APPLICATION NOTE

The Sciclone G3 NGSx iQ workstation combined with Roche® KAPA® HyperPlus™ kits.

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

Create an efficient automated library preparation solution

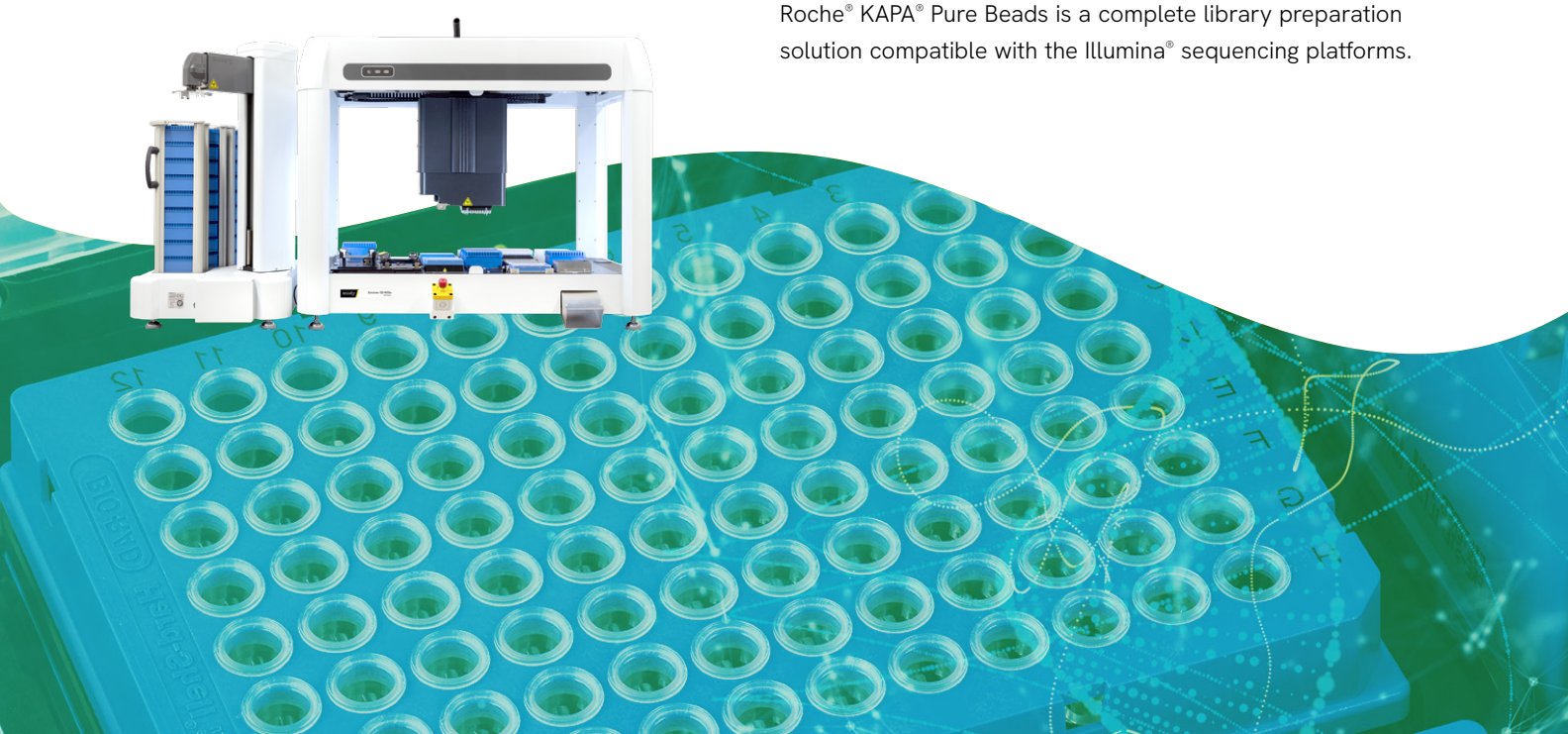
Designed with Next Generation Sequencing (NGS) in mind, the Sciclone® G3 NGSx iQ™ workstation provides automation of even the most complex NGS library construction workflows. High-quality NGS libraries are reliably created through:

- On-Deck Thermal Cycler (ODTC) capability
- Integrated tip storage with robotic tip transfer
- Highly reproducible liquid transfer technology
- On-deck thermal elements

This technology incorporated into the Sciclone G3 NGSx iQ workstation offers truly walk-away automation, allowing researchers to focus on intellectual contributions such as data analysis or experimental design. The Sciclone G3 NGSx iQ workstation upgrades existing applications for new innovative workflows using an OTDC with heated lid incubations, reducing or eliminating manual touch points without losing tip capacity. This upgrade involves integration of the Twister® III robot arm and an ODTC enabling complex processes such as:

- Automated cDNA synthesis in RNA-seq workflows
- Polymerase Chain Reaction (PCR)
- Fragmentation steps which occur at high temperatures
- Adenylation and end repair

Roche® KAPA® HyperPlus™ kit with Roche® KAPA® Adapters and Roche® KAPA® Pure Beads is a complete library preparation solution compatible with the Illumina® sequencing platforms.



When automated on the Revvity Sciclone G3 NGSx iQ workstation, users enjoy an interface-guided workflow set-up, modular sub-protocol step-ins, and advanced step tracking, in addition to the higher throughput and reduced variability afforded by automation. The yield and size of the libraries generated with the automated preparation were of an equivalent performance to manual preparation. Some fundamental advantages this automated method offers:

- Increase throughput capacity
- Greatly reduce hands-on time
- Curtail human error
- Remove inefficiencies due to wasted reagents
- Promote experimental reproducibility

Methods

Libraries were prepared on the Sciclone G3 NGSx iQ workstation with the Roche® KAPA® HyperPlus™ kit using 96 samples of 100 ng control DNA NA12878 (Coriell Institute) and 3 cycles of PCR amplification. Revvity's automated method run options for the Roche® KAPA® HyperPlus™ kit (Figure 1) allow for multiple conditions for processing samples such as:

- Number of columns
- Fragmentation time
- Number of PCR cycles
- PCR cycle duration
- Size selection ratios

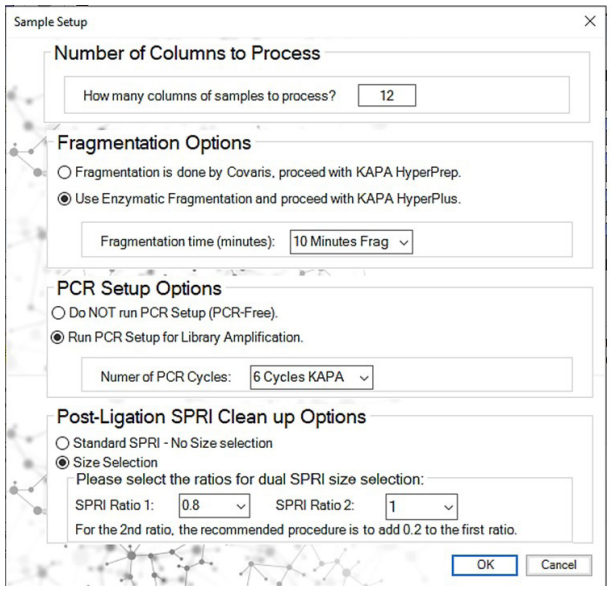


Figure 1. The Sciclone® G3 NGSx iQ™ workstation application run setup was used to determine the application setup including number of samples to process, fragmentation conditions, PCR options and size-selection bead volumes.

A separate user interface guides the researcher through the deck setup by following a series of pictures to place the correct plates and labware in the correct deck position (Figure 2). The sample set-up and automation run time is shown in figure 3.

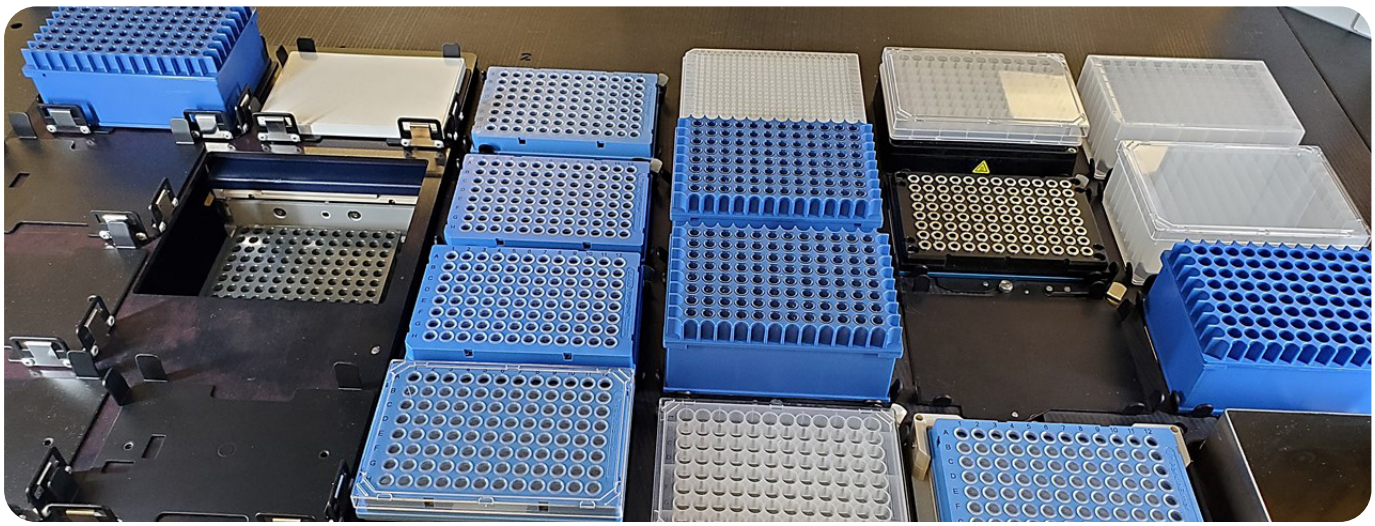


Figure 2. The Sciclone® G3 NGSx iQ™ workstation deck layout for application startup including the integrated ODTC and deck positions for the Twister® III robotic arm accessibility.

Following completion of the automated library preparation, the Thermo Fisher® Scientific Qubit® HS assay was used for quantification, and peak size distribution was determined by the LabChip® GX Touch™ nucleic acid analyzer with the NGS 3K assay (Figure 4).

Manual bench setup time: 30 min
Automation run total time: 3.5 hours
Fragmentation: 30 min
ER/AT: 30 min
Ligation and cleanup: 70 min
PCR: 35 min
Final purification: 45 min

Figure 3. Sample set-up and automation run time.

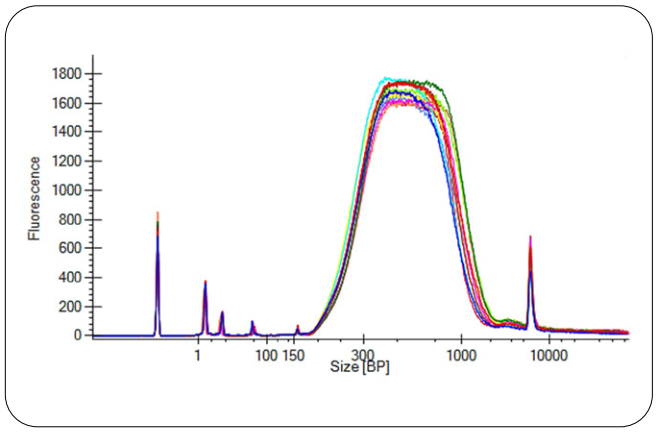


Figure 4. LabChip GX Touch nucleic acid analysis of 12 libraries generated across a 96-well PCR plate from the Roche® KAPA® HyperPlus™ kit.

Results

The yields and sizes of the NGS libraries generated with the Sciclone G3 NGSx iQ workstation were within the expected range of libraries prepared manually using the Roche® KAPA® HyperPlus™ kit and Roche® KAPA® Index barcodes. The automated library yields achieved 29.1 ± 4.8 ng/µL based on Thermo Fisher® Scientific Qubit® HS assay. The peak size distribution was 415 ± 74.7 base pairs (Figure 4).

Conclusion

The automated Roche® KAPA® HyperPlus™ kit protocol delivers uniform NGS libraries with yields and sizes consistent with manually prepared libraries. By automating it on the Sciclone G3 NGSx iQ workstation, a completely walk-away library prep solution is enabled to facilitate error minimization, reduce hands-on time, and increase throughput and reproducibility.

For more information about the Sciclone® G3 NGSx iQ™ workstation visit: www.revvity.com

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