Forensic Evaluation of the Verogen PrepStation and ForenSeq® Workflows for Implementation in Crime Laboratories

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OVERVIEW

This study evaluates the Verogen PrepStation and scripts as a low-cost automation solution for the library preparation of Next-Generation Sequencing DNA analysis using ForensSeq® workflows. In a series of experiments, library preparation on the PrepStation was compared to a manual preparation and tested for cross-contamination. The PrepStation was shown to perform similarly to the manual preparation, and showed no cross-contamination.

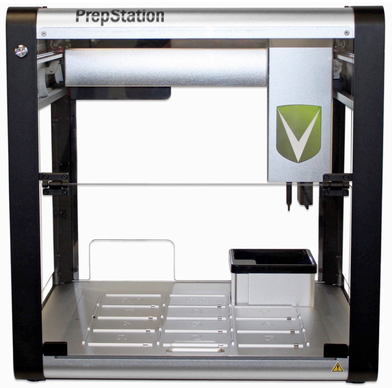


Figure 1: The Verogen PrepStation.

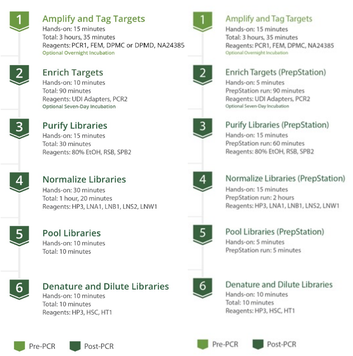


Figure 2: Workflow for library preparation of the ForenSeq® MainstAY kit performed manually (left) and using PrepStation (right).

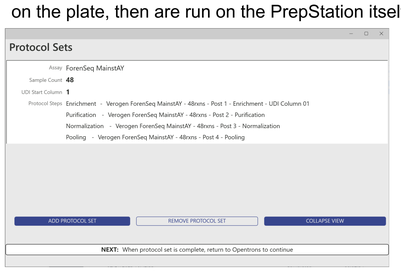
INTRODUCTION

Forensic DNA analysis is a rapidly evolving field with new techniques and technologies constantly being developed. While currently the established standard for DNA casework in crime laboratories is analyzing short tandem repeats (STRs) using capillary electrophoresis (CE), many in the field are beginning to look toward a newer technology called Next-Generation Sequencing (NGS).

While NGS offers many advantages over CE in the data it can produce, one major challenge of the NGS workflow is the time and labor required to prepare the runs. Library preparation for a run requires six major steps: amplification, enrichment, purification, normalization, pooling, and denaturation/dilution. Manual pipetting for each of these steps can be time-consuming and create opportunity for human error. Experience with the workflow can also be a major factor in the success of the sequencing run, especially in the purification and normalization steps, which involve binding and washing magnetic beads and may be unfamiliar to many laboratory analysts.

Recently Verogen has announced the PrepStation, a low-cost liquid-handling laboratory robot system designed to streamline NGS library preparation (**Figure 1**). PrepStation automates the most time-consuming and labor-intensive steps in the process (**Figure 2**)**,** minimizing both the hands-on time spent by the analyst and the potential for human error.

The PrepStation operates using an application that pulls the scripts optimized for the workflow of Verogen's kits for the specified numbers of samples. Scripts can be produced based on the number of samples being run at once (up to 48) and the position of the samples on the plate, then are run on the PrepStation itself using the Opentrons app (**Figure 3**).



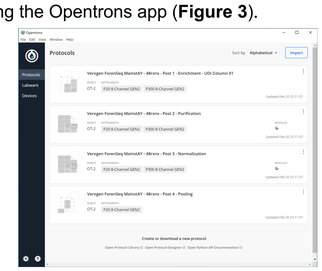


Figure 3: The PrepStation app (left) which pulls the scripts, and the Opentrons app (right) which executes them on the PrepStation.

METHODS

Library preparation of the ForenSeq® MainstAY Kit (Verogen) was tested in two experiments. PrepStation protocols for enrichment, purification, normalization, and pooling were generated using the PrepStation app for 48 samples. The deck layout of the robot was set up as shown below (**Figure 4**), according to each protocol. A calibration was performed as required before each PrepStation run. All sequencing was performed using the MiSeq FGx® Sequencing System, and data analysis was performed using the Universal Analysis Software v2.5 (Verogen).

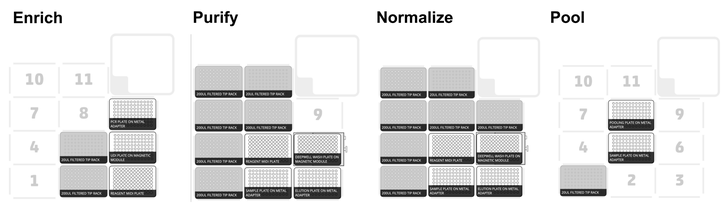


Figure 4: PrepStation deck layout for each step.

Experiment 1

The first experiment was a comparison of a PrepStation library preparation and a manual library preparation. Each preparation was 48 samples consisting of three replicates each of a positive control dilution series, seven single-source reference samples, and a negative control (**Table 1**). The first amplification step of each preparation was performed manually. Once each library preparation was complete, both were pooled together to be sequenced simultaneously according to the standard MainstAY protocol.

	1	2	3	4	5	6	7	8	9	10	11	12
A	POS 1ng	POS 1ng	POS 1ng	V01	V01	V01	POS 1ng	POS 1ng	POS 1ng	V01	V01	V01
B	POS 500pg	POS 500pg	POS 500pg	V02	V02	V02	POS 500pg	POS 500pg	POS 500pg	V02	V02	V02
C	POS 250pg	POS 250pg	POS 250pg	V03	V03	V03	POS 250pg	POS 250pg	POS 250pg	V03	V03	V03
D	POS 125pg	POS 125pg	POS 125pg	V04	V04	V04	POS 125pg	POS 125pg	POS 125pg	V04	V04	V04
E	POS 63pg	POS 63pg	POS 63pg	V05	V05	V05	POS 63pg	POS 63pg	POS 63pg	V05	V05	V05
F	POS 32pg	POS 32pg	POS 32pg	V06	V06	V06	POS 32pg	POS 32pg	POS 32pg	V06	V06	V06
G	POS 16pg	POS 16pg	POS 16pg	V07	V07	V07	POS 16pg	POS 16pg	POS 16pg	V07	V07	V07
H	POS 8pg	POS 8pg	POS 8pg	NEG	NEG	NEG	POS 8pg	POS 8pg	POS 8pg	NEG	NEG	NEG
	PrepStation						Manual Prep					

Table 1: Experiment 1 plate layout, with wells A1-H6 prepared by PrepStation and wells A7-H12 prepared manually.

Experiment 2

The second experiment was a test for cross-contamination using a checkerboard-patterned plate arrangement of positive and negative controls (**Table 2**). The plate set up and first amplification step was performed manually by Verogen, with the rest of the process performed at SHSU using the PrepStation.

	1	2	3	4	5	6
A	POS	NEG	POS	NEG	POS	NEG
B	NEG	POS	NEG	POS	NEG	POS
C	POS	NEG	POS	NEG	POS	NEG
D	NEG	POS	NEG	POS	NEG	POS
E	POS	NEG	POS	NEG	POS	NEG
F	NEG	POS	NEG	POS	NEG	POS
G	POS	NEG	POS	NEG	POS	NEG
H	NEG	POS	NEG	POS	NEG	POS
	PrepStation					

Table 2: Experiment 2 plate layout, in a checkerboard pattern to evaluate cross-contamination.

MANUAL COMPARISON

The PrepStation library preparation showed similar locus recovery to the manual preparation on both the dilution series and the reference samples (**Figure 5**). Both methods produced full recovery down to 63pg, with the exception of the 125pg dilution in the manual preparation showing a slight decrease that is most likely attributable to human error. Smaller input concentrations after that show decreased recovery at comparable rates between the methods.

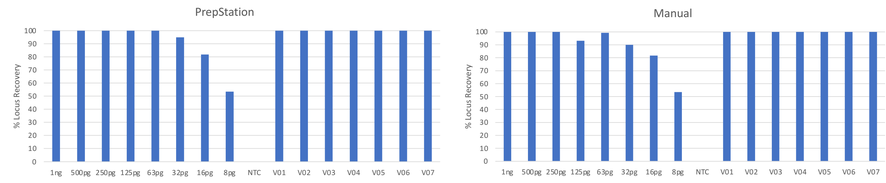


Figure 5: Locus recovery percentage of samples prepared by PrepStation versus those prepared manually. Each value represents an average of three replicates.

CROSS-CONTAMINATION

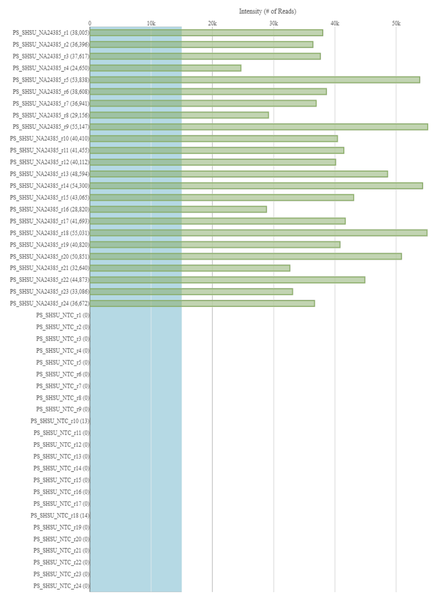


Figure 6: Total read count intensities for each sample in the checkerboard plate.

All of the positive control samples in the checkerboard plate produced read count intensities well over the 15,000 total sample read range (**Figure 6**). No reads were detected at any of the negatives, with the exception of NTC_r10 and NTC_r18.

The 11 allele at D5S818 seen in NTC_r10 is present in the positive, though the 10 allele at DYS460 seen in NTC_18 is not (**Figure 7**). The alleles' intensities of 13 and 14 reads are just above the base analytical threshold of 10 reads. We can conclude that these are most likely not true instances of cross-contamination due to the PrepStation. Rather, these alleles can likely be attributed either to contamination introduced during the manual PCR1 amplification step or to allele "drop-in", a phenomenon noted in forensic DNA interpretation where additional alleles may be occasionally be detected at low levels.

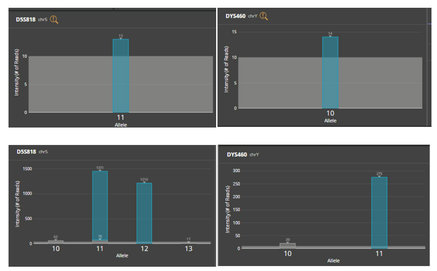


Figure 7: Allele reads detected in two of the negative control samples (top), and the corresponding loci in the positive control (bottom).

CONCLUSIONS

- The Verogen PrepStation and PrepStation app are simple to set up and use.
- PrepStation produced similar or better locus recovery compared to manual library preparation.
- The checkerboard run showed no cross-contamination issues attributable to the PrepStation.
- PrepStation offers effective low-cost automation for NGS library preparation in forensic DNA laboratories.

ACKNOWLEDGEMENTS

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