

Streamline Watchmaker's DNA Library Prep Kit for pre-fragmented DNA on the Sciclone G3 NGSx Workstation.



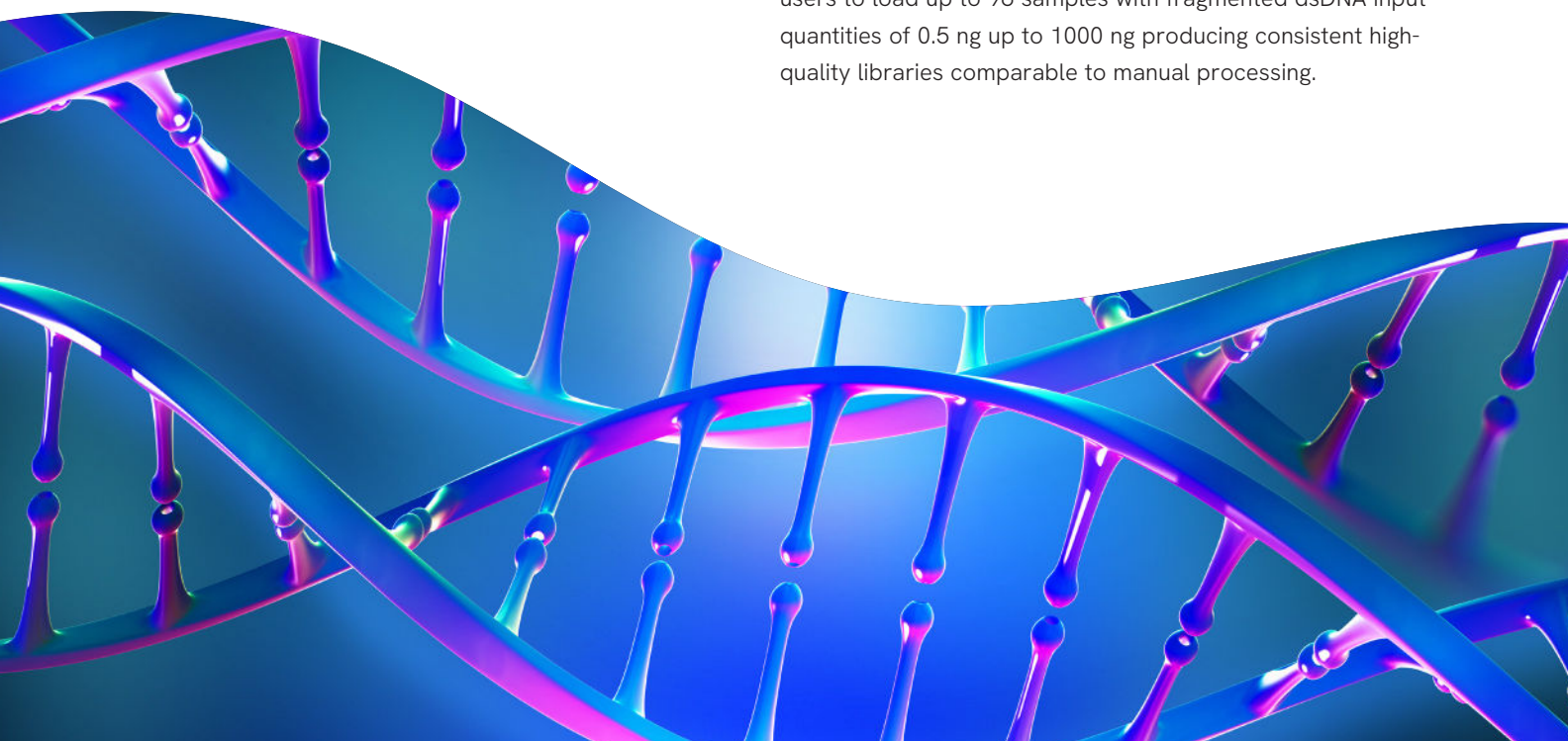
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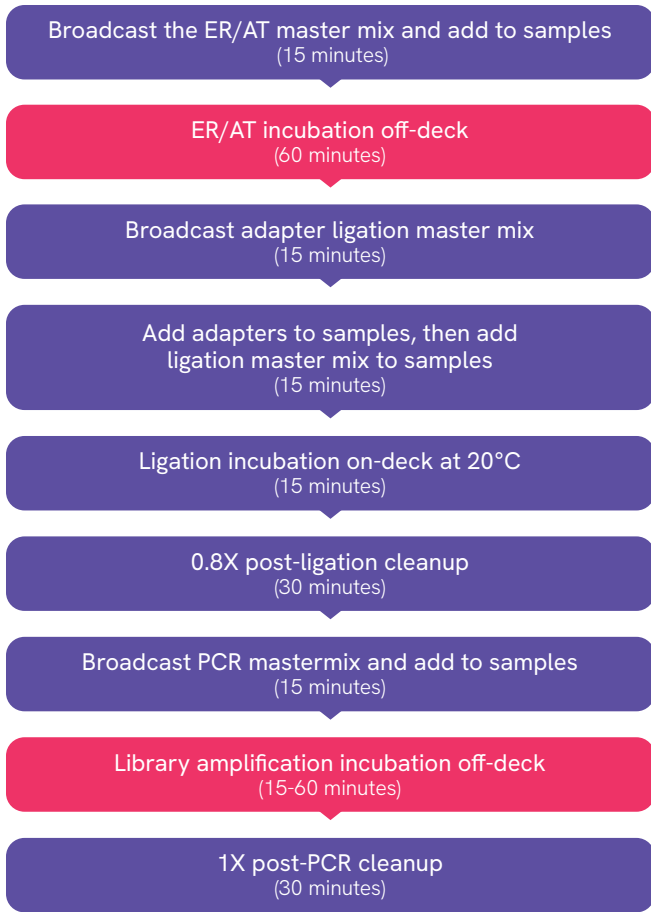
Introduction

Next-generation sequencing (NGS) plays a vital role in today's research and an efficient scalable workflow for library preparation is essential to producing high quality sequencing data. With an increase in high throughput demand, automation can help alleviate the bottleneck of sample preparation for sequencing.

Here we present the Watchmaker DNA Library Prep Kit automated on Revvity's Sciclone® G3 NGSx workstation. The automated workflow not only reduces overall hands-on time, but it also has the added benefits of minimizing human error and sample variability. Watchmaker DNA Library Prep Kits offer a robust library preparation solution from fragmented dsDNA templates. Fragmented dsDNA can be generated by biologically occurring DNA fragmentation, such as with cell-free DNA, mechanical shearing processes, or even enzymatic fragmentation methods. The Watchmaker DNA Library Prep Kit was developed to address the performance needs in NGS applications such as liquid biopsy, ultra-low variant detection, bisulfite sequencing, and hybrid capture. Watchmaker's kits include generous overages, combined reactions for simplified workflows, master mixed formulations for single reagent additions, and stable products all of which enable the use of automation.

By combining a high throughput automated solution with a robust library prep kit, this automated workflow enables users to load up to 96 samples with fragmented dsDNA input quantities of 0.5 ng up to 1000 ng producing consistent high-quality libraries comparable to manual processing.





Automated workflow features

- Reagent Workbook:** Includes master mix formulations, volumes, plate types and relevant deck locations of all reagent-containing consumables required to run (Figure 2)
- Push Button Protocols:** Allows users to choose between PCR vs. PCR-free workflows at the start of the run (Figure 3)
- Flexible User Interface:** Supports 1 - 96 sample processing, stubby vs. full-length adapters, dynamic bead cleanup ratios, and single-sided or double-sided post-ligation cleanup options (Figure 4)
- Deck Images:** Guides users through the placement of labware on-deck with simple step-by-step instructions including a final comprehensive image of the deck layout prior to starting (Figure 5)

Figure 1. Automated process for the Watchmaker DNA Library Prep Kit on the Sciclone® G3 NGSx Workstation. Total processing time for 96 samples ranges from 2.5 - 4 hours depending on whether PCR is performed and PCR cycling parameters. All automated steps are colored in purple and any manual interventions (for off-deck thermal cycling) are colored in pink.

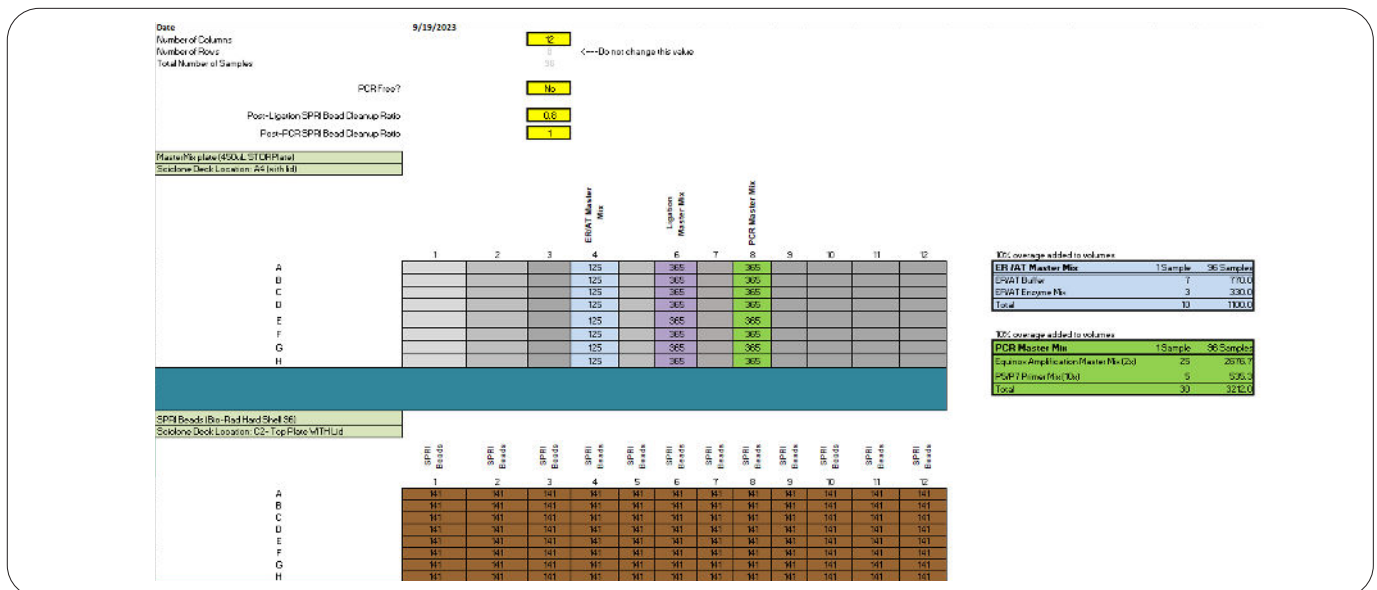


Figure 2. Watchmaker DNA Library Prep Kit reagent workbook available with the Sciclone® application.

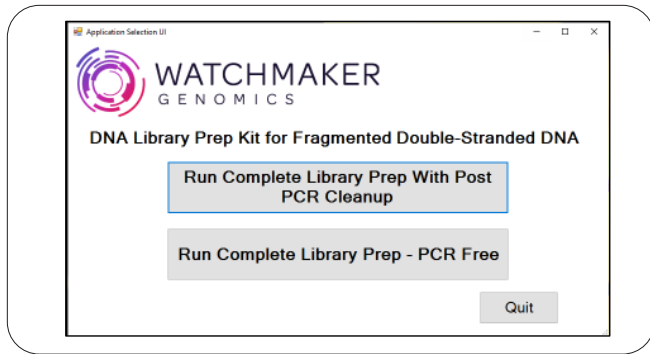


Figure 3. Application start screen presenting PCR vs. PCR-free workflow options.

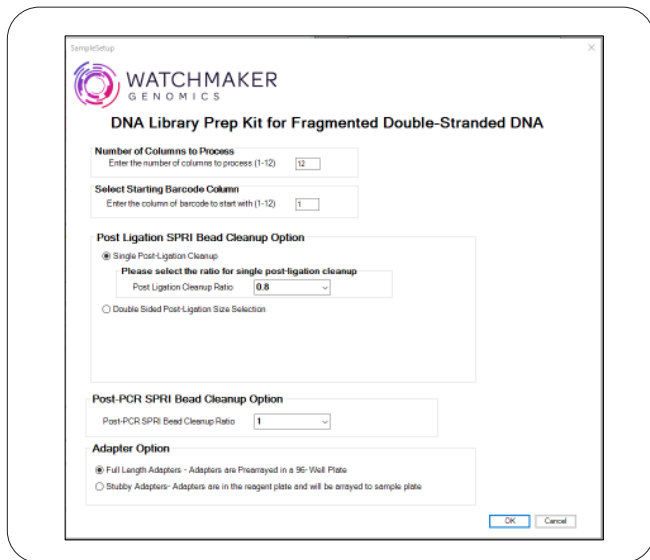


Figure 4. Flexible user interface including options for sample throughput, bead cleanups and adapter types presented at the start of the run.

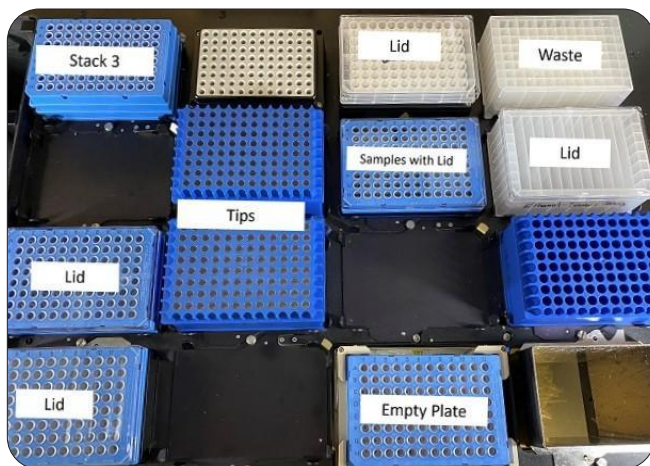


Figure 5. Comprehensive deck layout image when running the Watchmaker DNA Library Prep Kit application on the Sciclone® G3 NGSx (selected options include PCR and a single post-ligation cleanup).

Experimental design

To assess the performance of the automated workflow, a control sample, Genome in a Bottle (GIAB, NA 12878), was mechanically sheared to an average size of 226 bp (Figure 6) and diluted down to a final concentration 0.2 ng/μL. 8 manual and 48 automated libraries were prepared using 10 ng fragmented GIAB inputs following the standard workflow for the Watchmaker DNA Library Prep Kit. During ligation, 2 μM NEXTFLEX UDI Barcodes were used and a total of 8 cycles of PCR were performed on all samples. For the automated sample run, the 48 samples along with 48 No Template Controls (NTCs) were plated in a checkerboard pattern (Figure 7) to assess potential cross-contamination. For the manual sample plate, samples were plated in the first column in wells A1-G1 and a single NTC was included in well H1.

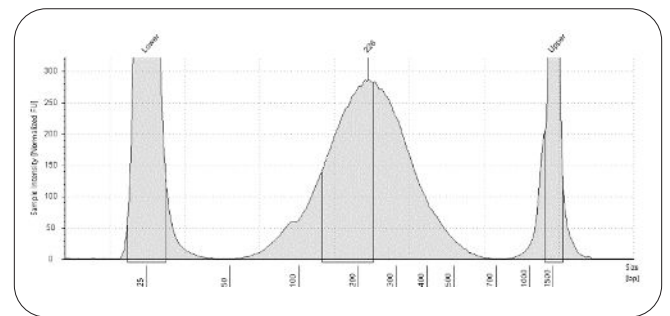


Figure 6. TapeStation D1000 trace of starting material, GIAB (NA 12878), mechanically sheared to average size of 226 bp.

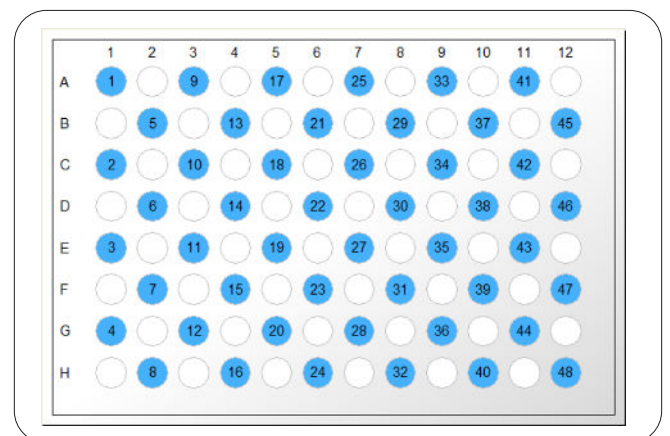


Figure 7. Automated sample plate layout showing checkerboard pattern. Wells in blue contain GIAB samples and all other wells contain NTCs.

Automated workflow performance

All final libraries were measured for both total library yield and average library size using the Qubit 1X dsDNA High Sensitivity assay and the LabChip® NGS 3K Assay with the X-Mark DNA LabChip®, respectively. Final yields between manual and automated library preps were comparable and there was no significant difference in average library size (Figure 8C, 8D). Additionally, there was no observed cross-contamination in the NTCs (data not shown), and there were no observed plate effects indicating library yield and sizes are reproducible across a 96 well plate (Figure 8A, 8B). Final library traces looked comparable between automated and manual processing with minimal adapter dimer present (Figure 9).

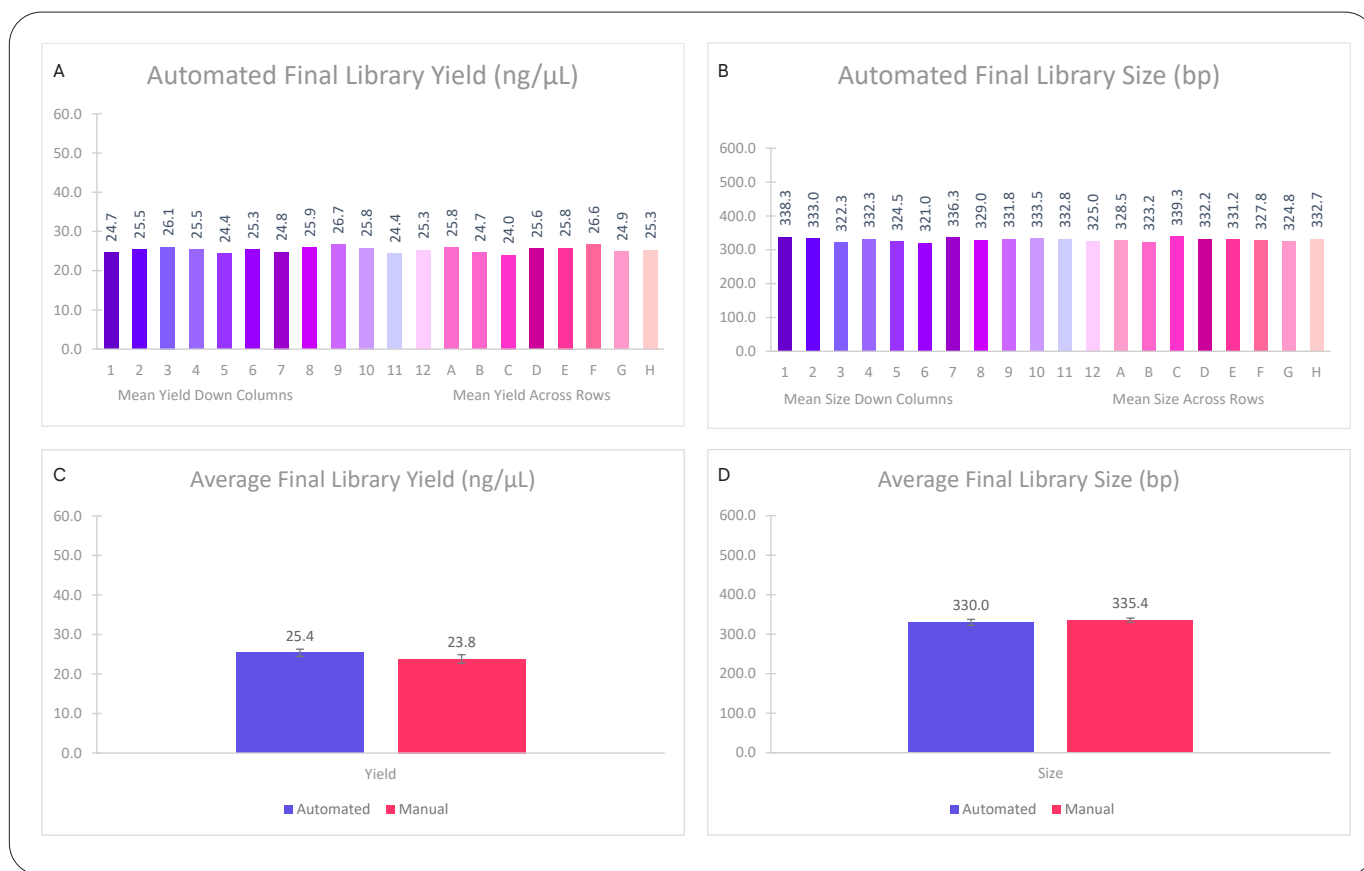


Figure 8. (A) Mean library yields down columns and across rows of the automated library prep plate (B) Mean library sizes down columns and across rows of the automated library prep plate (C) Comparison of manual and automated mean library yields (D) Comparison of manual and automated mean library sizes.

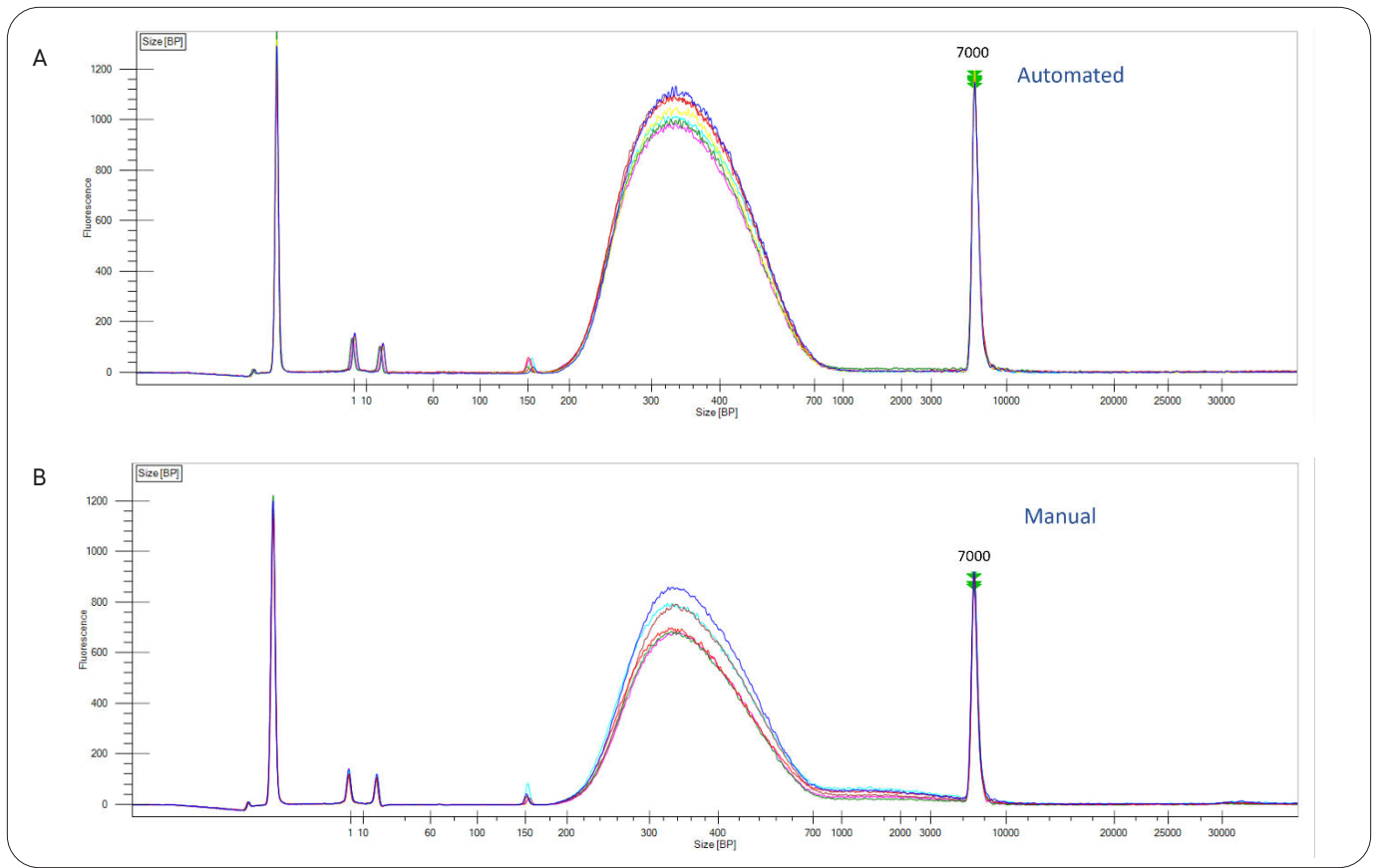


Figure 9. (A) Automated final library traces run on the LabChip® (B) Manual final library traces run on the LabChip® nucleic acid analyzer.

Conclusion

The automated method designed to run the Watchmaker DNA Library Prep Kit on the Sciclone® G3 NGSx workstation delivers a high throughput solution that is flexible, easy-to-use, and shows robust performance comparable to manual processing. To learn more about this automated solution or how to access the application please contact your local Watchmaker or Revvity representatives.

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