitochondrial DNA copy number exists between human cells. Hence, in this study, the development of a specific DNA assay for mitochondrial DNA provides a reliable determination of mitochondrial copy number. The testing of this novel assay has shown it is specific, sensitive and reproducible in DNA samples of artificially degraded qualities. As such, the incorporating of the DNA assay into workflows of mitochondrial DNA testing will improve the overall amplification of samples for downstream processes such as mitochondrial DNA sequencing.

Furthermore, the transition of mitochondrial DNA sequencing from capillary electrophoresis to massively parallel sequencing platforms is increasing the feasibility of typing multiple DNA fragments in a single reaction. This has led to the development of small PCR markers, which are capable of amplifying the entire mitochondrial genome even in challenging forensic samples. While the sequences of these PCR markers and panels are available for use, its full performance in amplifying compromised samples remains unknown due to the limited and usually specialist use of mitochondrial DNA testing and massively parallel sequencing in forensic laboratories. Therefore, the technical work carried out in this study tests the performance of the Early Access AmpliSeq™ Mitochondrial Panel (Applied Biosystems, CA, USA) for amplifying complete mitochondrial genomes in samples of degrading qualities. The testing of this Panel in compromised samples with limited STR success informs the use of the Panel in the mitochondrial testing of DNA in forensic laboratories. In this study, the typing of amplified DNA fragments in parallel reveals the recovery of complete mitochondrial DNA sequences in all samples. These samples were concordant to reference sequences and the HV1 and HV2 sequences provided by the ‘gold standard’ of capillary electrophoresis platforms. Importantly, the analysis of mitochondrial DNA sequences shows a capability to

resolve mitochondrial haplogroups and ancestries for familial matching. Overall, the results of this technical work confirms the Panel is able to amplify the complete mitochondrial genome of compromised samples for sequencing using massively parallel technologies. As such, this contributes to the use and validation of massively parallel sequencing technologies in forensic DNA testing.

Overall, the development of a novel DNA assay for the quantification of mitochondrial DNA and the technical testing of the Early Access AmpliSeq™ Mitochondrial Panel (Applied Biosystems, CA, USA) has contributed to improvement of mitochondrial DNA testing in compromised samples. Past use of mitochondrial DNA testing has provided identifications in cases of mass disasters, missing persons and historical remains. As such, the improvements to this process will continue to assist in these identifications.