ABSTRACT

X chromosomal short tandem repeat (STR) markers are recognised for its potential to provide valuable information in forensic and kinship testing. X-STR analysis is advantageous in cases where at least one female is involved and can be applied to cases of deficiency paternity, sibling or other disputed relationships, missing persons, immigrations and criminal incest. X-STRs also have a proven utility in tracing the sex biased demographic history among populations with complex admixture and geneflow patterns.

The scope of X-STRs however, is not yet incorporated into the practice of molecular forensics in Sri Lanka. As a result, many cases requiring the construction of complex pedigrees have ended inconclusively, in the last two-decade history of DNA typing in the country. Introduction of a suitable marker system for Sri Lankans is the first step in maximizing the power of this additional tool. However, there is only one widely accepted commercial kit currently available for X-STR typing; thus, most laboratories rely upon noncommercial in-house multiplex assays. As such, a novel single multiplex system that simultaneously amplify 16 X-STR markers consisting of four clusters of closely linked markers was developed and validated as per the recommendations of Scientific Working Group on DNA Analysis Methods.

This extensive developmental validation study investigated all the required aspects of an STR marker system. The assay was sufficiently robust and comparable to other commonly used commercial X-STR kits, including the ability to analyse mixed or deteriorated samples. To extend its application to routine analysis, allele and haplotype frequency databases were constructed based on a total of 838

samples, covering all four major ethnicities in Sri Lanka (Sinhalese, Sri Lankan Tamil, Indian Tamil and Moors), which demonstrated high discriminative power for all populations. The allele frequency distribution was also indicative of a subtle but statistically significant difference in Indian Tamils compared to Sinhalese and Moors. This observation contradicts with the genetic outlook portrayed by autosomal STRs and suggests a sex biased demographic history in Sri Lankans. Accordingly, use of a separate allele frequency database for Indian Tamils would be more appropriate for kinship analysis. Likewise, the pattern of linkage disequilibrium observed between the markers of linked clusters, differed substantially among the four ethnicities and recommend the use of different haplotype frequency databases for each ethnic group.

Since both linkage and mutation play an important role in the statistical calculations, a three-generation family study, comprising 162 grandsons, was conducted for Sinhalese. The results verified the population specific genetic distances between markers and the stability of haplotypes defined by the four clusters. The calculated marker specific mutation rates provide a framework to differentiate recombinations and mutations observed in pedigree analysis. Application of the novel 16 X-STR assay system to actual casework on complex kinship and forensic scenarios, created a more secure framework for interpreting results and produced reliable probabilities in all instances.

This research study on the whole has introduced X-STR analysis to the field of molecular forensics in Sri Lanka and has provided the necessary framework for its routine use. Considering the special applications of X-STR analysis in kinship and forensic case work, the outcome of this study is believed to significantly impact the criminal and civil justice system in Sri Lanka, through extending its boundaries.