VALIDATION STUDIES ON THE RELIAGENE Y-PLEX™ 6 STR AMPLIFICATION KIT

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The Centre of Forensic Sciences (CFS) has evaluated ReliaGene's Y-PLEX™ 6 kit which co-amplifies the following six Y-STR loci in a single PCR amplification: DYS393, DYS19, DYS389II, DYS390, DYS391 and DYS385. Internal validation studies were performed in accordance with the TWGDAM guidelines published in 1998. Following organic extraction, ultra filtration and quantification by Real-Time Quantitative PCR (QPCR), 31.25pg to 5ng of template DNA was amplified in a 25µl reaction volume. Laser activated fluorescence was detected during electrophoresis in 36cm, Long Ranger™ polyacrylamide gels on a ABI PRISM® 377 DNA Sequencer. DNA fragments were analyzed by Genescan® Analysis (v3.1.2) using the internal lane standard GS500 and the Local Southern Method for sizing. Peaks greater than 50 relative fluorescent units (rfu) and which fell within established allele categories were designated by Genotyper® (v2.5) based on allelic ladders supplied with the kit and run on each gel.

Results obtained with the Y-PLEX™ 6 kit were specific to higher-primate DNA. No alleles were detected when 5ng of DNA isolated from blood of a variety of non-primates was amplified. When high amounts of human female DNA (100-1000ng) were added to the PCR, no products were detected at DYS393, DYS19 and DYS389II. However, as many as 10 off ladder products were repeatedly detected within and outside of the allelic ladder ranges reported for DYS390, DYS391 and DYS385. More non-specific product was observed when 100-200ng of female DNA was amplified than when higher amounts of female DNA were amplified. In fact, when 500-1000ng of female DNA was amplified, there was inhibition of non-specific products.

The Y-PLEX™ 6 system produced complete profiles with 125pg of single source male DNA and partial profiles were detected with as little as 31.25pg. Identification of a full male profile in female/male DNA mixtures of up to 200:1 was likely and in mixtures of 500:1 a partial male profile was detected (male fraction kept constant at 1ng). Detection of alleles at DYS390, DYS391, and DYS385 was less likely. This observation might be due to female template competing for reaction components since many of the off ladder products seen earlier with the single source female samples were once again identified. When the amount of female DNA in the mixture was increased to 1000:1 the male fraction failed to amplify at any of the Y-STR loci. In addition to female/male mixtures, we assessed the sensitivity of the kit with male/male DNA mixtures ranging from 1:1 up to 16:1. The major source of male DNA in the mixture was maintained at 1ng, whereas the minor source of male DNA was sequentially reduced down to 62.5pg. We were consistently able to detect alleles from the minor contributor in a mixture of 8:1 (1ng/125pg). In male/male mixtures of 16:1 alleles from the minor contributor (at 62.5pg) were occasionally identified.

Ten non-probative casework samples from the epithelial fraction of vaginal swabs were amplified at 10ng in the Y-PLEX™6 system. Previous analysis of 1ng of DNA in the Profiler Plus™ STR system identified that 6 of 10 samples were female/male mixtures with the male being the minor contributor (approximately 100pg or less). We were able to obtain a Y-STR profile from 5 of these mixtures, 3 of which gave a result at all 6 loci. Of the remaining case samples examined 2 of 4 generated a Y-STR result, one at 6 loci the other at the DYS393 locus only. In none of these cases was a secondary male profile identified by STR analysis in Profiler Plus™.

To date we have typed 108 males from our Ontario population databases: 57 Caucasian, 23 East Indian and 28 Oriental males. Of the 108 haplotypes generated, 96 have been identified as unique. The most common alleles observed were as follows; DYS393 (13) at 61%, DYS19 (14) at 44%, DYS389II (29) at 41%, DYS390 (24) at 41%, DYS391 (10) at 56% and DYS385 (11) at 25%. The following off ladder alleles were identified; DYS389 (26), DYS390 (26), DYS385 (9, 16.3,20 & 21.3) In addition to haplotyping, the database samples were examined for the prevalence of n-4 stutter. As seen with autosomal STR

systems, all 6 loci demonstrated higher levels of stutter as the number of repeat units increased. The average stutter detected at each locus was as follows: DYS393 (7.1%), DYS19 (6.4%), DYS389II (13%), DYS390 (7.6%), DYS391 (5.9%) and DYS385 (6.4%). The highest level of stutter (17%) was observed at the DYS389II locus. However, at all other loci n-4 stutter was routinely less than 10%.