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DEVELOPMENT OF A LOW COST, MULTIPLEX PCR-BASED DIAGNOSTIC ASSAY FOR DOWN SYNDROME

A PROJECT REPORT PRESENTED BY

ANTOINETTE MARY RENUKA FERNANDO

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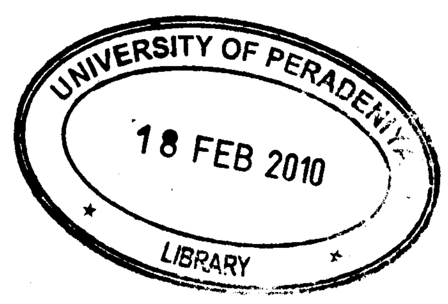

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ABSTRACT

DEVELOPMENT OF A LOW COST, MULTIPLEX PCR-BASED DIAGNOSTIC ASSAY FOR DOWN SYNDROME

RENUKA FERNANDO

Postgraduate Institute of Science

University of Peradeniya

Peradeniya

Sri Lanka

Down syndrome (DS) is a genetic disorder caused by the presence of all or part of an extra 21st chromosome, which occurs due to nondisjunction of the 21st chromosome during meiosis in either parent.

Traditionally, chromosome diagnosis was accomplished by karyotyping, where culture and analysis of fetal cells was time consuming and labour intensive. Molecular methods offer a faster, more effective alternative to karyotyping. Of the molecular methods that are available, multiplex PCR amplification of polymorphic allelic fragments located on chromosome 21, followed by polyacrylamide gel electrophoresis is the most cost effective.

The main objective of this study was to establish a low cost multiplex PCR method to analyze several chromosome 21 Short Tandem Repeats (STRs) in a single reaction and to develop a standard allelic ladder by which the repeat numbers of the STR alleles can be determined.

Peripheral venous blood samples were obtained from the suspected Down syndrome child and the parents, referred to Genetech laboratory by physicians. Genomic DNA was extracted from blood leucocytes by using Promega genomic DNA isolation kit. Six polymorphic STR markers were selected from non-overlapping regions of the 21st chromosome. The STR analysis at the six loci was performed in the form of duplexes, duplex I (D21S1440 and D21S1883), duplex II (D21S2055 and D21S1411) and duplex III (D21S1919 and D21S11). The PCR products were analyzed by 6% denaturing polyacrylamide gel electrophoresis, in which the STR alleles separated according to their sizes. These sample alleles were run along with allelic ladders, developed in the study, as size markers. The STR alleles were then visualized by silver staining. All three STR systems could be separated on a 6% polyacrylamide gel, thereby allowing all six loci to be separated on the same gel. K562 genomic DNA, universal standard, which was obtained from Promega Corporation, was used as a positive control. The bands obtained by amplification of K562 DNA were compared with the allelic ladder alleles to determine the unknown alleles of the ladder.

The sample alleles were analysed according to the number of bands produced by comparing their position with that of the allelic ladder alleles. For a heterozygous locus,

two bands were produced in a normal individual, and three bands were produced in the case of a Down syndrome patient.

The alleles obtained for the child were compared with that of the parents' to determine the parental inheritance.

It was found that duplex I, duplex II and duplex III could be amplified together simultaneously, thereby significantly reducing the time taken to generate the results. Stutter bands were observed with some of the STRs, but in most cases they were fainter than the correctly amplified alleles and could be distinguished unambiguously.

This method does not rely on specialized training and sophisticated machinery like in karyotyping. It is less time consuming and less laborious and useful for small volumes of samples from many kinds of tissues. The use of six polymorphic markers specific for chromosome 21 increased diagnostic confidence and informativeness. It can be used in Sri Lanka to rapidly detect trisomy 21 in a wide variety of samples; prenatal, post natal or even post mortem. This molecular technique could be used not only for diagnosis of Down syndrome but also for other chromosomal aneuploidies by changing the chromosome specific primers.