Technical Note

VEROGEN

Comparable performance of ForenSeq libraries prepared by manual methods and on the Verogen PrepStation

Introduction

DNA analysis of forensic samples is essential for solving criminal cases, property crimes and identifying unidentified human remains. Next-generation sequencing (NGS) offers multiple benefits such as the ability to analyze more forensic markers, provide better statistical discriminatory power, and support low input samples and mixtures. As an increasing number of global forensic laboratories implement NGS, it is important to enable the generation of high quality DNA data with an operationally efficient workflow that can reduce overall turnaround time, maximize reproducibility and minimize errors associated with manual handling of samples.

Automating the generation of NGS libraries allows laboratories to routinely batch samples while accounting for human variability and minimize sample-to-sample variance. Implementing an automation platform within a forensic laboratory requires extensive validation to ensure that it meets the high quality standards that are required by the justice system. This requires forensic laboratories to balance the capital expenditure costs associated with purchasing an automation platform with the cost, labor and time associated with validation.

The Verogen PrepStation provides a low-cost automation solution that supports low and high throughput laboratories, with out-of-the-box automation scripts that have been designed, developed and validated with the ForenSeq workflow. In addition, extensive support from a team of forensic scientists with forensically relevant validation plans make adoption of this platform easy and cost effective for all forensic laboratories irrespective of throughput considerations. This technical note summarizes data generated by Verogen PrepStation demonstrating the high quality of ForenSeq libraries generated with the Verogen PrepStation.

Figure 1: Verogen PrepStation for ForenSeq® Assays.

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No contamination of ForenSeq libraries generated on the Verogen PrepStation.

Contamination of forensic samples processed on an automation platform can compromise the DNA analysis. To assess the likelihood of contamination, two studies were performed. The first study evaluated 1ng of a male genomic DNA sample (NA18507) and 1 ng of a female genomic DNA sample (NA12878), plated in a 96-well checkerboard layout format. The second study evaluated 1 ng of positive control DNA (NA24385), provided in the ForenSeq MainstAY Library Prep Kit, and a negative template control (NTC), also plated in a 96-well checkerboard layout format. In both studies, half the plates were manually processed using the ForenSeq MainstAY Library Prep Kit[1] and the other half of the plate was processed using the Verogen PrepStation and the ForenSeq MainstAY Library Prep Kit. Figure 2 illustrates the plate layouts. Analysis of the data after library preparation and sequencing demonstrated no contamination across the male and female samples in both the manual and automated studies. Similarly, no contamination was detected in any of the NTC samples that were processed with the positive control samples using the Verogen PrepStation, demonstrating that the Verogen PrepStation can process up to 48 samples with minimized risk of contamination.

Reproducible performance at low inputs of DNA within and across automation platforms.

To assess the reproducibility of the Verogen PrepStation platforms, ForenSeq MainstAY Kit libraries were prepared using 1 ng 500 pg, 250 pg, 125 pg and 63 pg positive control DNA (NA24385) on two PrepStation instruments (PS-1 and PS-2). Loci detected above the AT threshold were reproducible across the two PrepStation platforms tested (Fig 3A). A similar study was performed to asess column-to-column variability within a PrepStation. Figure 3B summarizes the results from this within-platform study for 3 input DNA levels (1 ng, 125 pg and 63 pg) showing high reproducibility within the columns of a PrepStation. In addition, the results demonstrate that the samples are processed consistently between columns one to six.

These studies suggest little variation and a high degree of reproducible performance within and between Verogen PrepStations with very low inputs of DNA, supporting its use in forensic casework.

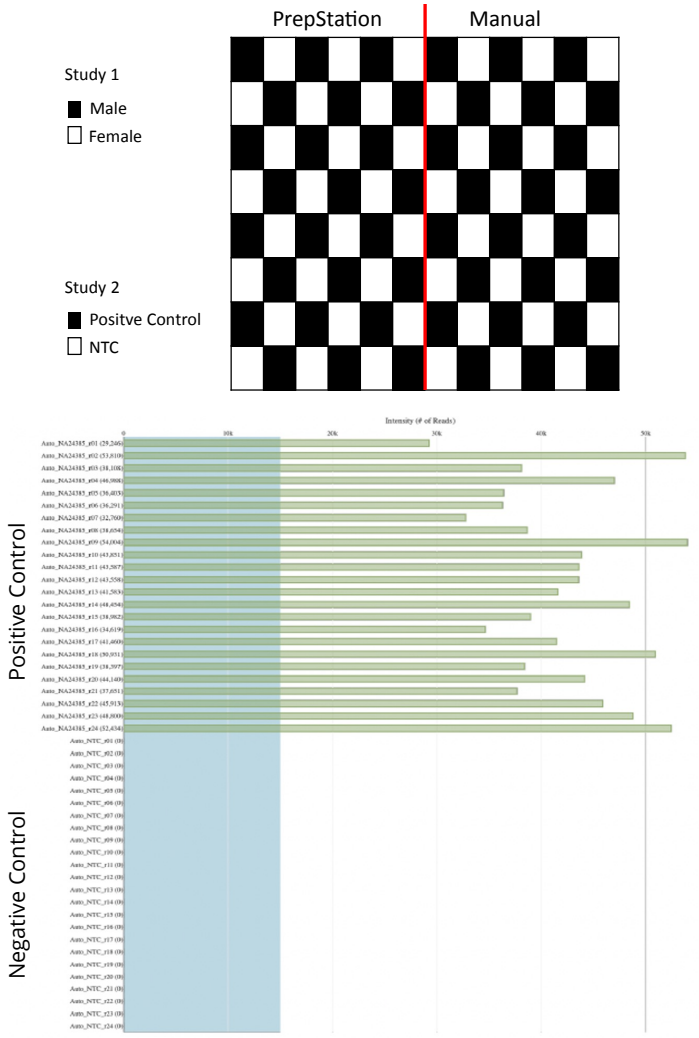

Figure 2: (Top) Plate set-up for contamination study. (Bottom) Total sequencing reads per sample above analytical threshold as measured for Study 2. (Data for Manual samples not shown.)

Highly concordant results across manual and automated workflows.

The ForenSeq chemistry is a highly sensitive workflow demonstrating high locus call rates and full profiles with low inputs of DNA. To enable the adoption of the Verogen PrepStation, studies were conducted to evaluate the concordance of the calls generated by the automated workflow against call generated by the manual workflow. Libraries were prepared manually or with the PrepStation with Control DNA (NA24385) at the following inputs: 4 ng, 2 ng, 1 ng, 500 pg, 250 pg, 125 pg, 62 pg, 31 pg, 16 pg and

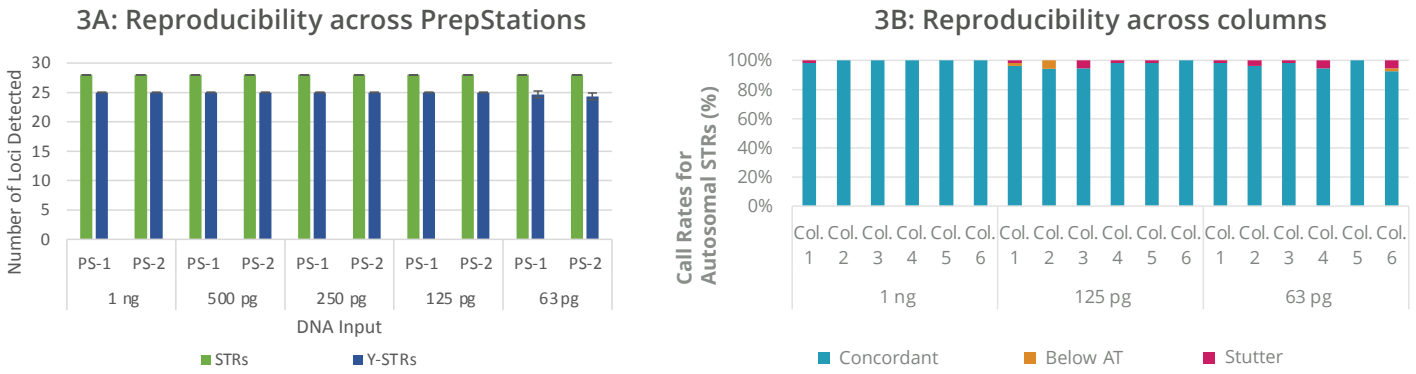

Figure 3: Libraries prepared on Verogen PrepStation are highly reproducible across PrepStations (left) and across columns (right). Autosomal STR data are shown.

8 pg in quadruplicates. At DNA inputs of 63 pg or higher, all expected alleles for control DNA NA24385 are detected for both automated and manual library preparation methods. At inputs below 63 pg, some alleles are below the AT for libraries prepared either on PrepStation or prepared manually. Figure 4 summarizes the results from the study.

Conclusion

Automation of library preparation for NGS workflows has the potential to streamline a time consuming and labor intensive process, while generating high quality results that have the additional advantage of minimizing any human factors introduced during the manual workflow. The studies presented in this technical note describe the successful testing of the Verogen PrepStation forensic. During the course of this study, no well-to-well or sample-to-sample carry over was observed and no contamination detected in any of the negative controls. These results show that a forensic lab can use the Verogen PrepStation to generate high-quality libraries with reproducible results even from samples at low inputs or smaller batch sizes.

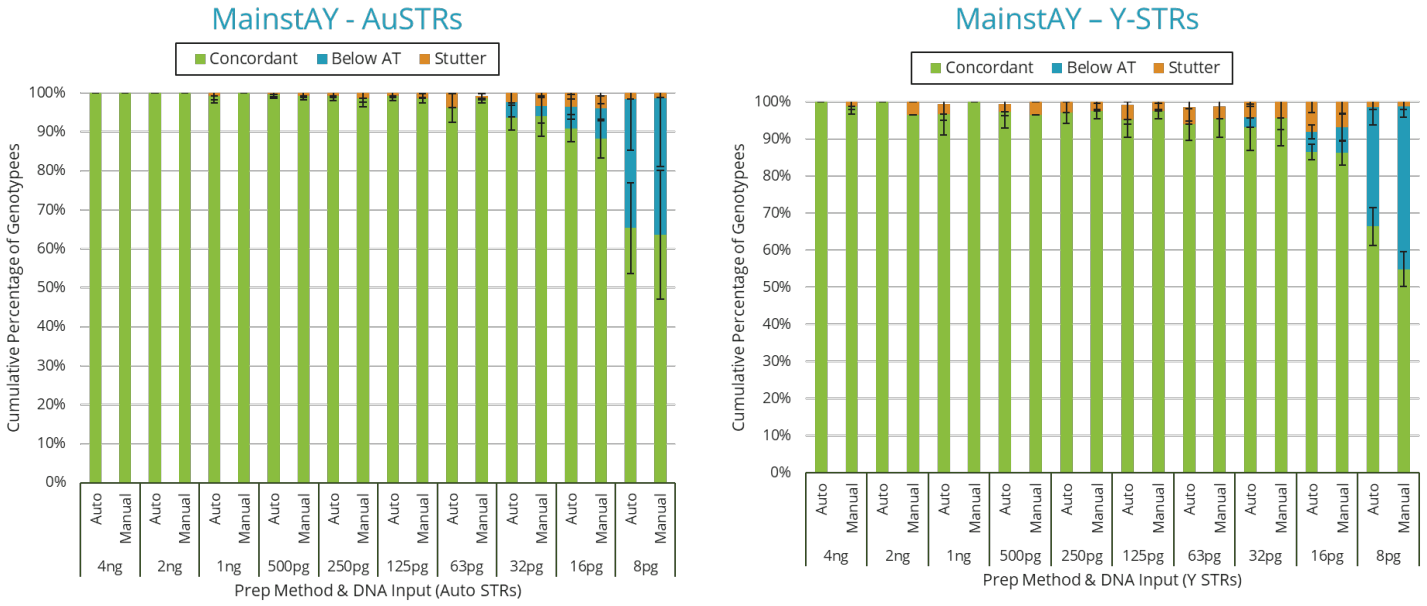

Figure 4: MainstAY Au-STRs (Left) and Y-STRs (Right) exhibit high call rates when processed on PrepStation compared to manual workflow.

References

1. Kathryn M. Stephens*, Richelle Barta, Keenan Fleming, Juan Carlos Perez, Shan-Fu Wu, June Snedecor, Cydne L. Holt, Bobby LaRue, Bruce Budowlea. (2022). Developmental Validation of the ForenSeq™ MainstAY kit, MiSeq FGx® Sequencing System and ForenSeq™Universal Analysis Software (Manuscript Submitted for Publication)

Product documentation is available for download at **www.verogen.com/support**

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