Comprehensive Evaluation of the ForenSeq™ DNA Signature Prep Kit on the MiSeq FGx™ Next-Generation Sequencing System

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Introduction

Capillary electrophoresis (CE) is the current standard for human identification in forensic DNA laboratories. CE uses fragment analysis to analyze short tandem repeats (STRs). These are the mainstay markers for human identification due to their highly polymorphic and discriminatory power among individuals.

Next-Generation Sequencing (NGS) has grown in popularity in recent years. This is due to its enhanced capability to multiplex and sequence hundreds of forensically relevant markers including STRs and single nucleotide polymorphisms (SNPs). Sequencing of individual STR fragments make it possible to distinguish same-sized alleles that have internal sequence variations and can also aid in linking stutter to its parent allele based on the sequence motif. The ForenSeq™ DNA Signature Prep Kit for the MiSeq FGx™ NGS system is an assay that targets 27 autosomal STRs, 24 Y-STRs, 7 X-STRs and 94 identity SNPs, and can include 22 phenotypic SNPs, and 56 biogeographical ancestry SNPs through an additional primer set (Primer Set B).

This study aimed to evaluate the performance of the ForenSeq™ DNA Signature Prep Kit (Primer Set B) in single source and mixture analysis. In addition, a concordance study was be performed between NGS and CE platforms.

Methods

Sample Collection

Buccal swabs from two males and two females were collected.

Extraction

EZ1® Advanced XL (QIAGEN) using the EZ1® DNA Investigator Kit

Quantification

7500 Real-Time PCR (Applied Biosystems™) system using the Quantifiler™ Trio DNA Quantification Kit

Sample Preparation

Single source and mixture samples were prepared for fragment analysis and sequencing. A serial dilution from 1 ng to 31 pg was prepared using one male sample (Male 1). Mixtures were prepared as shown in Table 1 with a target DNA input of 1 ng.

PCR Amplification

GlobalFiler™

CE

Applied Biosystems™ 3500 Genetic Analyzer

Library Preparation

ForenSeq™ DNA Signature Prep Kit (primer set B).

NGS

MiSeq FGx™ (Illumina) System

Table 1: Mixtures at varying contributor ratios.

Mixtures	Ratios
Two Person (Male:Male and Male:Female)	1:1, 1:5, 1:10, 1:20, 1:50, 1:100
Three Person (Female:Male:Male)	1:1:1, 5:1:1, 10:5:1, 20:5:1
Four Person (Female:Female:Male:Male)	1:1:1:1, 5:5:1:1, 10:5:1:1

Results

F1:M1 20:1

Figure 1A: D8S1179, TH01, and TPOX loci for the M1:F1 1:20 sample run on the MiSeq FGx™. M1 = Male 1, F1 = Female 1.

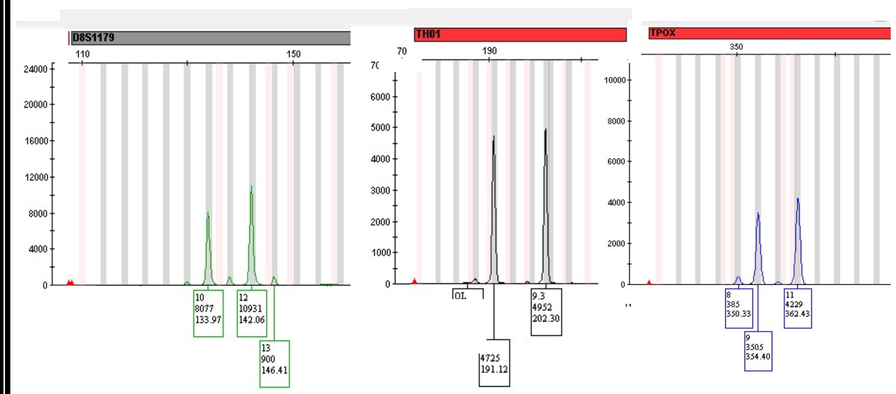

Figure 1B: D8S1179, TH01, and TPOX loci for the M1:F1 1:20 sample run on the 3500 genetic analyzer. M1 = Male 1, F1 = Female 1. Major contributor should show up as 10, 12 at D8S1179, 7, 9.3 at TH01, and 9, 11 at TPOX. Minor contributor should be seen as 12, 13 at D8S1179, 9.3 at TH01, and 8 at TPOX.

F1:M1:M2 10:5:1

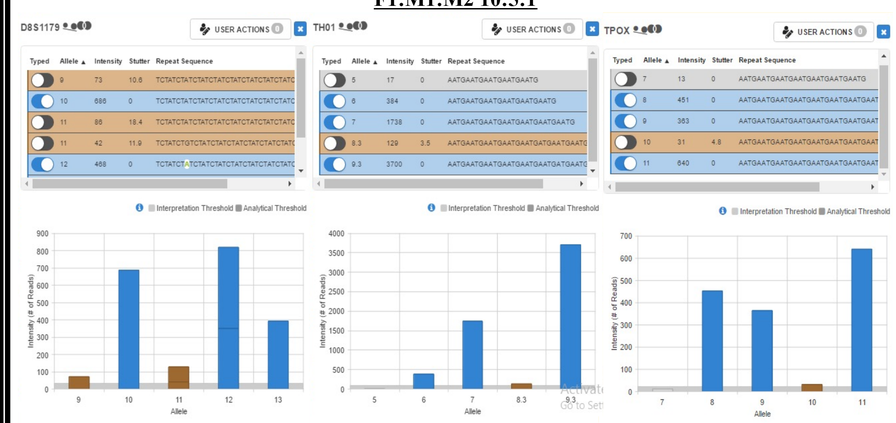

Figure 2A: D8S1179, TH01, and TPOX loci for the F1:M1:M2 10:5:1 sample run on MiSeq FGx™. M1 = Male 1, M2 = Male 2, F1 = Female 1.

Results

F1:M1:M2 10:5:1

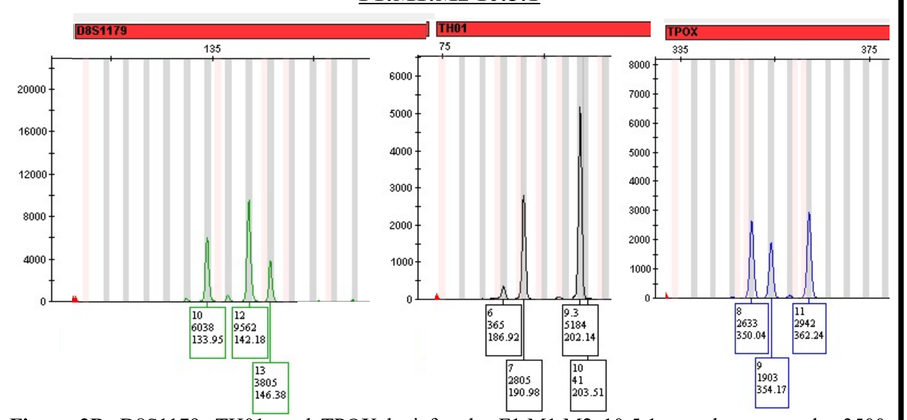

Figure 2B: D8S1179, TH01, and TPOX loci for the F1:M1:M2 10:5:1 sample run on the 3500 genetic analyzer. M1 = Male 1, M2 = Male 2, F1 = Female 1. Major contributor should show up as 10, 12 at D8S1179, 7, 7, 9.3 at TH01, and 9, 11 at TPOX. Minor contributors should be seen as 12, 13 and 10, 13 in D8S1179, 9.3 and 6, 10 in TH01, and 8 and 8, 11 in TPOX.

Table 2. Genotype of donor(s) from two-person and three-person mixtures at D8S1179, TH01, and TPOX. M1 = Male 1, M2 = Male 2, F1 = Female 1.

Mixture Ratio	Locus	M1		F1		M2	
M1:F1 1:20	D8S1179	12	13	10	12		
	TH01	9.3		7	9.3		
	TPOX	8		9	11		
F1:M1:M2 10:5:1	D8S1179	12	13	10	12	10	13
	TH01	9.3		7	9.3	6	7
	TPOX	8		9	11	8	11

Discussion and Conclusions

In this initial mixture evaluation, the number of alleles detected in single and mixture source samples run on the 3500 Genetic Analyzer and the MiSeq FGx™ NGS system were counted and reported. In particular, a full concordance of STR allele call was observed for the major and minor DNA contributors of the whole mixture samples tested between the CE and NGS platform. In addition, all alleles of the minor DNA contributors falling into stutter range position of the parental alleles were confirmed by sequencing for the two-, three- and four person DNA mixture series.

This preliminary study showed full concordance of genotypes between CE and NGS platforms for single source and mixture samples. Further analyses are ongoing to evaluate the sensitivity of the ForenSeq™ DNA Signature Prep Kit and the detection of isoalleles. This may assist with mixture deconvolution, enabling the inclusion or exclusion of reference profiles from mixture samples.

References

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