Sensitivity Comparison of the Illumina® ForenSeq™ DNA Signature Prep kit and the Applied Biosystems® GlobalFiler™ kit

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Abstract

After attending this presentation, attendees will be better informed regarding the level of sensitivity of the ForenSeq™ DNA Signature Prep kit compared to traditional genotyping methods.

The presentation will impact the forensic science community by describing the benefits of using next generation sequencing (NGS)-based methods for analysis of low template forensic samples.

Currently forensic DNA analysis is typically performed using capillary electrophoresis. However, next generation sequencing (NGS) enables an increase in the breadth and depth of information obtained from each sample. Megaplex kits target a large number of DNA markers including globally recognized short tandem repeats (STRs) and single nucleotide polymorphisms (SNPs). SNPs are desirable targets for forensic analyses because they have low mutation rates, and can be recovered from highly degraded samples due to their short lengths. Additionally, some SNPs are interrogated to build a phenotypic profile of an individual that can be used to generate investigative leads. NGS technologies also enable an analyst to pool several samples together to run simultaneously, ultimately reducing per sample costs.

The Illumina® ForenSeq™ DNA Signature Prep kit targets a total of 231 STRs and SNPs. Conversely, the Applied Biosystems® GlobalFiler™ kit targets 24 STRs. Both kits are designed to perform optimally with approximately 1 ng of input DNA. However, due to the chemistry of the Illumina® MiSeq FGx™ Forensic Genomics System, high-quality data can still be recovered with significantly lower inputs.

For this study, buccal swabs were obtained from five donors following approved IRB protocol. DNA was extracted from each buccal swab using the QIAGEN EZ1 Advanced XL, and DNA Investigator kit. Extracts were then quantified using the Applied Biosystems® QuantiFiler™ Trio DNA Quantification kit. A serial dilution was performed on each buccal swab sample to obtain a range of DNA concentrations (1.0 ng/µL – 12.5 pg/µL) commonly encountered in the forensic laboratory. Extracts were amplified using either the Illumina® ForenSeq™ or Applied Biosystems® GlobalFiler™ kit. Amplified products were run on respective sequencing instrumentation. Complete profiles were obtained for inputs of 1 and 0.5 ng using both methods. At lower DNA inputs, samples amplified using the ForenSeq™ kit led to a higher percentage of allele recovery than those amplified with the GlobalFiler™ kit. Similar results were obtained when looking strictly at the 59 STRs that are targeted in the ForenSeq™ kit versus the 24 STRs in the GlobalFiler™ kit. Again, at input levels of 1 and 0.5 ng, both methods resulted in full profiles. At the three lowest inputs, the ForenSeq™ kit also led to a higher percentage of allele recovery than the GlobalFiler™ kit.

The Illumina® ForenSeq™ kit is significantly more sensitive than the Applied Biosystems® GlobalFiler™ kit. As a result, there is an increase in the amount of data recovered from low quantity samples using NGS-based sequencing methods. Future studies will include assessment of both methods using extracts obtained from forensically relevant samples such as calcified tissues and mock casework samples.

Introduction

The Illumina® MiSeq FGx™ is a next generation sequencing platform that has been created specifically for the field of forensic science and has been fully validated according to the Science Working Group on DNA Analysis Methods, or SWGDAM[1]. This project aims to assess the sensitivity of the Illumina® ForenSeq™ DNA Signature Prep kit and MiSeq FGx™ Forensic Genomics System, and directly compare it to traditional DNA analysis methods, such as the Applied Biosystems® GlobalFiler™ kit and 3500 xL Genetic Analyzer. There have been previous studies that have focused on the sensitivity of the MiSeq FGx™ system, but there are very few studies that directly compare the next generation sequencing methods to traditional capillary electrophoresis methods when it comes to forensically relevant samples.

The Illumina® ForenSeq™ DNA Signature Prep kit, targets 230 loci. These loci are as follows; 27 global autosomal STRs, 24 Y-STRs, 7 X-STRs, 94 identity informative SNPs, 22 phenotypic SNPs, and 56 biogeographical ancestry SNPs[1]. The ForenSeq™ DNA Signature Prep kit not only targets a large range of loci, but it also is designed to process challenging samples such as degraded DNA. While many of the STRs that are targeted in the ForenSeq™ DNA Signature Prep kit may be affected, the SNPs that can be processed are less than 125 base pairs which makes it much easier to target loci in degraded DNA. This kit is also helpful when it comes to mixture samples. This is likely due to the sensitivity of sequencing by synthesis, as well as the number of markers that it includes[2].

Conversely, the Applied Biosystems® GlobalFiler™ kit is used with capillary electrophoresis by way of the 3500 xL Genetic Analyzer, which is the traditional sequencing method used in this project. It targets 21 autosomal STRs, 1 Y-STR, and 2 sex-determining markers (Amelogenin and Y-indel)[3].

For both sequencing methods, buccal swabs from five donors were serially diluted to 1.0 ng/µL, 0.5 ng/µL, 0.1 ng/µL, 50 pg/µL, and 12.5 pg/µL. All five samples for each of the dilutions were run on both instruments to generate results.

Methods

Illumina® ForenSeq™ DNA Signature Prep kit and MiSeq FGx™ Forensic Genomics System

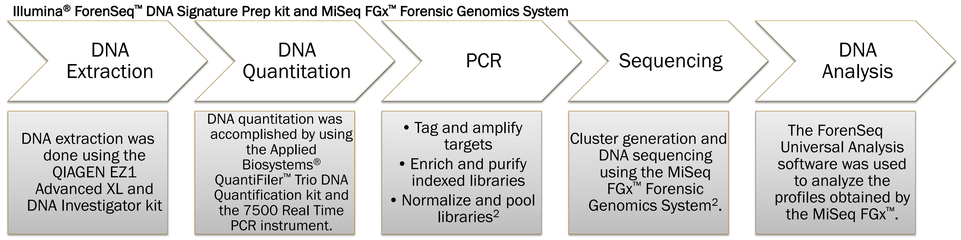


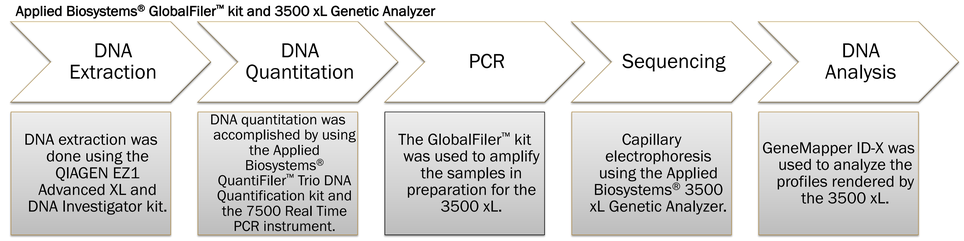
Table 1. ForenSeq DNA Signature Prep Kit Loci[1]

Type of Marker	Number of Markers	Amplicon Size Range (bp)
Global Autosomal STRs	27	61-467
Y-STRs	24	119-390
X-STRs	7	157-462
Identity SNPs	94	63-231
Phenotypic SNPs	22	73-227
Biogeographical Ancestry SNPs	56	67-200

Table 2. GlobalFiler kit Loci[3]

Type of Marker	Number of Markers	Amplicon Size Range (bp)
Global Autosomal STRs	21	60-475
Y-STRs	1	355-400
Sex-determining	2	65-110

Applied Biosystems® GlobalFiler™ kit and 3500 xL Genetic Analyzer



Future Work

- ❑ Experiment with different extraction methods for bones and compare the discrimination powers of the methods used for this experiment
- ❑ Complete runs by extracting DNA from cremated remains using a modified Prepfiler protocol
- ❑ Examine sensitivity when using mock casework samples such as degraded bone, teeth, and hair

Conclusion

- ❑ After obtaining profiles of the five diluted buccal swab extracts, the results of each method were compared.
- ❑ The Illumina® ForenSeq™ kit has the ability to target more than the 24 loci targeted by the GlobalFiler™ kit, precisely 230 total markers.
- ❑ Overall, the Illumina® MiSeq FGx™ showed a higher allele recovery than the Applied Biosystems® 3500 xL Genetic Analyzer based on results shown in Figure 2.

Results and Discussion

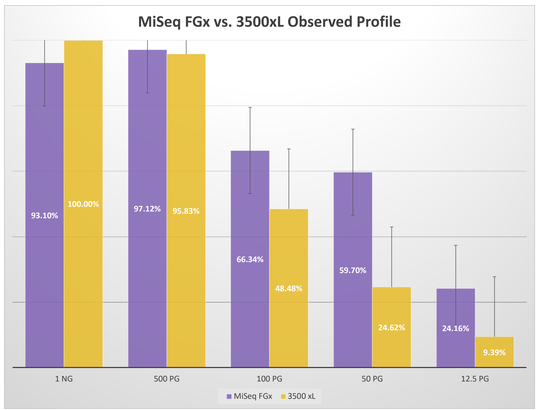


Figure1. Observed profiles generated by both instruments for all possible markers.

For both types of sequencing instruments, the expected results were seen and complete profiles were able to be recovered at 1.0 and 0.5 ng. At lower quantities, slight differences in the recovered profiles were observed with the MiSeq FGx rendering more sensitive results.

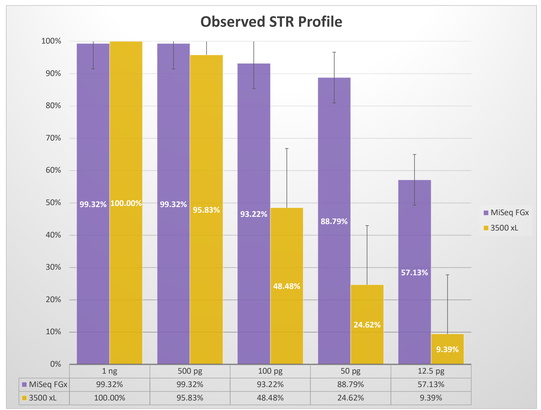


Figure 2. Observed profiles generated by both instruments for only STR targets (MiSeq FGx-59, 3500 xL-24).

Similar to the results of Figure 1, at 1.0 and 0.5 ng full profiles were observed for only STRs targeted for each. There was a higher allele recovery for the MiSeq FGx, than the 3500 xL.

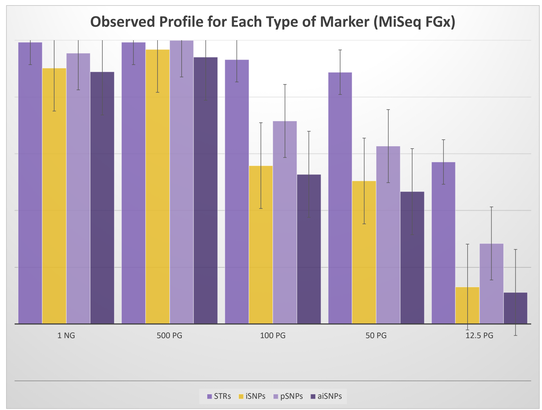


Figure 3. Observed profiles generated by the MiSeq FGx for each type of marker.

The results shown in the figure above, are not as expected. Given that SNPs are much shorter in length than STRs, it was expected that there would be higher recovery of the SNPs targeted at lower quantities than STRs. However, STRs were recovered at higher levels for the lower quantities than the SNPs.

References

[1] Jäger A, et al. Developmental Validation of the MiSeq FGx Forensic Genomics System for Targeted Next Generation Sequencing in Forensic DNA Casework and Database Laboratories. Submitted for publication, 2016.

[2] Illumina, Inc. (n.d.). ForenSeq™ DNA Signature Prep Kit Data Sheet.

[3] Applied Biosystems by Thermo Fisher Scientific (n.d.). GlobalFiler® PCR Amplification Kit (200 Reactions).

Acknowledgements

I would like to thank Western Carolina Department of Chemistry and Physics for assisting with experimental execution and providing reagents necessary for this experiment.