Development and validation of miniSTR typing capabilities to aid in the analysis of degraded DNA evidence in Sri Lankan forensic casework

A thesis submitted for the Degree of Doctor of Philosophy

Nakandala Darshana Suraj Goonawardhana

Faculty of Science

University of Colombo

August 2016

ABSTRACT

Individual identification using highly polymorphic short tandem repeat markers (STRs) has become one of the key advancements in forensic investigations today. A comparison of several such markers on each individual's genome with that of the reference samples allows sensitive and accurate identification of the donor whose biological sample is associated with a crime or the accurate identification of biological relationships between individuals. However, biological evidence is not often stable when shed to outside environment from a living body, and results in the degradation of DNA. In such circumstances, DNA analysis using conventional STR analysis becomes challenging and the problem further exacerbates when larger sized PCR fragments are targeted. Numerous studies have shown that targeting miniaturized fragments (miniSTRs) can increase the success rates in retrieving information from such degraded biological evidence.

As such, one of the aims of this study was to design miniSTR primers for 9 STR loci that are routinely used in forensic identification /parentage analysis, and assess the advantage of using miniSTRs for degraded DNA samples in Sri Lankan conditions. The study also included the development of a 13 miniSTR panel to be used for routine DNA typing of degraded samples in Sri Lanka. An evaluation was also performed for the use of modified dinucleotide STR markers, with a view to using these to generate extremely small PCR fragments from degraded DNA.

Miniaturized PCR products for routinely used autosomal STR loci CSF1PO, TPOX, THO1, D7S820, D13S317, D16S539, vWA, F13A01 and D8S1179 were produced by designing PCR primers close to the core repeat region of each of the 9 STR loci. The primer sets were designed to form PCR multiplexes that can be analysed with capillary electrophoresis in a single injection and also as three multiplexes in silver staining based manual genotyping. Six STR loci TPOX, THO1, D7S820, D13S317, vWA, F13A01 were used to evaluate the detection level of conventional and miniSTR systems for simulated blood and seminal fluid stains stored in different storage and environmental conditions through a period of 145 days. The overall results generated for six STR loci tested revealed that the use of miniSTR system has a significant ability to retrieve information from samples exposed to harsh conditions such as 100% humidity, uncontrolled outside environment and samples exposed to sunlight.

The three novel miniSTR loci D4S2632, D6S2436 and D19S589 were identified from human genome searches and were tested on a sample of the Sri Lankan population. Allele frequency databases were established using 431 blood samples obtained from three Sri Lankan populations groups, the Sinhalese, Sri Lankan Tamils and Sri Lankan Moors. These miniSTR loci are unlinked from the CODIS marker panel and are able to generate PCR amplicons within the range of 108-180, 64-100 and 98-126 for STR loci D4S2632, D6S2436 and D19S589 respectively. A statistical analysis of the population data demonstrated that these 3 novel miniSTR loci possessed the characteristics that qualified them to be used as forensic STR markers.

A set of ten highly polymorphic set of 10 miniSTR markers were selected from already available data in order to create a panel of miniSTR markers that would better suit the population genetic makeup of Sri Lanka. These 10 mini STRs were also designed as multiplex panels and important forensic parameters for the Sri Lankan population was derived for these loci, thereby resulting in a new panel of 13 miniSTR loci that can be used for DNA typing in Sri Lanka.

In addition, a new approach "micro-STRs" was proposed through this study for the forensic community by performing a genome-wide search to short list dinucleotide STR loci with interrupted repeats which are capable of generating DNA typing results with minimal stuttering which is known to be associated with dinucleotide STR analysis. Six selected

dinucleotide STR loci were evaluated for the stuttering effect and were found to contain 12.3 to 18.4% of stuttering which is acceptable compared to the results discussed in other studies with STR loci without uninterrupted repeats.

In conclusion, this study has achieved the objectives of enhancing DNA typing capabilities of forensic DNA analysis in Sri Lanka by improving, developing and validating the use of miniSTR markers to complement the existing STR marker panel. This study also demonstrated the extent to which DNA degradation occurs under Sri Lankan conditions, and established the feasibility of using miniSTRs for DNA typing of evidence that is subject to such environmental insult. It also expands the understanding of the requirement of good practices in collection, preservation, transportation of biological evidence. A panel of highly discriminating miniSTR markers were developed and shown to be suitable for forensic DNA typing purposes in Sri Lanka, thereby expanding the DNA typing capabilities in Sri Lanka. In addition, the concept of using interrupted dinucleotide "microSTR" markers was proposed and shown to be feasible for potential use in forensic DNA typing.