DEVELOPMENT OF A NOVEL X CHROMOSOMAL STR BASED DACAPLEX PCR ASSAY FOR THE KINSHIP ANALYSIS OF THE SINHALESE POPULATION

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The X Chromosome is of great importance to molecular forensics due to its unique inheritance pattern and recombination restricted to female individuals. With the use of X chromosomal STR markers (X-STR), it is possible to solve certain challenging kinship cases that other commonly used forensic markers (autosomal STR, Y-STR) cannot resolve efficiently. Being located on the same chromosome, X-STR markers tend to exhibit linkage groups that are inherited together from a single parent, and thus can be considered stable haplotypes. So far, more than 45 X-STR makers have been studied in various populations worldwide, which have been used to design numerous multiplex PCR systems. At present, X-STR analysis has not been established for the Sri Lankan population. As such, the aim of this study was to develop a powerful automated multiplex X-STR assay, appropriate for Sri Lankans and to test its applicability using a sample of the Sinhalese population.

Ten previously published X-STR makers covering three tightly linked clusters (DXS10079-DXS10074-DXS10075 on Xq12; DXS6801-DXS6789-DXS6809 on Xq21 and DXS7424-DXS101-DXS7133 on Xq22 and DXS8378 on Xp22) were selected based on their molecular weights to optimise a single decaplex PCR for automated fragment analysis. In-house constructed allelic ladders were validated (against 9947A and K562 standard DNA) and sequenced to confirm the repeat numbers. The amplified products were subjected to capillary electrophoresis (ABI 3500 Genetic Analyzer; Applied Biosystems) at Genetech, Colombo and were analyzed by GeneMapper IDX software (Applied Biosystems). The applicability of the 10 X-STR multiplex system was tested on 100 Sinhalese individuals (50% male) using finger pricked blood. DNA was extracted using the chelex method. Allele frequencies were generated for the 10 markers using Arlequin 3.5.2.

The novel decaplax assay is optimized to use only 0.5 ng DNA compared to 1-5 ng of DNA used in routine forensic practice. Genotyping data showed a high polymorphism with respect to all the selected loci allowing for a large haplotype diversity. Numbers of alleles observed for each cluster was; DXS10079-DXS10074-DXS10075:9-8-7; DXS6801-DXS6789-DXS6809:6-9-10; DXS7424-DXS101-DXS7133: 9-11-6. Six alleles were observed for DXS8378. Further none of the alleles in the selected 10 X-STR loci exceeded 50%

frequency, reflecting the usefulness and validity of these loci in kinship analysis. In order to confirm its general applicabilityto the Sri Lankan population, it would be necessary to extend the assay to cover other ethnic groups in Sri Lanka.

Keywords: Recombination, Multiplex PCR, Linkage, Haplotypes, Alleles

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