APPLICATION NOTE

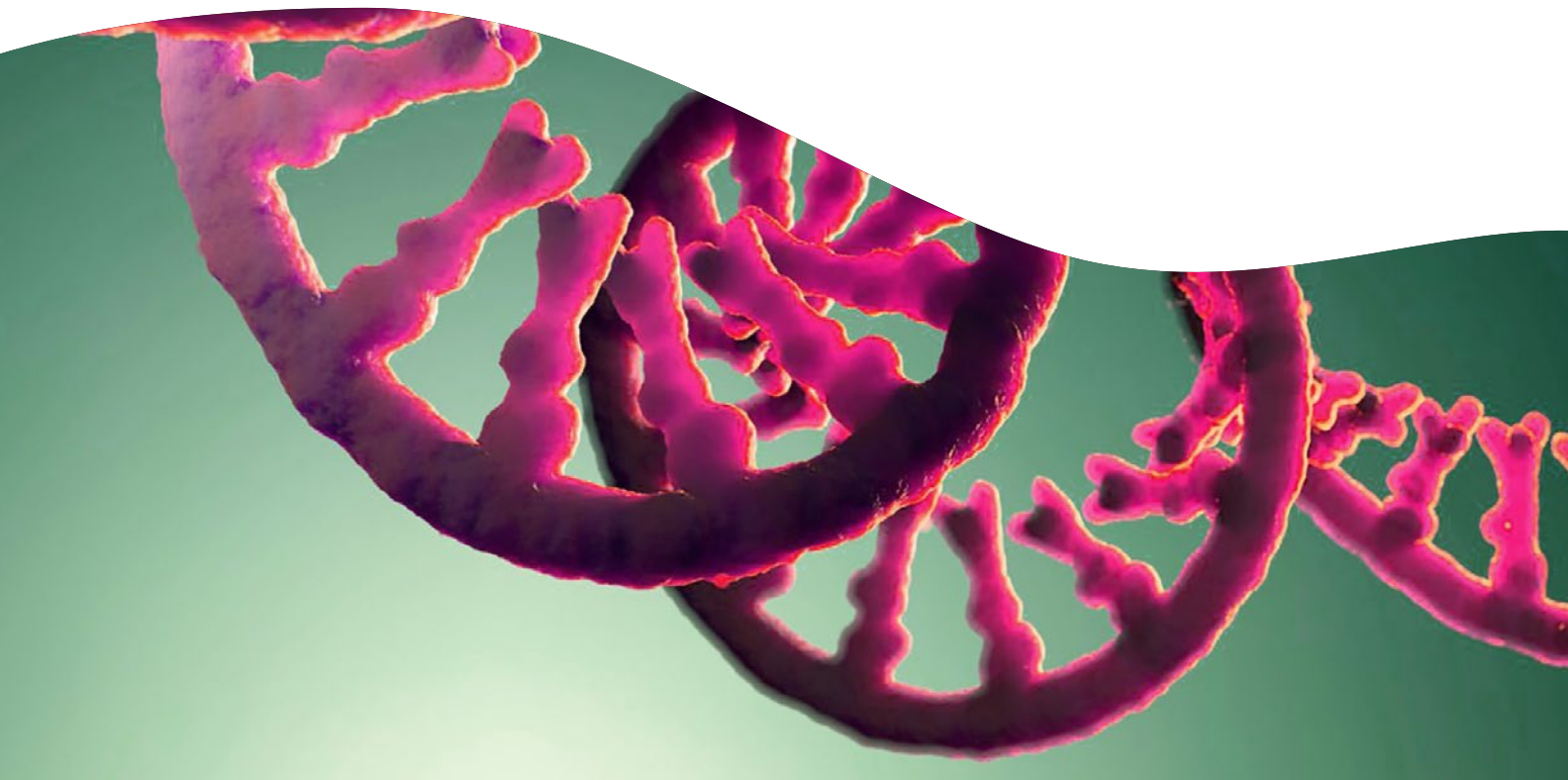
Automated Watchmaker® mRNA Library Prep Kit on Sciclone™ G3 NGSx

Introduction

mRNA sequencing enables robust and high-resolution gene expression analysis, which is crucial for understanding the functional implications of genetic variations. This technology helps researchers decipher biological processes and advance research in fields such as medicine, agriculture, and environmental science. However, mRNA library preparation is typically time and labor intensive – requiring multiple days to complete.

By streamlining and automating the workflow, we have significantly reduced the hands-on time and total turnaround time for library preparation. The Watchmaker mRNA Library Prep Kit is designed for rapid library construction using as little as 2.5 ng of total RNA. The Sciclone™ G3 NGSx workstation provides automated liquid handling for up to 96 samples, featuring a user-friendly interface that supports various workflow options. Together, these solutions can produce 96 libraries in as little as 5 hours.

For research use only. Not for use in diagnostic procedures.



Experimental Setup

To assess the performance of the automated workflow, we performed a high-throughput run with 48 control samples and 48 NTCs as the plate. The starting material, Universal Human Reference RNA (UHRR), was diluted down to 1.03ng/µL for a total of 50 ng input per library with the following library construction parameters per Watchmaker's mRNA Library Prep User Guide: 2µM full length adapters, 0.7X first post ligation bead ratio, 1X second post ligation cleanup, 12X PCR cycles, and 1X post-PCR cleanup bead ratio. For the automated sample plate, the 48 samples along with 48 No-Template Controls (NTCs) were plated in a checkerboard pattern (Figure 1) to assess cross-contamination. Final library yields and sizes were measured using the Qubit® dsDNA High Sensitivity Assay and the LabChip™ NGS 3K Assay.

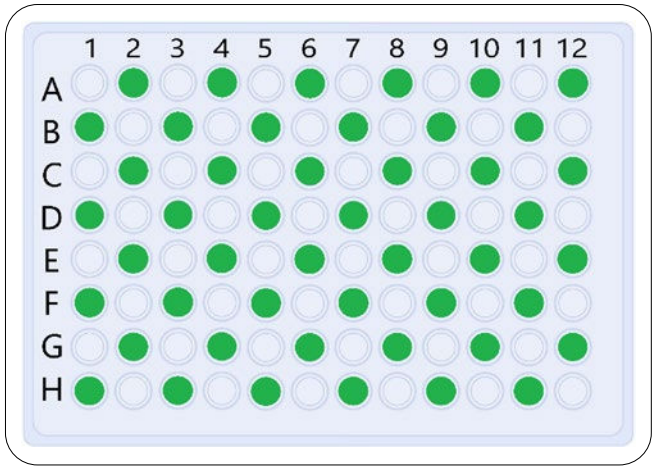


Figure 1. Checkerboard pattern showing the wells in green containing UHRR samples. NTCs are shown in grey.

Application Method Steps

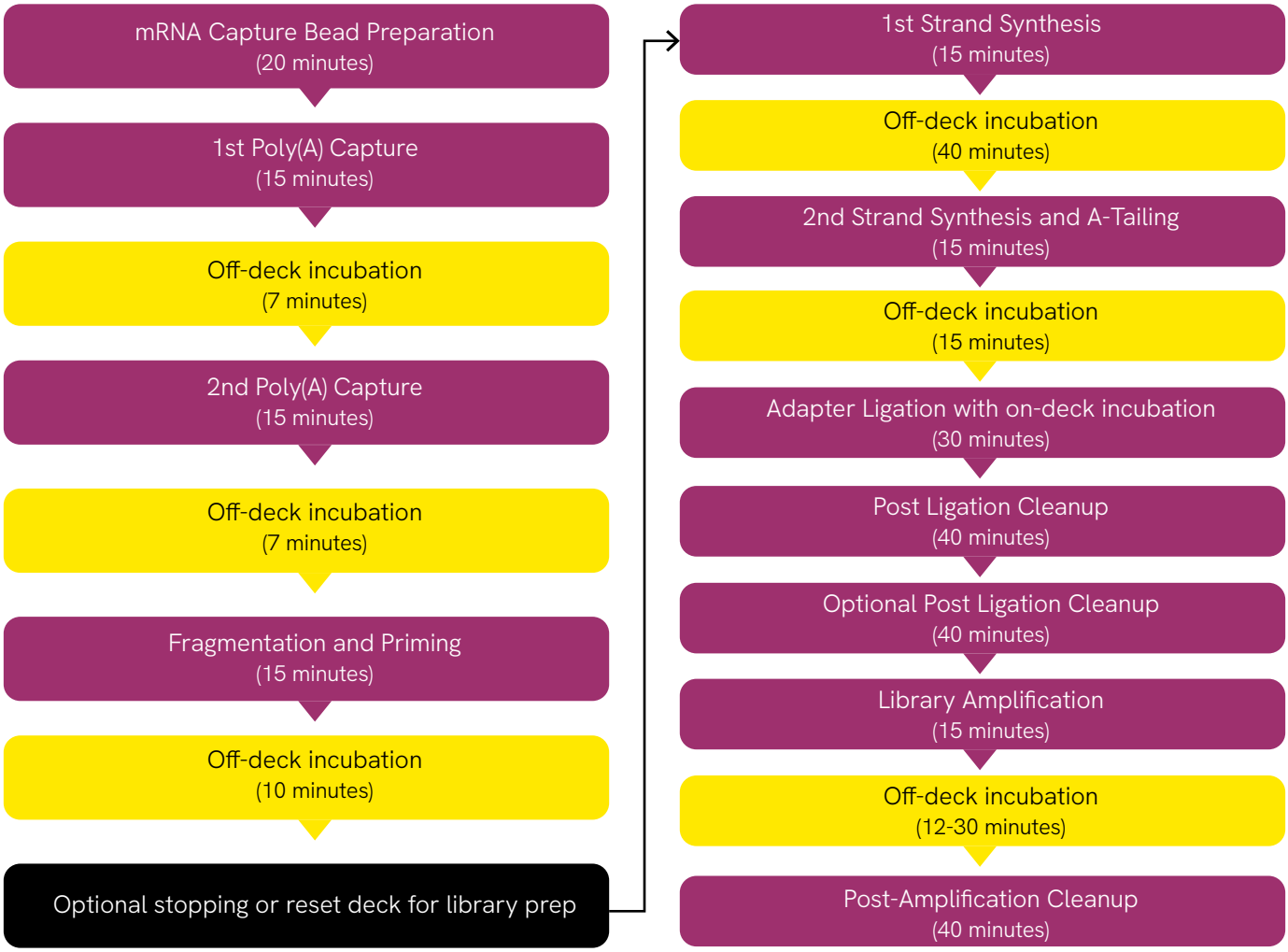


Figure 2. Watchmaker's mRNA Library Prep Kit on the Sciclone G3 NGSx Workstation. Total automated processing time for 96 samples is between 5-6 hours depending on number of ligation bead cleanups and library amplification time. All automated steps are colored in purple and any manual intervention is colored in yellow.

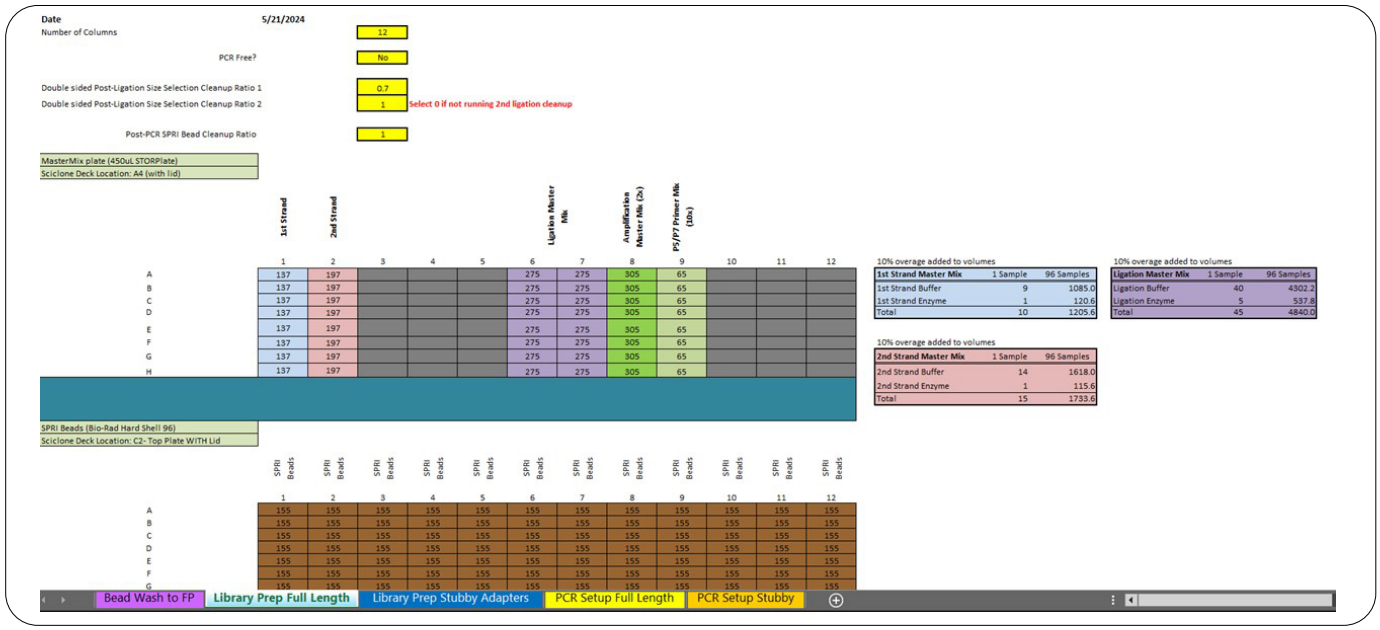


Figure 3. Reagent workbook available with the Sciclone application for Watchmaker's mRNA Library Prep Kit.

To start the automated workflow, the user follows the reagent workbook that details plate type, deck location and reagent volumes, calculated from user input (Figure 3). The user will then proceed to start the application, triggering the application user interface for the user to choose the desired workflow, (Figure 4), numbers of columns to process, bead cleanup ratios and adapter options (Figure 5). Based on the option the user has chosen, the application will begin to guide the user through placement of labware with deck images (Figure 6).

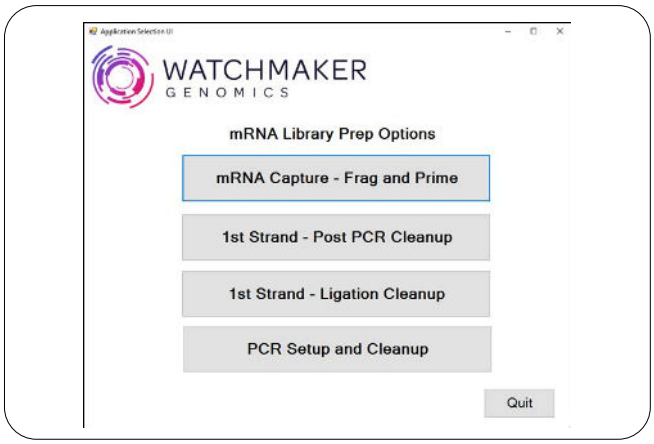


Figure 4. User interface to select desired application protocol at the start of the software.

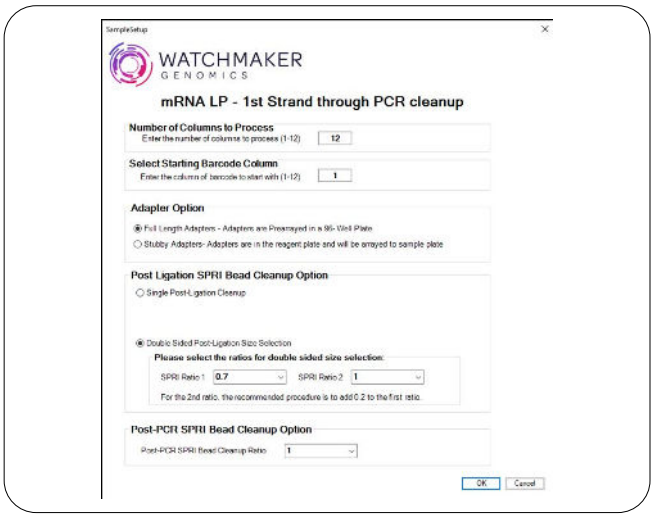


Figure 5. Sample setup prompt for users. Includes bead cleanup options and adapter options.

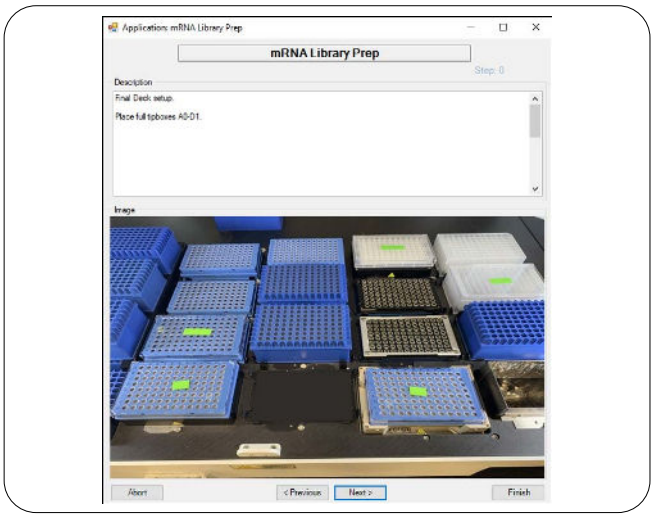


Figure 6. Full deck layout of the application using single post ligation cleanup option.

Results

Final libraries were comparable between the manual and automated methods with no observed cross-contamination (Figures 7, 8 and 9). No obvious plate effects were observed, displaying comparable performance across the high-throughput run.

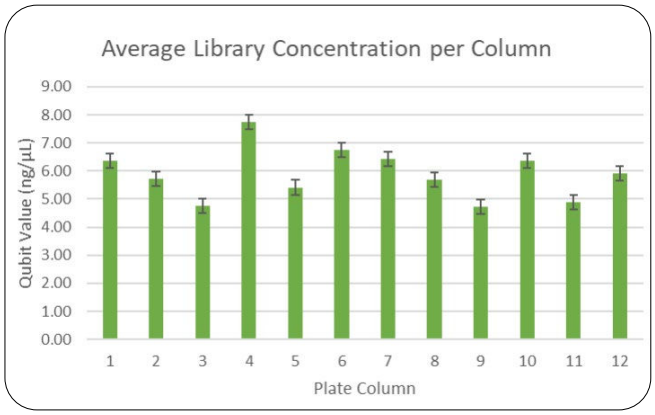


Figure 7. Qubit® high sensitivity final library yields per column.

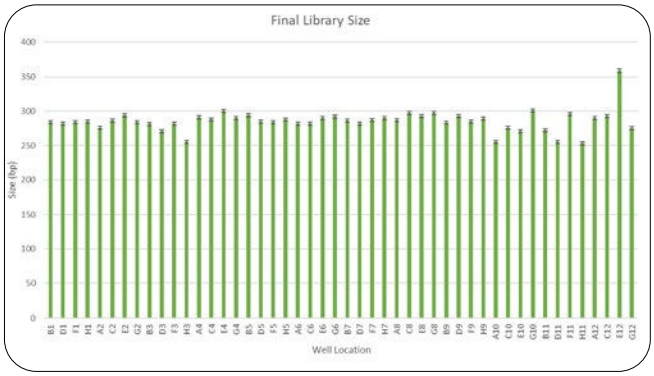


Figure 8. LabChip size of final automated workflow libraries.

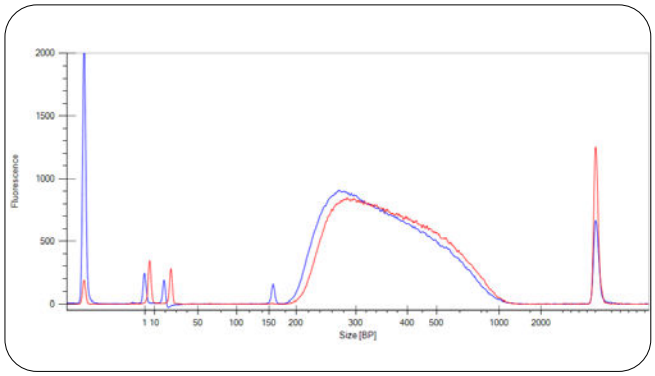


Figure 9. Electropherogram of final library construction (red: automation, blue: manual). Final library sizes were assessed using the LabChip NGS 3K Assay.

Conclusion

The automation workflow of the Watchmaker mRNA Library Prep Kit on the Sciclone NGSx workstation delivers a high-throughput solution that is easy-to-use, reduces human error and hands on time. Please contact your local Watchmaker Genomics Scientific Support team at **support@watchmakergenomics.com** or Revvity representative for more information.

For research use only. Not for use in diagnostic procedures.

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com