

## VALIDATION AND OPTIMIZATION OF RELIAGENE'S Y-Plex™ 6 KIT FOR PROCESSING NUCLEAR CASE SAMPLES AT THE ARMED FORCES DNA IDENTIFICATION LABORATORY

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The Y-Plex™ 6 kit is a Y-STR identification kit marketed by Reliagene Technologies that allows for the identification of the male component from evidentiary samples, and the identification of paternally related individuals. This kit utilizes primers designed to amplify the DYS393, DYS19, DYS389II, DYS390, DYS391, DYS 385 loci located in the NRY region of the Y chromosome. The objective of the experiments and results described below was the validation of the Y-Plex™ 6 kit on the ABI PRISM®377 DNA Sequencer for implementation into casework at the Armed Forces DNA Identification Laboratory (AFDIL). The validation process included an intra-gel and inter gel precision study, sensitivity study, the successful typing of male DNA from; chelex extracted blood samples, organically extracted blood and tissue samples and Qiagen extracted blood samples, reproducibility, the ability to identify paternally related individuals, specificity, and the ability to distinguish between male: male and male: female mixtures. All experiments were performed according to the protocol set forth in the Y-Plex™ 6 Instruction Manual v3.0.

The precision of the Y-Plex™ 6 STR system was determined for 36cm well to read 5% Long Ranger, 6M Urea gels using GeneScan® 500 Internal Size Standard and analyzed on two different ABI 377 DNA Sequencer. Results demonstrated that the Y-Plex 6 ladder had a greater than 99.9% precision within and between gels arguing that there was little to no variation in the running accuracy of gels between the two 377's. In addition there was no size standard deviation greater than 0.10 bp for any specific locus.

The sensitivity of the Y-Plex™ 6 typing system was evaluated by running serial dilutions of the Genomic male kit control (CCL-256.1) DNA. The male control DNA was independently evaluated 4 times by amplifying the following concentrations: 10ng, 5ng, 2.5ng, 1ng, 0.5ng, 0.25ng, 0.1ng, 0.05ng. Results demonstrated that the Y-Plex™ 6 typing system gave consistent full profiles at concentrations from 0.5ng-10ng of input DNA when amplified at 30 cycles. At DNA concentrations higher than 2.5 ng there was an increase in –A peaks as well as pull-up from the Fam into the TAMARA. It should be noted that the Y-Plex6™ kit has primer imbalance that leads to imbalanced peak heights for the loci at DNA concentrations of 0.5-5ng and relatively quick drop out for DYS389II and DYS391 at DNA concentrations below 0.5ng when compared to the other alleles. To try and increase the sensitivity of the Y-Plex6™ kit, the dilutions described above were amplified with either increased TaqGold (10 units instead of 5) at 30 cycles or the suggested 5 units of TaqGold amplified at 33 cycles instead of 30 cycles. It was observed that adding additional cycles is preferable to adding more Taq-Polymerase, which actually caused inhibition. On average, samples amplified at 33 cycles had a nine-fold increase in RFU strength and the sensitivity of the kit increased from 0.5ng to 0.1ng.

Non-probative Qiagen Robot extracted blood punches; chelex extracted tissue, and organically extracted bone samples that gave full, partial, and no profiles with AmpFISTR® Profiler Plus were evaluated by multiple individuals. The results obtained from the Y-Plex kit were reproducible and in concordance with the Profiler Plus results for the same samples, including previously typed female samples that did not give results when tested with Y Plex. When variation was observed it was due to one technician amplifying more template than the other. In addition to non-probative case samples, the Y-Plex™ 6 kit was also used to test paternity on nine father/son samples and sibling relatedness for a single sibling pair. Results showed that the Y-Plex™ 6 kit confirmed paternally related individuals.

The ability of Y-Plex™ 6 kit to distinguish a male: female mixture, a male: male mixture where the two males differed at 2 out of the 6 loci, and a male: male mixture where the males differed at all 6 loci were evaluated at several mixture ratios. Results demonstrated that the Y-Plex™ 6 kit is specific for male DNA and does not cross-react with female DNA in a known mixture of male and female DNA even when 10ng

of female DNA was used. However, it has been observed that as little as 1ng of female case sample DNA as well as the female kit positive have cross-reacted with certain lots of Y-Plex™ 6 kits, but in no instance was a typeable profile obtained. The cross reactive peaks sized as off ladder alleles and are consistently at 171, 217, 256 and 270 bp. Similarly, an upper ratio limit of 4:1 was established to distinguish a full profile in a mixture of individuals differing at 2 out of 6 loci and 3:2 for individuals differing at all 6 loci. In addition, mixtures were detectable at the 5:1 ratios for both male:male mixtures. We are currently evaluating what the lower ratio limit (*i.e.* 10:1, 20:1) that can be detected by the Y-Plex™ 6 kit.

The stutter percent for all six loci were evaluated and results demonstrated that the average stutter and maximum stutter for each locus was comparable to Reliagene's reported stutter percentages. In addition, a minus 2 stutter was observed at DYS19 on the 377 instruments used for this validation that was not in Reliagene's reported stutter percentages. The Y-Plex™ 6 STR system has proven to be acceptable for analyzing forensic DNA case samples at the Armed Forces DNA Identification Laboratory.

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