AN INTERNAL VALIDATION OF THE PROMEGA POWERPLEX® Y23 ON EXTRACTED DNA AND FTA SAMPLES

Li Min Chua[1], Jun Yu Lee[1] , Nicole An Ting Tang[1] , Terenze Yao Rui Ong[1] , and Christopher Kiu Choong Syn[1,2]

[1]DNA Profiling Laboratory, Applied Sciences Group, Health Sciences Authority
[2]Department of Biological Sciences, National University of Singapore

Y-STR analysis has gained an important foothold within the forensic community, as it is not only applicable to forensic casework, but also to genealogical research and kinship analysis. The PowerPlex® Y23 (PPY23) kit that was recently introduced to the forensic community serves to boost the discriminating power of Y-STR analysis as it includes 6 additional markers (DYS576, DYS481, DYS549, DYS533, DYS570 and DYS643) on top of the existing 17 markers in the AmpFLSTR® Yfiler® PCR Amplification Kit. In this validation study, the robustness of the PPY23 kit in amplifying both liquid DNA and reference blood samples on FTA™ cards was examined. A statistical study was also performed on the local Chinese population in Singapore using the PPY23 kit.

Sensitivity studies performed on extracted liquid DNA showed that 150 pg of DNA was sufficient to obtain full DNA profiles using 30 PCR amplification cycles. A reduction of the PCR amplification cycle to 28 required 500 pg of DNA to be used before complete DNA profiles could be generated. The PPY23 kit was also tested for its cross-reactivity to female DNA. Male and female DNA were mixed in different proportions, before they were amplified using the PPY23 kit. Full male DNA profiles could be obtained from a mixture comprising of 62.5 pg of male DNA and 100 ng of female DNA (in a 1:1600 ratio). We were also able to obtain full profiles even when 500 pg of male DNA was present in an excess of 1 µg of female DNA (in a 1:2000 ratio). Additionally, the PPY23 kit was also tested for its ability to detect the alleles present in a male-male DNA mixture at varying concentrations.

The PPY23 kit was also validated for its use in the direct amplification of FTA™ reference blood samples using a reduced volume protocol. 0.75 mm punches of the reference blood samples on FTA™ cards were taken and amplified using a reduced volume of 15 µL at 26 cycles of PCR amplification. Allele peak heights observed generally range from 1000 RFU to 12000 RFU. In addition, allele calls were consistent even at a reduced volume, with complete profiles obtained; suggesting that the system is robust even when used at reduced volumes. These results further support the use of a reduced amplification volume for the direct amplification of FTA™ reference blood samples using the PPY23 kit.

Lastly, the allelic frequencies of the Y-STR markers in the PPY23 kit were also examined for the local Singapore Chinese population. A total of 200 samples were processed and analyzed to obtain the respective allele and genetic frequencies for application to forensic casework.