Intra- and Interlaboratory Comparison Study of Peak Height and Area Ratios Within Short Tandem Repeat (STR) Loci of Single Contributor Samples Employing the Promega *GenePrint*® Powerplex™ 1.1 and the Hitachi FMBIO® Fluorescence Imaging Systems

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Interpreting genotype profile information can be difficult when band patterns do not conform to the basic premise that two bands correspond to a heterozygotic and one band to a homozygotic genotype. Commonly encountered samples (degraded or mixed DNA) and amplification imbalances or anomalies like stutter can produce indeterminate band patterns. These patterns become more complex with the multiplexing of loci. Interpreting these multiple band profiles can be aided by comparing fluorescent signal intensities as detected and measured through fluorescence imaging systems, such as the Hitachi FMBIO®. Establishing a range of typical allele ratios within a locus from intact, single contributor (non-mixture) samples can guide the interpretation of these more complex profiles.

This study was designed to help users of the Hitachi FMBIO® system in their interpretation of profiles by comparing typical allele ratio ranges (including n-4:n) from a number of laboratories. Scanned gel image files were collected from each participating laboratory. Gel analysis was performed independently by two analysts according to a standardized protocol. Peak height (OD) and area (IOD) data were analyzed for bands in each locus using StaR Call™ software. For this study, the StaR Call™ software was modified to calculate any OD or IOD ration (smaller fragment: larger fragment) within each locus as a percentage.

Percentage frequencies were grouped by locus and allele pair for each gel. For each laboratory in the study, percentage data were tabulated by locus and allele pair from all the gels submitted by that lab. Finally, percentage data were compared between different labs to assess overall variability.