FLUORESCENT MULTIPLEX PCR FOR SIMULTANEOUS ANALYSIS OF SEVEN STRs ON THE Y-CHROMOSOME

M.S. Figueiredo, A.C. Pacheco, K. Miyashiro, and M.A.D. Oliveira
Molecular Biology Section, Laboratorio Fleury, São Paulo, Brazil

The analysis of DNA polymorphisms on the Y-chromosome is a powerful tool currently employed for human population studies, forensic investigations, and paternity testing. We present here a rapid test based on a Multiplex PCR procedure to simultaneously evaluate seven distinct loci on the Y-chromosome.

Specific primer pairs for the amplification of the polymorphic *loci* DYS19, DYS388, DYS389, DYS390, DYS391, DYS392, and DYS393 were synthesized being one primer of each pair labeled at the 5' extremity with the fluorescent dyes 6-FAM or HEX. Primer sequences were those described by de Knijff et al. (*Int. J. Legal Med*. 110: 134, 1997) except for two of the primers that were lightly modified in order to equilibrate the melting temperatures. Multiplex PCR was performed with DNA extracted from blood samples and the PCR products were analyzed using the ABI Prism® 310 Genetic Analyzer (PE Biosystems).

After minor adjustments in primer concentrations and PCR program, a balanced amplification across all *loci* could be obtained. For the seven *loci*, the allele size (in base pairs) was exactly within the expected range. Mendelian inheritance of full haplotypes was observed in all sets of samples from two-generation relatives. As expected, the haplotypes from non-relatives were distinct. The test was 100% reproducible and robust under the conditions evaluated.

The Multiplex PCR described in this study is very informative, rapid to perform, and easy to interpret. It has been used successfully in some complex paternity testing cases involving a deceased alleged father or cases producing low CPI in which the child is male.