Executive Summary

Title: Validation of the PowerPlex Y23 Amplification Kit for Casework Samples using the Applied Biosystems 3500xl Genetic Analyzer

Purpose: To verify the performance characteristics of the Y23 Amplification Kit and validate it for usage in DNA casework.

Background: Currently the DNA Casework Unit (DCU) uses the AmpFLSTR™ Yfiler™ kit for the generation of Y-STR profiles. The Yfiler™ kit amplifies 17 Y-STR loci and is currently validated on the Applied Biosystems 3130xl genetic analyzer. Newer Y-STR amplification kits that include a larger number of YSTR loci are available. Given the larger number of markers, these assays offer greater discriminatory power in Y-STR testing. The PowerPlex® Y23 assay, which targets 23 Y-STR markers, shows a marked increase in haplotype diversity and discriminatory power when compared to 17-marker systems such as Yfiler™. In a survey of nearly 20,000 samples representing 129 global populations, 93% of the PowerPlex® Y23 profiles were observed only once in the total dataset, as compared to 78% of Yfiler™ profiles (Purps et al. 2014). Given the high levels of haplotype resolution across global populations, 23-marker Y-STR testing is likely to offer substantial value in the FBI's Y-STR casework.

With the aims of confirming the suitability of the PowerPlex® Y23 kit for DNA casework use and improving the sensitivity to low-level male contributors, a series of preliminary method development studies were conducted. These tests were aimed at establishing cycle number and target DNA input parameters that would improve the sensitivity of Y-STR testing in the FBI's DNA casework. The manufacturer's recommended cycle number of thirty and target input of 0.5 ng was selected for subsequent validation studies conducted in accordance with SWGDAM Validation Guidelines for DNA Analysis Methods and the Quality Assurance Standards for Forensic DNA Testing Laboratories.

Internal Validation:

Precision and Accuracy

The precision and accuracy of the PowerPlex® Y23 assay were established with twenty-four (24) separate allelic ladder preparations and multiple injections in both Quantico (QCO) and Huntsville (HSV). Across both laboratories, the minimum observed precision in sizing was 99.92% (HSV lab), and the maximum observed deviation in size was 0.069 bp (again, observed in the HSV lab). Three times the maximum observed standard deviation of 0.069 bp across the

two laboratories results in a value of 0.207 bp, indicating that the precision of the assay should consistently permit the distinction of alleles differing by 1 bp in size.

Accuracy was tested via two experiments in which dilutions of the same three male samples were amplified in duplicate. The experiments were performed by different scientists and run on different capillary electrophoresis instruments. Across the experiments, amplification duplicates, scientists and instruments, allele calls were repeatable, reproducible and accurate.

Sensitivity and Stochastic Effects

In both QCO and HSV, assay sensitivity was evaluated with amplifications of 1 ng, 0.5 ng, 0.25 ng, 0.12 ng, 0.06 ng, 0.03 ng and 0.01 ng of undegraded DNA. In QCO, 286 amplifications from 13 donors were evaluated. In HSV, 176 amplifications from 8 donors were tested. In both laboratories, full Y23 profiles were consistently obtained with total inputs down to 0.12 ng. At 60 pg, approximately 1% allelic loss was observed in both laboratories. At 30 pg, 6.4% (HSV) and 10% (QCO) allelic loss were observed; and at 10 pg, 25% (HSV) and 51% (QCO) allelic loss were observed.

Dye specific analytical thresholds were developed using sensitivity data developed in QCO. Electropherograms were analyzed at an RFU of 1, and all authentic alleles, pull-up peaks, spikes and other obvious artifacts were removed to assess background signal. These analyses led to the establishment of the following ATs: blue – 100, green – 125, yellow – 135, and red – 135. To determine the appropriateness of these ATs for the HSV lab, baseline signal from a subset of HSV data was assessed. The results were highly consistent with the QCO results, supporting the use of the same ATs across laboratories.

The average peak height ratio (PHR) for the DYS385 locus was evaluated using QCO amplifications of 0.01 ng to 1 ng of DNA (as noted above). The average PHR across all DNA inputs was 86%. As expected, however, average PHRs decreased as DNA input decreased, with allelic dropout observed in some amplifications of 0.30 ng and lower. Among the 12 samples and 260 amplifications in which two distinct alleles were expected at DYS385a/b, 22 dropouts were observed across the 520 alleles expected. As dropout was not observed when partner alleles were greater than 872 RFUs, a stochastic threshold of 950 RFUs was recommended for this locus. When the stochastic threshold of 950 was applied to the HSV sensitivity data, all heterozygous samples had detectable companion alleles above the AT.

Contamination and Cross-Contamination

Sporadic drop-in and robotic cross-contamination were assessed using the dye-specific analytical thresholds (ATs) established during validation. For drop-in, rates observed in QCO and HSV were very similar. In QCO, rates of 0.28% per locus and 6.4% per amplification were observed. In HSV, rates of 0.34% per locus and 6.9% per amplification were observed. With respect to potential cross contamination and the target input of 0.5 ng, two instances were observed in HSV and no instances were observed in QCO. The low levels of sporadic drop-in

and possible robotic cross-contamination suggest that 1) the PowerPlex® Y23 kit reagents are appropriate for use with the amplification of casework samples and 2) the risk of cross-contamination is low with the validated, automated laboratory workflow.

Mixtures

A total of ninety-six (96) two and three person mixtures, representing three different DNA inputs (1 ng, 0.5 ng, and 0.01 ng) and various mixture ratios were typed and successfully analyzed with PowerPlex® Y23. Peak height ratios at loci with unshared alleles were indicative of the approximate ratio of DNA contributors, and amplifications of various mixture ratios and DNA inputs suggest that the system can be used to distinguish between male components in a mixture. For apparent two person mixtures with alleles exceeding the analytical threshold at all loci, a peak height ratio of <60%, minor to major, permitted correct deduction of the major contributing haplotype.

At the target input of 0.5 ng, minor contributors were regularly detectable in apparent 2P mixtures at ratios as low as 1:99, while at 0.1 ng, evidence of the minor was only detectable down to 1:19 or 1:9, depending on the specific mixture tested. For apparent 3P mixtures, the major contributor could be deduced with a peak height ratio of <60% for both minor peaks relative to the major in amplifications of 0.5 and 1 ng, but not in 0.1 ng amplifications. It is therefore recommended that if major contributing haplotypes are deduced in 3P mixtures, it be done with caution, particularly when DNA inputs are known to be low (i.e., less than the target input of 0.5 ng).

Case-Type Samples

Amplification of twenty (20) mock evidence extracts and five (5) skeletal remains extracts, representing a variety of casework scenarios, demonstrated that mixed and single source samples can be successfully typed with PowerPlex® Y23. For those samples with known Y-STR haplotypes, the Y23 results obtained from the mock samples were consistent with the known profiles.

SRM

A NIST SRM demonstrated correct Y23 typing results and performed as expected.

Conclusion:

The experiments performed here demonstrate the efficacy, accuracy, reproducibility, sensitivity, and reliability of the PowerPlex® Y23 assay. In addition, they serve to define the conditions under which reliable and reproducible Y haplotypes can be obtained from the range of sample types, quantities and qualities typically encountered in DNA casework at the FBI Laboratory. The totality of the internal validation studies supports the suitability of the PowerPlex® Y23 assay for the FBI's Y-STR casework at both the Quantico and Huntsville facilities.

Supporting Documentation:

An experimental roadmap, experiment summaries and corresponding supporting data are located in the Validation of the PowerPlex® Y23 Chemistry for DNA Casework electronic notebook.

Technical Publications and/or Studies:

Thompson J et al. Developmental validation of the PowerPlex® Y23 System: a single multiplex Y-STR analysis system for casework and database samples. Forensic Sci Int Genet. 2013; 7:240-50.

Technical Manual. PowerPlex® Y23 System for Use on the Applied Biosystems® Genetic Analyzers. Promega Corporation. Revised 04/21.