APPLICATION NOTE

Roche KAPA EvoPlus library prep kit automated on Revvity's Sciclone NGSx workstation.

In a collaboration between Roche and Revvity, a KAPA EvoPlus Kit workflow for WGS library prep was successfully automated on the Sciclone® G3 NGSx workstation. Quality metrics demonstrate that the automated method generates libraries with consistent concentration and size across the plate without significant edge effects or measurable cross contamination.

Introduction

The automation of the Roche KAPA EvoPlus Kit on Revvity's Sciclone™ NGSx offers high throughput, rapid and reliable NGS library construction with reduced hands- on time and fewer touchpoints. The Roche KAPA EvoPlus Kit is an enzymatic fragmentation DNA library preparation solution designed for whole-genome (WGS), whole-exome (WES), and targeted sequencing applications performed on Illumina® sequencing systems.

The novel formulation of the KAPA EvoPlus Kit enables robust fragmentation in the presence of EDTA and other buffer components, and the ReadyMixes (in ready-to-use tubes or a plated format) are stable at room temperature for 24 hours; thus, they are automation-friendly.

This app note demonstrates the successful automation of KAPA EvoPlus Workflow on the Sciclone G3 NGSx from Revvity. The automated method can process up to 96 samples in 3.5 hours, is compatible with either tubed or plated reagent format options and offers multiple bead cleanup options. In addition, this method can be used with two different index strategy options – full-length UDI Adapters or UDI Primer Mixes in combination with universal adapters (Figure 1).

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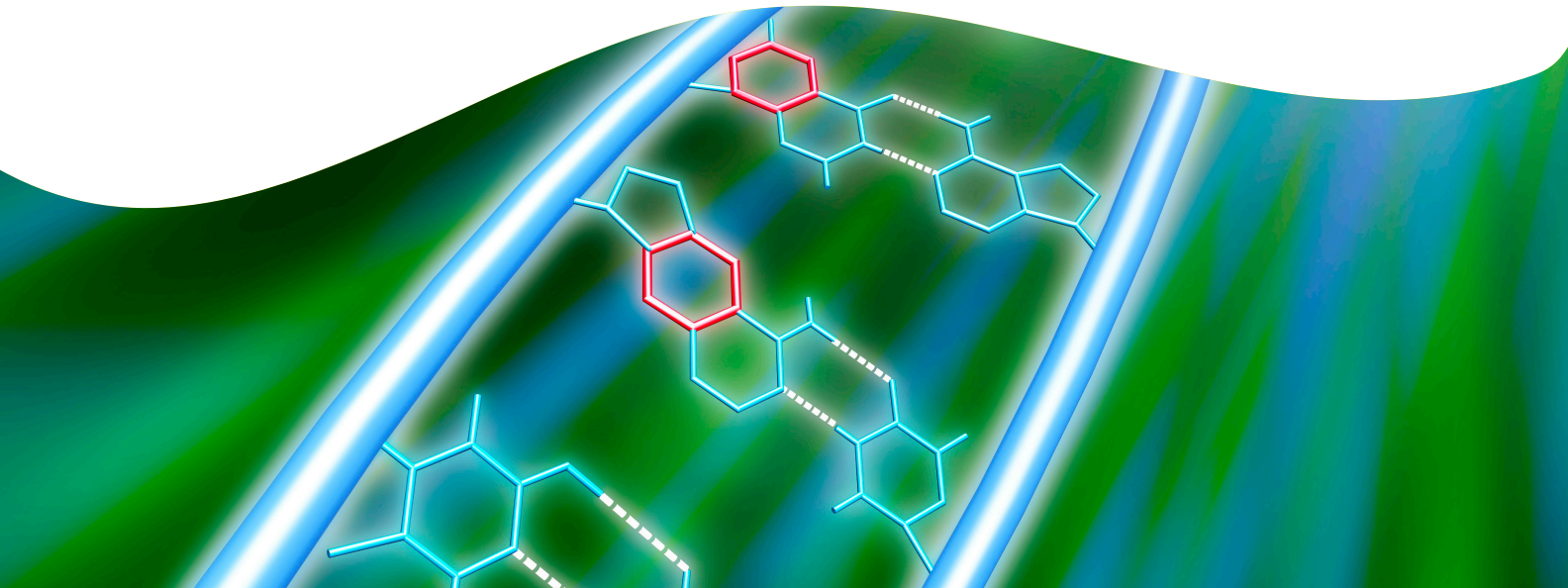

Methods

Automated library preparation for 96 samples on the liquid handler takes about 3.5 hours, including the off-deck incubations (Figure 2). Users can select a PCR-free workflow for DNA inputs > 75 ng. The workflow consists of following steps (Figure 1):

1. Fragmentation and A-tailing
2. Adapter Ligation
3. Post Ligation Cleanup/Optional Double-sided Size Selection
4. PCR Amplification (Optional with input > 75 ng)
5. Post-Amplification Cleanup/Optional Double-sided Size Selection

The automated application was developed to allow the user to choose from any of the available protocols when starting a run on the Sciclone G3 NGSx with the simple Guided User Interface (GUI). The GUI (Figure 3) assigns values to variables within the application that trigger the specific protocol steps and identifies the deck consumables required to process the starting samples. The application provides the user with deck images and text on how to properly set up the Sciclone deck (Figure 4). All reagents were placed on ice or at room temperature as per Roche KAPA EvoPlus Kit instructions. Each consumable required on deck for a setup was filled with volumes of reagent or ReadyMix provided by the application workbook (Figure 5).

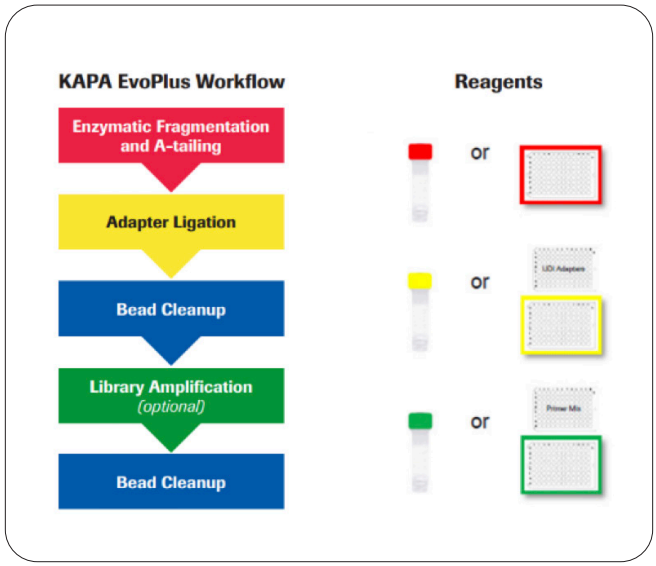

Figure 1: The KAPA EvoPlus workflow. Two reagent formats are available to choose from: Tubed or plated format. The workflow is compatible with two different indexing strategies: full-length UDI Adapters, or KAPA Universal Adapters with KAPA UDI Primer Mixes and KAPA Library Amplification Primer Mix (10x) (sold separately).

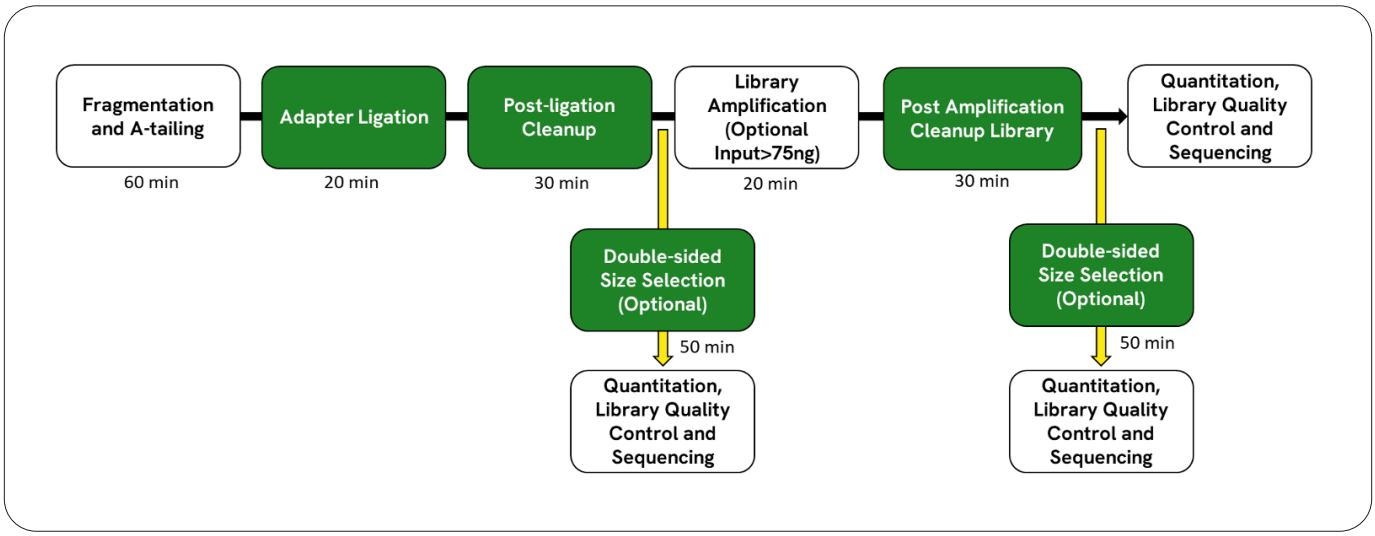

Figure 2: Roche KAPA EvoPlus workflow along with the time required to complete each step. Green blocks represent on-deck incubations and white blocks represent steps that require off-deck thermocycler incubations.

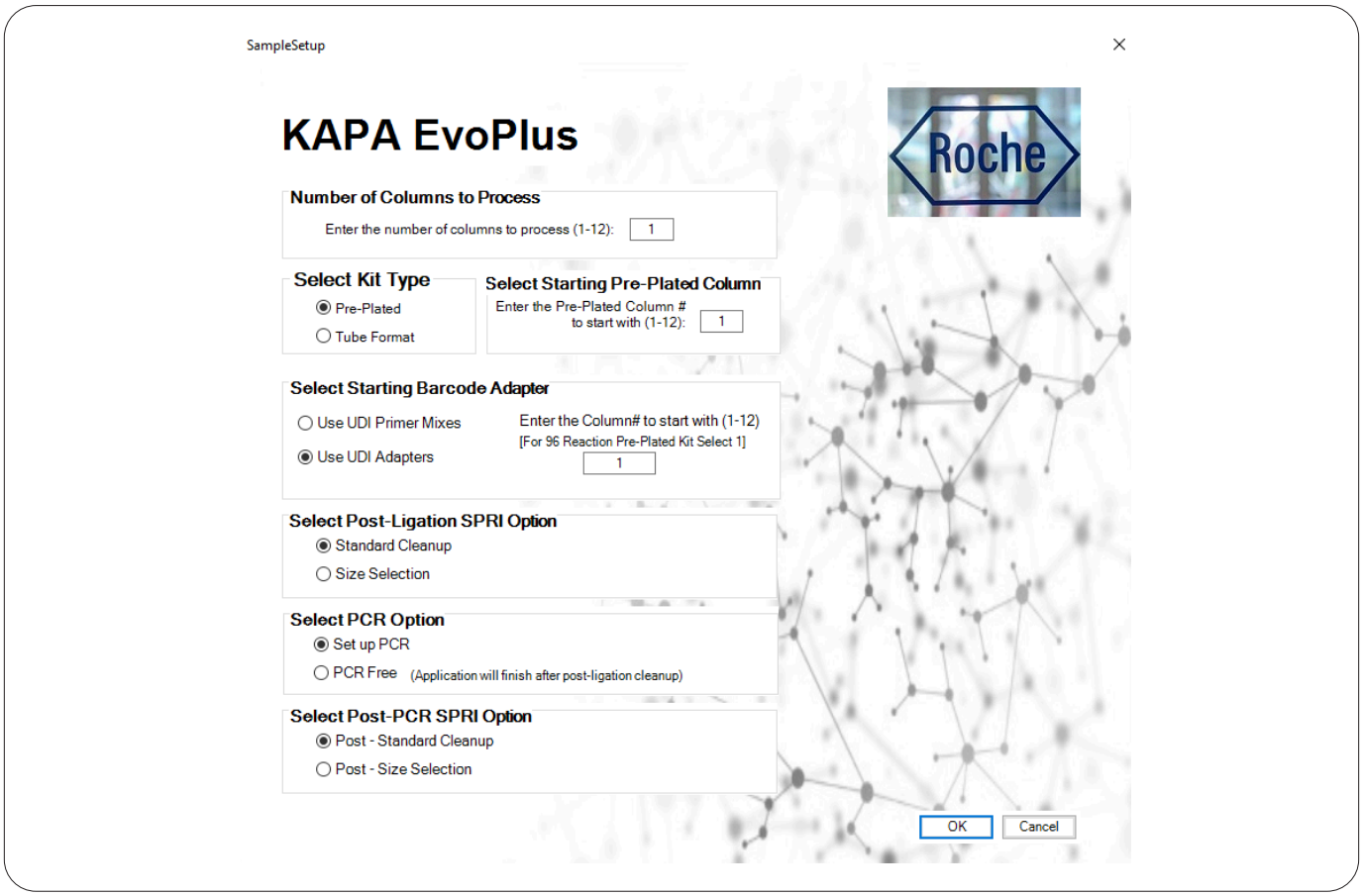

Figure 3: Guided User Interface (GUI) for the Automated KAPA EvoPlus Workflow. Based on the selection made, required reagents and consumables will be described during the method setup.

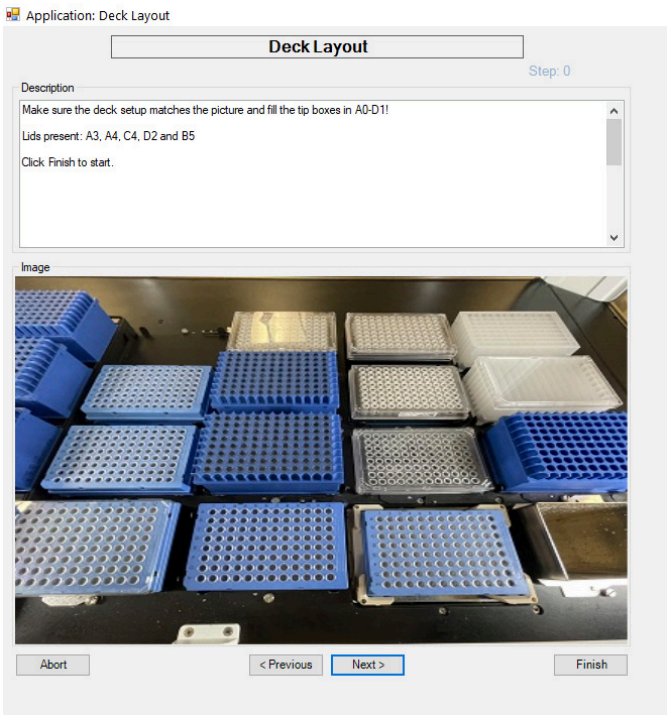

Figure 4: Deck layout to start the Roche KAPA EvoPlus application on Sciclone NGSx workstation. This setup is for a method employing pre-plated ReadyMixes, UDI Primer Mixes and Double-sided Size selection with PCR Amplification.

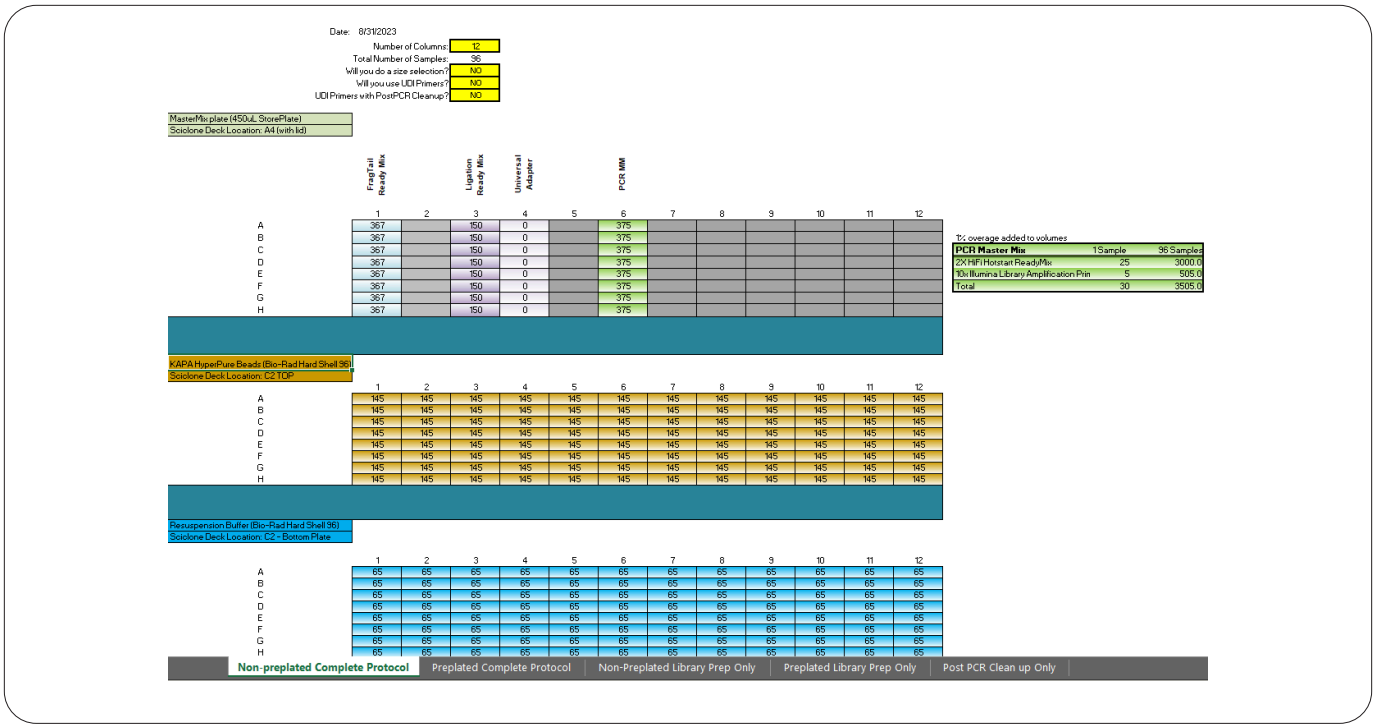

Figure 5: The Excel workbook for setting up the KAPA EvoPlus application on Sciclone NGSx workstation.

Two high-throughput experiments were performed on Sciclone G3 NGSx. Both experiments were conducted with 96 samples (81 samples of 10 ng human genomic DNA input and 15 negative controls of H_2O) to study the plate effects and cross-contamination. The sample plate format is shown in Table 1. During both the experiments, all samples were fragmented for 20 minutes, ligated for 15 minutes, and PCR-amplified for 5 cycles. The final libraries were assessed for total yield by Qubit® and for fragment size distribution by the LabChip™ GX Touch™.

In Experiment 1, the libraries were generated following the KAPA EvoPlus protocol for pre-plated reagents and the KAPA Unique Dual-Indexed (UDI) Adapters. In Experiment 2, libraries were generated following tubed reagents protocol and the KAPA UDI Adapters.

Table 1: Sample input (ng) for Experiment 1 and 2 by well position in a 96-well plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	10	10	10	10	10	10	10	10	10	10	10	10
B	10	0	10	10	10	0	10	10	10	0	10	10
C	10	10	10	0	10	10	10	0	10	10	10	10
D	10	0	10	10	10	0	10	10	10	0	10	10
E	10	10	10	0	10	10	10	0	10	10	10	10
F	10	0	10	10	10	0	10	10	10	0	10	10
G	10	10	10	0	10	10	10	0	10	10	10	10
H	10	10	10	10	10	10	10	10	10	10	10	10

Results

The Sciclone NGSx workstation was used to prepare libraries using 10 ng gDNA with KAPA UDI Adapters. The automated method using pre-plated reagents produced libraries averaging 5.55ng/ul with a CV of 11.08%. No edge effects were observed (Figure 6) when 96 samples (81 with 10 ng gDNA and 15 negative controls) were processed.

The 81 samples of 10 ng gDNA input yielded an average concentration of 3.3 ng/ul with a CV of 7.6% across the plate using tubed reagents. Both pre-plated and tubed reagents resulted in an expected fragment size of ~230 bp (Figure 7). No measurable cross-contamination was observed, indicated by 0 ng/ul concentration from the negative controls across the plate (Figure 6).

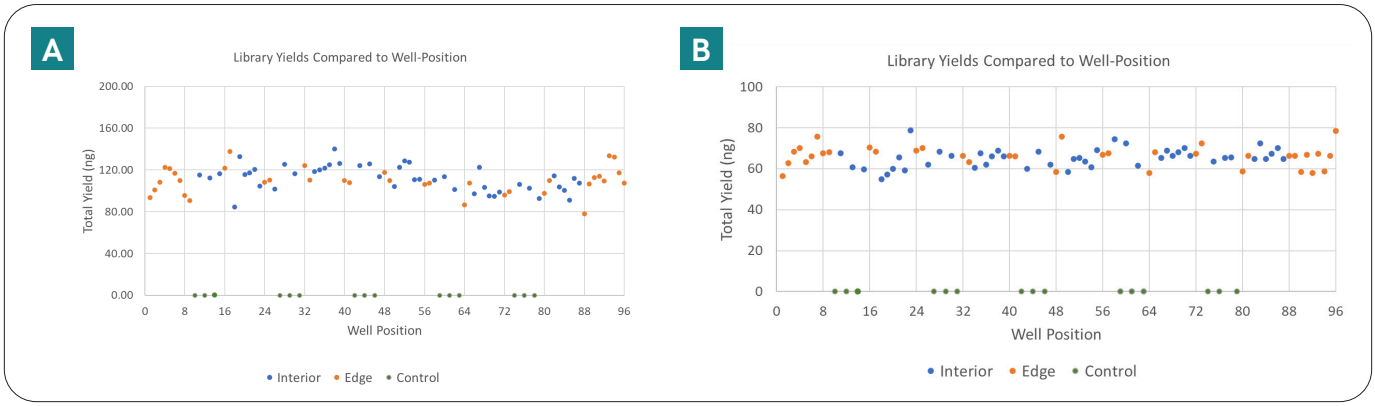

Figure 6: Well position and concentration of 96 samples (81 with 10 ng gDNA and 15 negative controls) prepared on the Sciclone G3 NGSx workstation. (A) is experiment 1 using pre-plated reagents, while (B) is experiment 2 with tubed reagents. In a 96-well plate, Well A1 is position 1, well H12 is position 96 and all other positions are likewise identified in a sequential columnar manner. Wells on the edge (orange) are compared to wells on interior positions (blue).

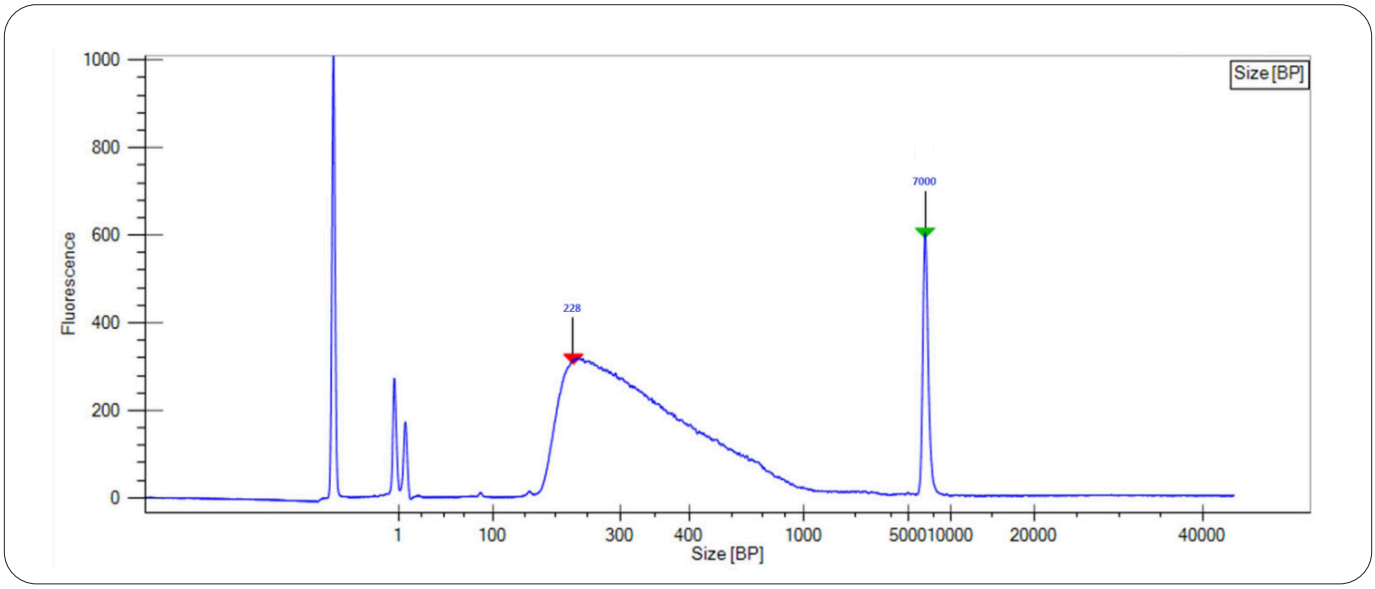

Figure 7: Example Labchip trace of KAPA EvoPlus libraries generated from the automated method using 10 ng input demonstrated the expected size (~230 bp). Trace is of a library produced with the pre-plated reagent workflow.

Conclusion

In a collaboration between Roche and Revvity, a KAPA EvoPlus Kit workflow for WGS library prep was successfully automated on the Sciclone™ G3 NGSx workstation. Quality metrics demonstrate that the automated method generates libraries with consistent concentration and size across the plate without significant edge effects or measurable cross contamination.

Data on file.

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