Comparable performance of ForenSeq libraries prepared manually and on the Opentrons Prepstation platform

Introduction

DNA analysis of forensic samples is essential for solving criminal cases, property crimes and identifying 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 Opentrons Prepstation platform 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. This technical note summarizes some of the high quality data generated by for ForenSeq libraries using the Opentrons Prepstation for library preparation.

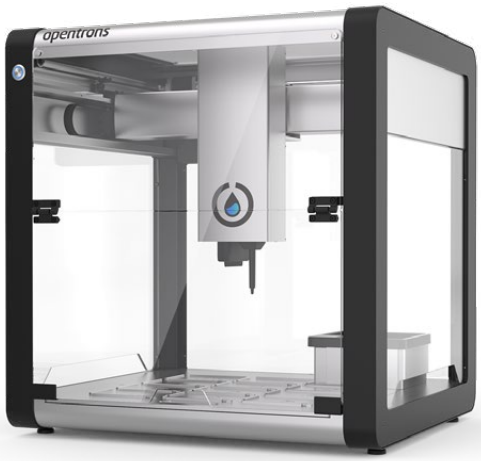

Figure 1.
Opentrons Prepstation automation platform.

No contamination of ForenSeq libraries generated on the Opentrons Prepstation

Contamination of forensic samples processed on an automation platform can compromise DNA identification. 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 and the other half of the plate was processed using the Opentrons Prepstation and the ForenSeq MainstAY Library Prep Kit. Figure 2 (top) 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 Opentrons Prepstation, demonstrating that this automation platform can be used to 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 across Opentrons Prepstation platforms, ForenSeq MainstAY libraries were prepared using 1 ng 500 pg, 250 pg, 125 pg and 63 pg input positive control DNA (NA24385) on two Opentrons Prepstation instruments (PS-1 and PS-2). Loci detected above the analytical threshold (AT) were reproducible across the two platforms tested (Fig 3A). A similar study was performed to assess column-to-column variability within an Opentrons 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 an Opentrons 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 Opentrons Prepstation instruments, with very low inputs of DNA – supporting its use in forensic casework.

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 Opentrons Prepstation, studies were conducted to evaluate the concordance of calls generated by the automated workflow against calls generated by the manual workflow. Libraries were prepared manually or with the Opentrons 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 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 were below the AT for libraries prepared either on the Opentrons Prepstation or prepared manually. Figure 4 summarizes the results from the study.

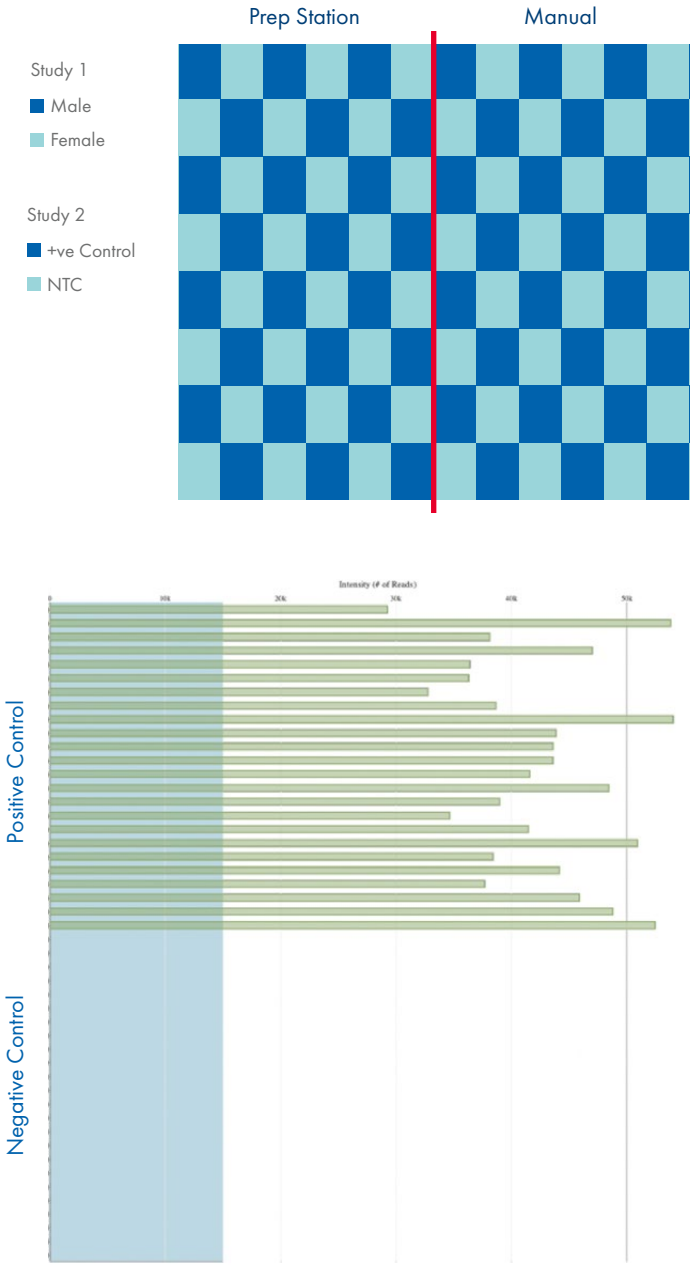

Figure 2.
(Top) Plate set-up for contamination study. (Bottom) Total sequencing reads per sample above analytical threshold as measured for Study 2 (data for Manual samples not shown).

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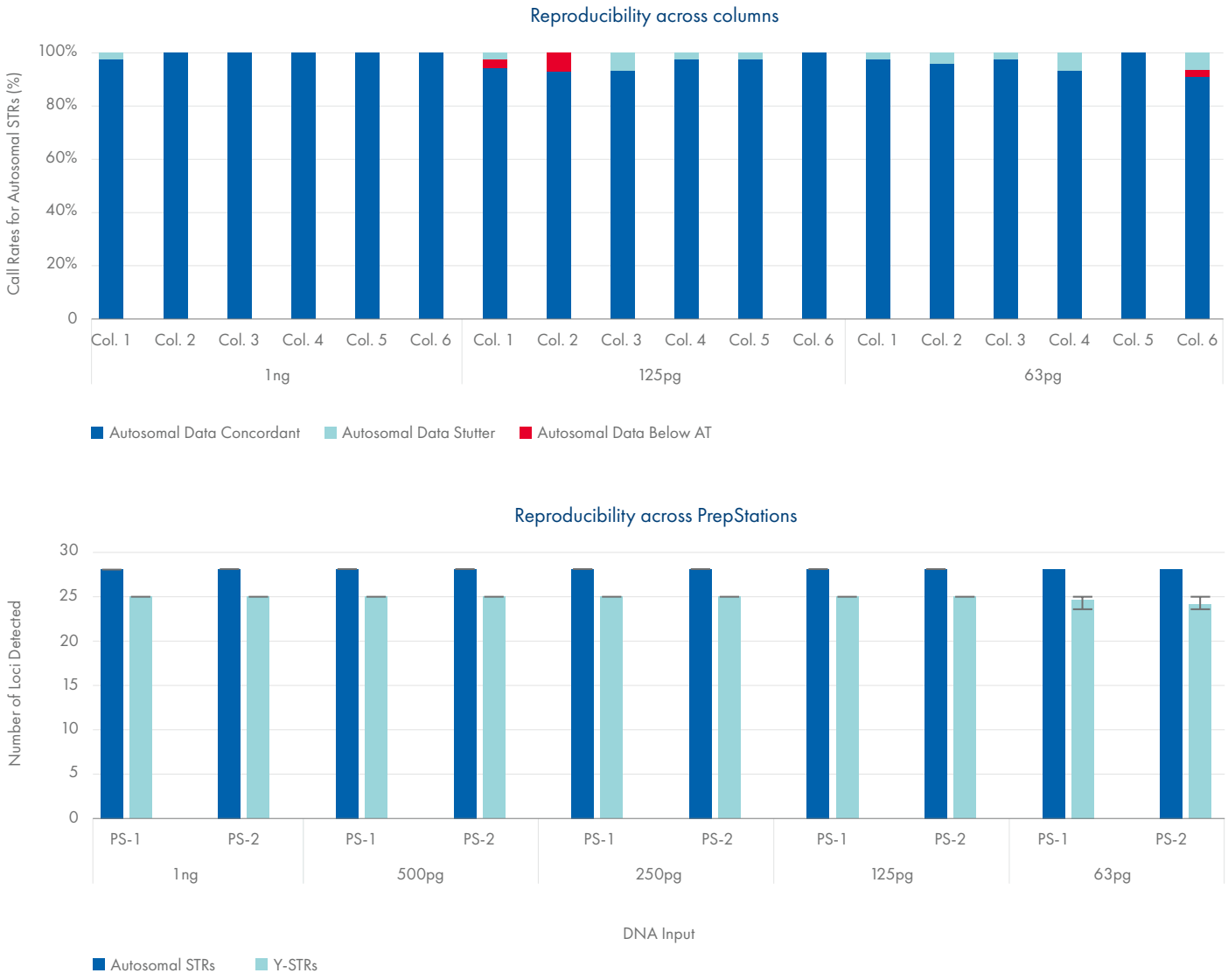

Figure 3.
Libraries prepared on the Opentrons Prepstation are highly reproducible across instruments (above) and across columns within the same instrument (below). Autosomal STR data shown.

Conclusion

Automation of library preparation for NGS workflows has the potential to streamline a time consuming 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 Opentrons Prepstation platform for ForenSeq library preparation.

During the course of this study, no well-to-well or sample- tosample carry over was observed and no contamination detected in any of the negative controls. These results show that a forensic lab can use the Opentrons Prepstation to generate high-quality libraries with reproducible results even from samples at low inputs or smaller batch sizes.

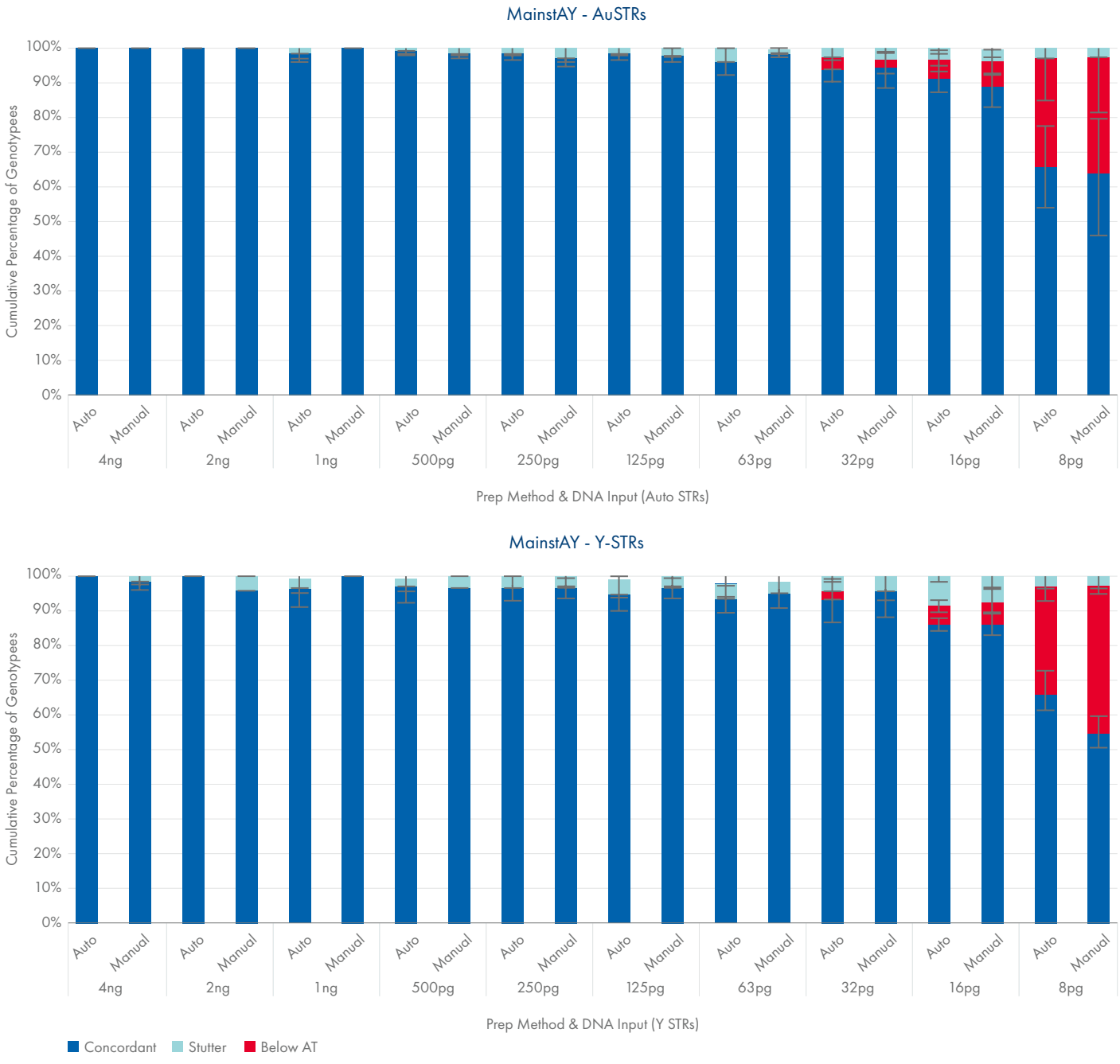

Figure 4.
MainstAY Au-STRs (above) and Y-STRs (below) exhibit high call rates when processed on the Opentrons Prepstation compared to manual workflow.

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Product documentation is available for download at **www.qiagen.com/contact_prepstation**

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)