Evaluation of the ForenSeq MainstAY Kit with Challenging Samples

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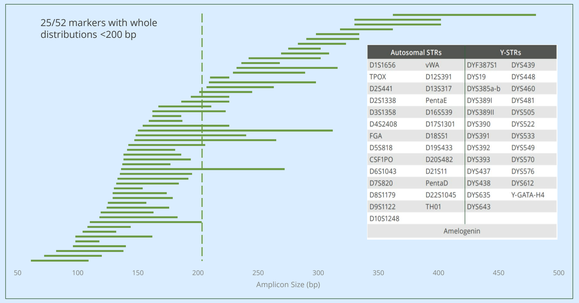
INTRODUCTION

Short Tandem Repeat (STR) kits coupled with capillary electrophoresis are still the gold standard for human identification in forensic laboratories. The discriminatory power of these kits is effective for most cases with high quality DNA. However, there continue to be examples where recovered DNA may not be sufficient to produce STR profiles due to low quantity, low quality, degradation, or mixtures[1].

Sensitivity of the MiSeq FGx™ (Verogen, San Diego, CA) system has been previously demonstrated with degraded or damaged DNA[2]. Challenging samples collected from skeletal remains, environmentally aged samples, sexual assault kits, or historical cases can benefit from Next Generation Sequencing (NGS).

The ForenSeq MainstAY Kit (Verogen) is a NGS option that puts forensic laboratories at the focus of its development. This new NGS assay from Verogen focuses on identification and only contains autosomal and Y- STRs (52 total) (**Figure 1).** This decrease in markers compared to the ForenSeq DNA Signature Prep Kit (Verogen) allows for improved amplification success and more robust data.



Autosomal STRs		Y-STRs	
D1S1656	vWA	DYF387S1	DYS439
TPOX	D12S391	DYS19	DYS448
D2S441	D13S317	DYS385a-b	DYS460
D2S1338	PentaE	DYS389I	DYS481
D3S1358	D16S539	DYS389II	DYS505
D4S2408	D17S1301	DYS390	DYS522
FGA	D18S51	DYS391	DYS533
D5S818	D19S433	DYS392	DYS549
CSF1PO	D20S482	DYS393	DYS570
D6S1043	D21S11	DYS437	DYS576
D7S820	PentaD	DYS438	DYS612
D8S1179	D22S1045	DYS635	Y-GATA-H4
D9S1122	TH01	DYS643	
D10S1248			
Amelogenin			

Figure 1. Amplicon sizes of ForenSeq MainstAY Kit

In this study, we evaluated the ForenSeq MainstAY Kit. The kit's performance regarding sensitivity, repeatability, and challenging samples was evaluated. Previously collected capillary electrophoresis (CE) data for the challenging samples was used for comparison purposes.

MATERIALS AND METHODS

Challenging Samples and DNA Extraction:

- Skeletal samples (n=14) extracted using Prepfiler® BTA Forensic DNA Extraction Kit
- Embalmed tissue (n=3) extracted using QIAamp® DNA FFPE Tissue Kit
- Decomposing muscle tissue (n =3) extracted using QIAamp® DNA Investigator Kit
- Aged saliva (n=2); blood (n=4);rooted hairs (n=2) extracted using EZ1 DNA Investigator Kit
- Touch DNA collected from handled rifle magazines using nylon FLOQSwabs™ (n=2) and extracted using the QIAamp DNA Investigator kit

DNA Quantification and STR Analysis

- DNA extracts were quantified with the Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher Scientific) and amplified with with the Investigator® 24plex QS PCR Amplification Kit (QIAGEN) or GlobalFiler™ PCR Amplification Kit (Thermo Fisher Scientific).
- Amplified products were separated and detected on a 3500 Genetic Analyzer. Data were analyzed on GeneMapper ID-X v.1.4.

ForenSeq MainstAY Kit Evaluation Parameters

Repeatability
- Four repetitions of 12 pristine DNA extracts and controls were prepared for a 64-sample run on a standard flow cell.

Sensitivity
- Two sets of serial dilutions (1ng – 8pg) of 2800M DNA (Promega) were prepared.

Inter-individual
- Two examiners (E1 and E2) performed a sensitivity run using 4 repetitions (2 per dilution) for a 32-sample run on a standard flow cell.

Challenging Samples
- 30 samples were tested with 2 controls for a 32-sample run using the MainstAY chemistry on a standard flow cell. Results were compared with CE data.

**Data Analyzed using ForenSeq Universal Analysis Software v2.1

RESULTS AND DISCUSSION

Repeatability

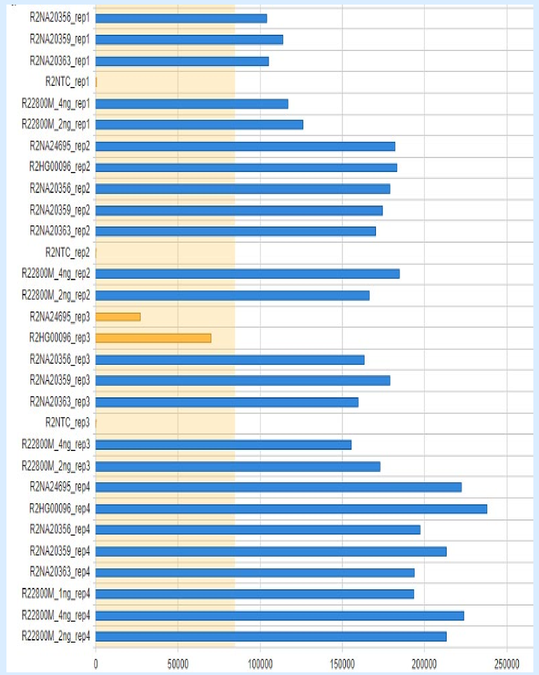


Figure 2. Read intensities from repeatability evaluation. Only 30/64 samples shown. There were only two instances of inconsistent repeatability. Two samples (R2NA24695 and R2HG0096) each had one replicate (rep3) that had much lower reads counts than other replicates due to a pipetting error.

Challenging Samples Study

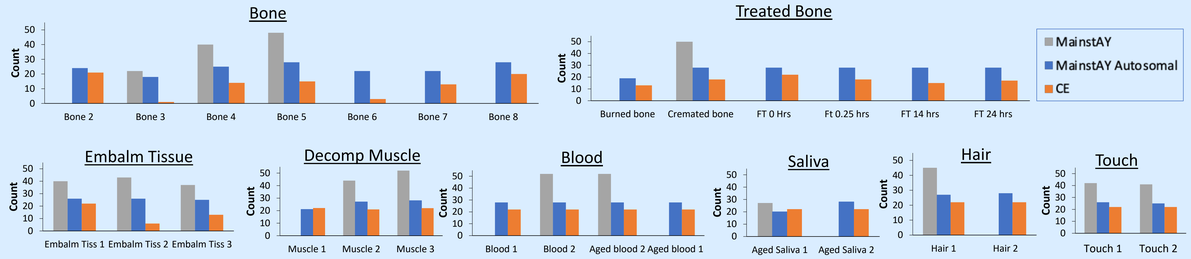


Figure 4. MainstAY and CE comparison. Gray bars include Y-STRs allele counts seen in male samples. *Bone 2-7 CE data are from GlobalFiler, All others use Investigator 24Plex. Bone 1 recovered no alleles for either method and is not displayed.

Samples < 1ng	Input (pg)
Bone 1	3.71
Bone 2	104.92
Bone 3	9.02
Bone 4	15.48
Bone 5	35.36
Bone 6	9.40
Bone 7	150.68
Aged blood1	488.20
Touch1	38.32
Touch2	34.18
Burned bone 1	688.29

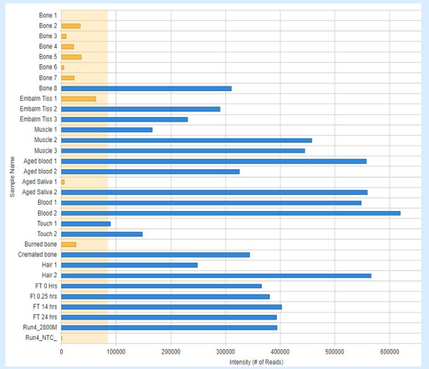


Figure 5. Read intensities from challenging samples evaluation. Table shows samples with less than 1ng input. Most samples with less than 1 ng input failed to reach manufacturer's recommended intensity threshold, except for Touch Samples 1 and 2. *Pipetting error with aged saliva.

Repeatability
- Highly repeatable technique with 58/60 samples above quality threshold (**Figure 2**).

Sensitivity and Inter-Individual Variability
- The MainstAY Kit provides quality data at low concentrations of 8pg with 40% – 50% of alleles recovered across two examiners (**Figure 3**).
- Most common cause of variability in 7/64 replicates was pipetting error (**Figure 3**).

Challenging Samples
- Compared to traditional CE methods, most samples saw improvement with the MainstAY chemistry (**Figure 4**).
- 8/30 samples had reduced autosomal STR recovery with MainstAY compared to CE methods (**Figure 4**).
- Even with low sample input (**Figure 5**), high allele recovery was seen due to mean amplicon size of 235bp for the 52 loci.

Sensitivity and Inter-Individual Variability

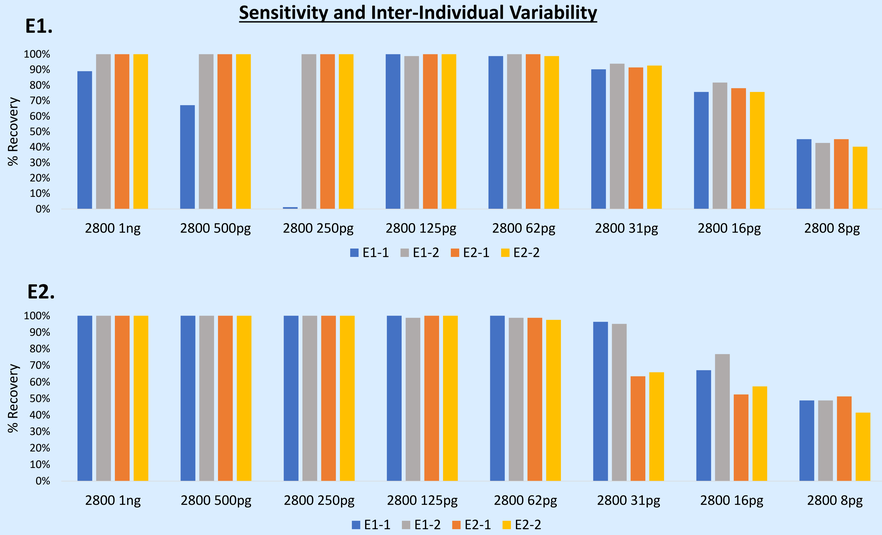


Figure 3. Sensitivity and examiner comparisons. Examiner E1 prepared blue and green dilutions, Examiner E2 prepared orange and yellow. Each examiner prepared four replicates from the serial dilution of 2800M from 1ng to 8pg.

CONCLUSIONS

- Overall, quality testing with MainstAY demonstrated the chemistry to be highly sensitive and reproducible, with consistent results observed in four repetitions of 12 samples, including controls.
- Dropout was seen starting with input DNA of 31pg, but approximately 40-50% of loci were still recovered at 8pg.
- With challenging samples, MainstAY method improved or obtained comparable results to CE in 73% of samples, and when comparing all alleles (autosomal and Y) this increased to 93%.
- Y-STRs give possible mixture information if there are male inclusions.
- Time and cost are comparable to CE methods, and MainstAY can be a tool for challenging samples.

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