Validation of the *GenePrint*™ PowerPlex™ and FFFL STR Multiplex Systems

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We have developed a multiplex system, the *GenePrint*™ PowerPlex™ System combined with the Amelogenin-TMR System, which allows simultaneous amplification, detection, and analysis of 8 polymorphic STR loci (CSF1PO, TPOX, TH01, vWA, D16S539, D7S820, D13S317, D5S818) and the gender identification locus, amelogenin. The loci detected in this 2-color fluorescent system provide balance of alleles within and between loci and ample amplification product for allele determination using 1 ng of DNA template. The *GenePrint*™ Fluorescent STR System F13A01, FESFPS, F13B and LPL (FFFL) allows simultaneous amplification, detection, and analysis of 4 polymorphic STR loci in a one color fluorescent system. While providing high discrimination power, the loci included in these systems have been selected for the minimum of artifacts produced, including stutter bands, and microvariant alleles.

The loci described have been extensively evaluated for sequence variation and the paucity of microvariant alleles contained within these systems. Allelic ladders have been created as size standards for each locus. Each component fragment of the allelic ladders has been characterized by sequence analysis. In addition, these loci generate little or no stutter band artifact (an artifact of the PCR process using repeated sequences) as compared with other candidate loci.

This report includes evaluation of these systems for their performance under stressed conditions not recommended for use and for the effects of thermal cycle variations as recommended by The Working Group on DNA Analytical Methods (TWGDAM).

Also discussed will be results of an extensive population survey of over 600 individuals used to determine allele frequencies in three racial groups, the independence of the inheritance of alleles in one-locus and pair-wise locus combinations for evaluation of Hardy-Weinberg Equilibrium, and calculation of matching probability. Matching probability exceeds 1 in 114,000,000 for the PowerPlex™ System alone and is greater than 1 in 303,000,000,000 in each racial group when used in combination with the 4-locus FFFL Multiplex System to evaluate 12 loci in two amplification reactions.

STR System	Matching Probability		
	African-Amer.	Caucasian-Amer.	Hispanic-Amer.
PowerPlex™ System (8 STRs)	1 in 2.74×10^8	1 in 1.14×10^8	1 in 1.45×10^8
PowerPlex™ System plus FFFL (12 STRs)	1 in 4.61×10^{12}	1 in 3.03×10^{11}	1 in 4.75×10^{11}