SDPD Forensic Science Section Applied Biosystems 3130 Genetic Analyzer Validation

Validation Summary

Introduction

Since the implementation of Short Tandem Repeat (STR) DNA testing at the San Diego Police Department (SDPD) in 1999, we have been analyzing amplified DNA products using the Applied Biosystems 310 Genetic Analyzers. With the growing demand for DNA testing at the SDPD, the number of samples requiring analysis has steadily grown. In order to accommodate the increased number of samples being processed in the laboratory, the Forensic Biology Section has sought to increase their capacity for analysis through a number of means, including technologically. The Applied Biosystems 3130 Genetic Analyzer is one solution to increasing sample throughput.

The 3130 Genetic Analyzer operates on the same basic principles as he 310 Genetic Analyzers. However, some fundamental differences exist between the two platforms, especially with respect to fluorescent detection. Some of the basic differences between the platforms include the 4-capillary arrays and 96-well sample racks allow for increased sample throughput in less time than the conventional single capillary platform. The new automated polymer delivery system will reduce maintenance time and eliminate manual syringe washing and filling. Some of the more integral differences deal with the detection system. In the 310 the detection of fluorescence from the STR fragments is accomplished in parallel with the excitation beam. In the 3130 in contrast, the detection of the fluorescent STR fragments is done in perpendicular to the excitation beam. Additionally the excitation beam is split with a beam splitter before passing through the capillary array from alternate sides to obtain more even excitation across the array. The detection of the fluorescent molecules in the STR identification method and directly linked with the sensitivity of the platform, and is therefore critical to investigate prior to implementing the 3130 Genetic Analyzer into forensic casework. Additionally, the new analysis software called GeneMapper ID, which replaces all functions of GeneScan and Genotyper, should reduce analysis time (please see the GeneMapper^{TM} ID validation for more details).

The validation of the 3130 Genetic Analyzer by the SDPD forensic biology section was based on the DAB/SWGDAM Quality Assurance Standards for Forensic DNA Testing Laboratories. The 3130 Genetic Analyzer was evaluated

Materials and Method

Data from five Identifiler casework run folders from two different 310s were converted to .fsa files using a conversion program supplied with GeneMapper ID, to render the data compatible with GeneMapper ID installed on a PC computer. The raw collection data was then analyzed using GeneMapper ID with parameters equivalent to those used with GeneScan and Genotyper. Results for up to five alleles per DNA locus were tabulated and exported to Excel worksheets so that the GeneMapper ID analyzed data could be compared to the previous GeneScan/Genotyper

analyzed data with respect to genotyping, allele sizing and peak heights. Using a similar approach, data from several PowerPlexY casework run folders and validation studies were converted, combined and analyzed with GeneMapper ID for comparison to previous GeneScan/Genotyper analyzed data.

Results

The five Identifiler casework run folders contained data from approximately 190 samples, allelic ladders and controls (positive, negative and reagent blanks). The PowerPlex Y data was from 20 samples, ladders and controls.

Genotypes

In all samples, the major allele assignments were identical whether analyzed using GeneScan/Genotyper or GeneMapper ID. When a 'no match' was indicated, this was explainable by one of two factors:

1) An allele or artifact with a peak height close to the 75rfu peak detection threshold was missing from one or other analysis due to drop-out. (Note: some of the GeneScan/Genotyper data was analyzed using a peak detection threshold of 50rfu, which explained why the same peak was not detected using GeneMapper ID with a peak detection threshold of 75rfu.)

2) An unfiltered artifact, such as a stutter peak, was labeled (often as an OL Allele?) by one or both analyses.

Allele Size

When an allele peak (and in some cases artifact) was labeled by both analyses, the difference in a base-pair sizing between GeneScan/Genotyper and GeneMapper ID was calculated. Average base-pair sizing differences within a casework run folder (or for the PowerPlex Y samples) did not exceed 0.055 base-pairs. The maximum base-pair size difference observed was 0.33 base-pairs.

Peak Height

When an allele peak (and in some cases artifact) was labeled by both analyses, the difference in peak height between GeneScan/Genotyper and GeneMapper ID was also calculated. Average peak height differences within a casework run folder (or for the PowerPlex Y samples) did not exceed 47rfu. The maximum peak height difference observed was 236rfu.

Conclusions

For both Identifiler and PowerPlex Y amplified DNA samples, the final DNA results were equivalent in allele assignments, and comparable in allele base-pair sizing and peak heights whether the collection data was analyzed using GeneScan and Genotyper on a Macintosh platform or using GeneMapper ID on a PC platform.