APPLICATION NOTE

Roche® KAPA HyperCap Workflow v3 using KAPA HyperPrep or KAPA HyperPlus Kit automated on the Sciclone® G3 NGSx and iQ™ workstations.

Introduction

Target enrichment of Next-Generation Sequencing (NGS) libraries limits the breadth of library content to regions of interest. Reducing library diversity concentrates data collection to relevant biological information, which also relieves sequencing and computational demands per sample. This approach increases feasibility for large-scale studies and seqencing-based diagnostic tools by maximizing resources for data generation, analysis, and storage.

The KAPA HyperCap Workflow v3 from Roche combines the high conversion rate from KAPA HyperPrep or KAPA HyperPlus library preparation kits with the uniform target capture from the KAPA Target Enrichment kit to produce high-quality enriched NGS libraries. This workflow accommodates input of mechanically sheared gDNA (KAPA HyperPrep) or can use an upfront enzymatic fragmentation module for intact gDNA (KAPA HyperPlus). The target enrichment component captures regions of interest using an in-solution probe hybridization process compatible with a range of standardized and custom probe panels, including the KAPA HyperExome human exome panel.

This workflow was automated on the Revvity Sciclone G3 NGSx and NGSx iQ Workstations. These liquid handling workstations are designed for high-throughput, rapid and reliable sequencing library preparation, and reduce the overall operational cost, error rate and sample variability, allowing an operator to reliably process up to 96 samples at a time. The Sciclone G3 NGSx iQ workstation further reduces required user intervention by utilizing an integrated on-deck thermocycler and robotic tip storage and waste management system.

Sciclone® G3 NGSx iQ™ Workstation

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

The KAPA HyperCap Workflow v3 automated on the Sciclone G3 NGSx and iQ workstations has been demonstrated to reduce hands-on time, increase throughput processes, and generate reproducible experimental data. Sequencing data of low-throughput and high-throughput sample processing reveals consistent target capture specificity and uniformity, with minimal PCR duplicates on both the Sciclone G3 NGSx and NGSx iQ workstations.

Experimental setup

Enriched sequencing libraries were generated in a low-throughput (16 samples) format on both the Sciclone G3 NGSx and NGSx iQ workstations and in a high-throughput (96 samples) format on the Sciclone G3 NGSx workstation. Each experiment followed the standard KAPA HyperCap Workflow v3. Libraries were generated from 100 ng of intact NA12878 human gDNA with the KAPA HyperPlus kit, using the enzymatic fragmentation module prior to adapter ligation. Pre-Capture libraries were combined into pools of 8 column-wise, equally contributing to a total 1.5 µg of pooled libraries input for target capture, for which the KAPA HyperExome probe panel was used. Pre-capture libraries and post-capture pools were quantified using the Qubit™ dsDNA HS Assay kit and a Qubit™ 2 fluorometer, and fragment distributed was analyzed using the LabChip® GXII Touch™ HT instrument. A subset of post-capture pools from the low-throughput (1 pool - 8 samples) and high-throughput (12 pools - 96 samples) conditions were further quantified with the KAPA Library Quantification Kit (ABI Prism™ qPCR Master Mix) on a StepOnePlus™ Real-Time PCR System and sequenced on an Illumina® NextSeq® 500 instrument with high-output flowcells, generating 75 bp paired-end reads. Sequencing data was down-sampled to 30 M paired-end reads for each sample.

Methods

KAPA HyperCap Workflow v3 on the Sciclone NGSx workstation consists of six steps, while the Sciclone G3 NGSx iQ workstation completes the workflow in four steps:

Table 1: List of steps needed to complete the KAPA HyperCap Workflow v3 on the NGSx and NGSx iQ workstations.

Sciclone G3 NGSx workstation	Sciclone G3 NGSx iQ workstation
1. Library preparation	1. Library preparation, PCR and Post-PCR purification
2. Pre-capture purification	2. Normalization and pooling
3. Normalization and pooling	3. Hybridization setup
4. Hybridization setup	4. Target capture, PCR and Post-PCR purification
5. Target capture	
6. Post-capture purification	

These steps are visually shown in Figure 1.

Setup of the automated methods are directed by prompts in the User Interface and an excel workbook. The user has the option to select the number of columns of 8 samples to process as well as additional parameters. For example, during the Library Preparation step the user can select the leftmost column of the UDI Primer Mixes plate to be used for barcoding the libraries (Figure 2). The User Interface will also prompt the user to set up the deck accordingly (Figure 3). Master mixes and reagents are prepared following the guidance provided by the excel workbook (Figure 4). For the normalization and pooling steps, the user will fill the concentration of the sample, source and destination sample well in the excel workbook.

Once saved, information will be automatically imported into the application for Normalization and Pooling. Throughout the protocol, master mixes are stored at 4°C on a CPAC tile on the deck, and run time is optimized by pre-broadcasting master mixes into the indicated number of wells in an intermediate plate during incubations. With the Sciclone G3 NGSx workstation, longer temperature-controlled incubations, such as End-Repair and A-Tailing and PCR amplification, are performed with off-deck thermocyclers, thus requiring additional user intervention. The Sciclone G3 NGSx iQ workstation, however, uses the integrated on-deck thermocycler, thus reducing required hands-on time. The recommended 16-20 hour hybridization incubation is performed with an off-deck thermocycler for both workstations.

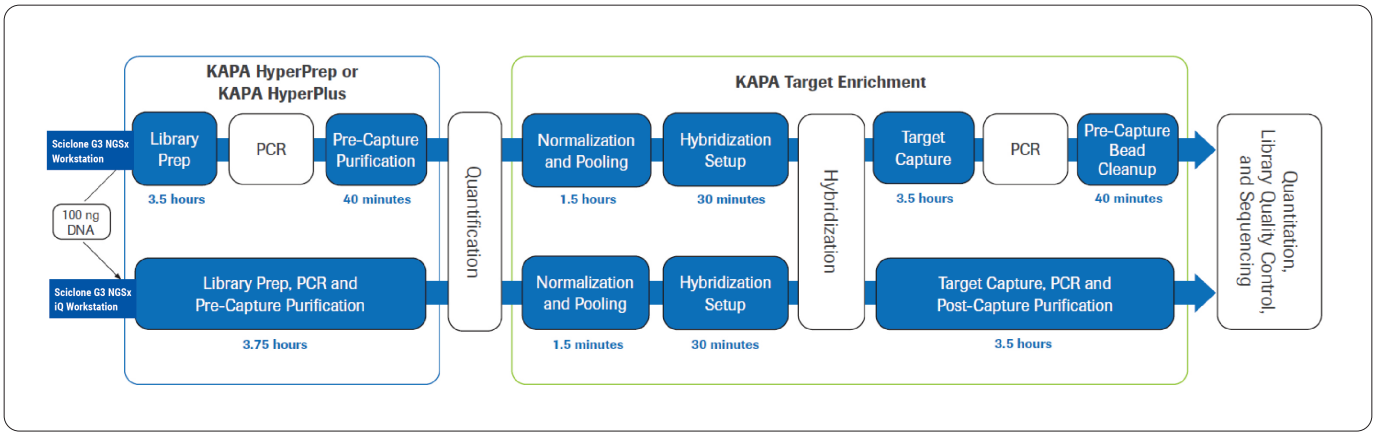

Figure 1: The KAPA HyperCap Workflow v3 implemented on the Sciclone G3 NGSx (top) and NGSx iQ (bottom) workstations include library prep using either the KAPA HyperPrep or HyperPlus kit (encircled in blue) followed by selective capture using the KAPA Target Enrichment kit (encircled in green). Blue blocks indicate on-deck processes, and the white blocks identify actions occurring off-deck.

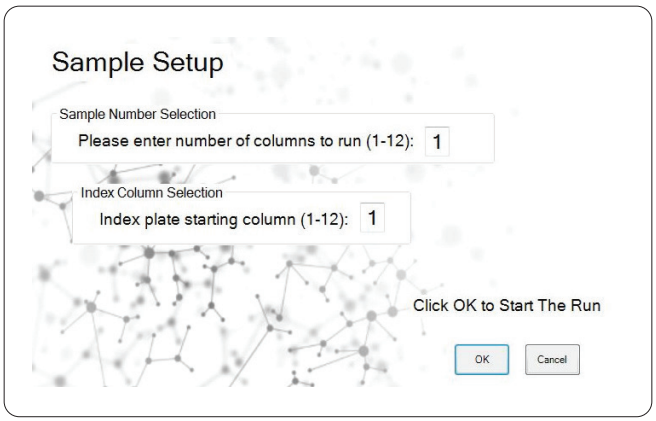

Figure 2: Example of the User Interface and how the user will select run parameters, such as the sample number and Index column.

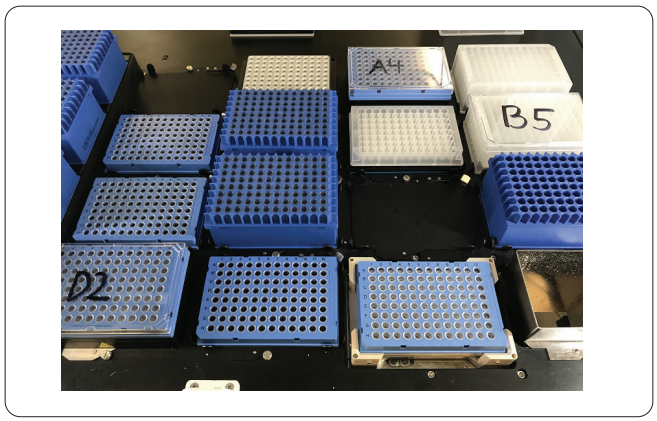

Figure 3: Example of the deck layout for the setup for the KAPA HyperPlus protocol, the first step of the KAPA HyperCap workflow v3, on the Sciclone G3 NGSx workstation.

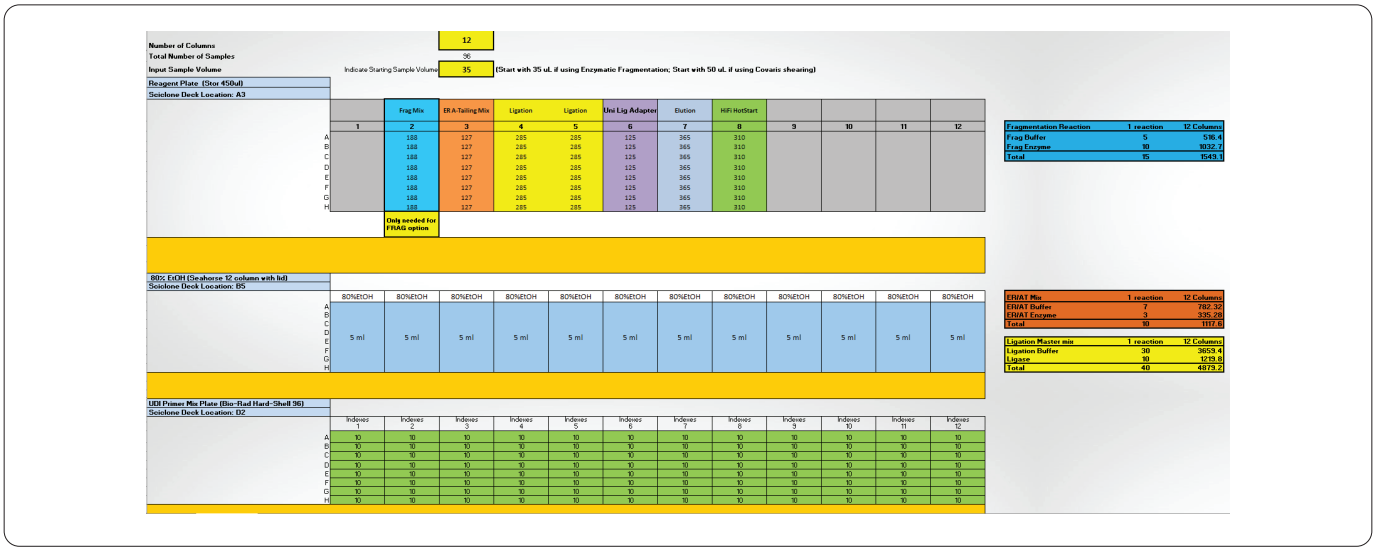

Figure 4: The excel workbook describes how the master mixes and reagent plates should be prepared, in this example, the KAPA HyperPlus protocol on the Sciclone G3 NGSx workstation.

Results

Libraries were generated on the Sciclone G3 NGSx and NGSx iQ workstations under low-throughput (16 replicate samples) and high-throughput (96 replicate samples) conditions. Pre- and post-capture quality metrics showed that all libraries were within acceptable ranges regardless of throughput or workstation (Figure 5). Average pre-capture yields for low-throughput processing (5.8 µg) on the Sciclone G3 NGSx iQ were comparable to that of the low-throughput (5.2 µg) and high-throughput (4.9 µg) processing on the NGSx workstation. While the average post-capture yields varied between the Sciclone G3 NGSx (2.3 µg for low-throughput and 3.5 µg for high-throughput) and NGSx iQ (1.2 µg) workstations (Figure 5A), all libraries were well above the recommended minimal yield for successful post-capture libraries, which is 100 ng according to the KAPA HyperCap Workflow v3. Furthermore, pre- and post-capture average fragment sizes remained consistent across all conditions on both workstations (Figure 5B), demonstrating reproducible fragmentation from the enzymatic fragmentation module in the KAPA HyperPlus kit.

Variability between replicates was minimal within each condition. For example, CV values for pre-capture library yields were 3.2% (Sciclone G3 NGSx workstation - low-throughput), 5.8% (Sciclone G3 NGSx workstation - high-throughput) and 4.2% (Sciclone G3 NGSx iQ workstation-low-throughput). Mapping pre-capture library yields across the 96-well plate from the NGSx high-throughput condition illustrates consistent performance with little-to-no plate effects (Figure 6A). Likewise, pre-capture fragment distribution was also reproducible across all 96 wells (Figure 6B).

Analysis of sequencing data across all experimental conditions shows that at least 75% of reads were mapped to targeted regions of interest. Mapped reads were supported with an average of >50 X coverage, and read coverage was evenly distributed: over 85% of mapped reads have at least 30x coverage (Figure 7A). The targeted regions were also uniformly represented across the data, as is shown by the Fold 80 Base Penalty averaging ~ 1.5 or less. Furthermore, the libraries contained a minimal percentage of duplicated reads (Figure 7B), indicating rich and diverse libraries unencumbered by PCR duplicates.

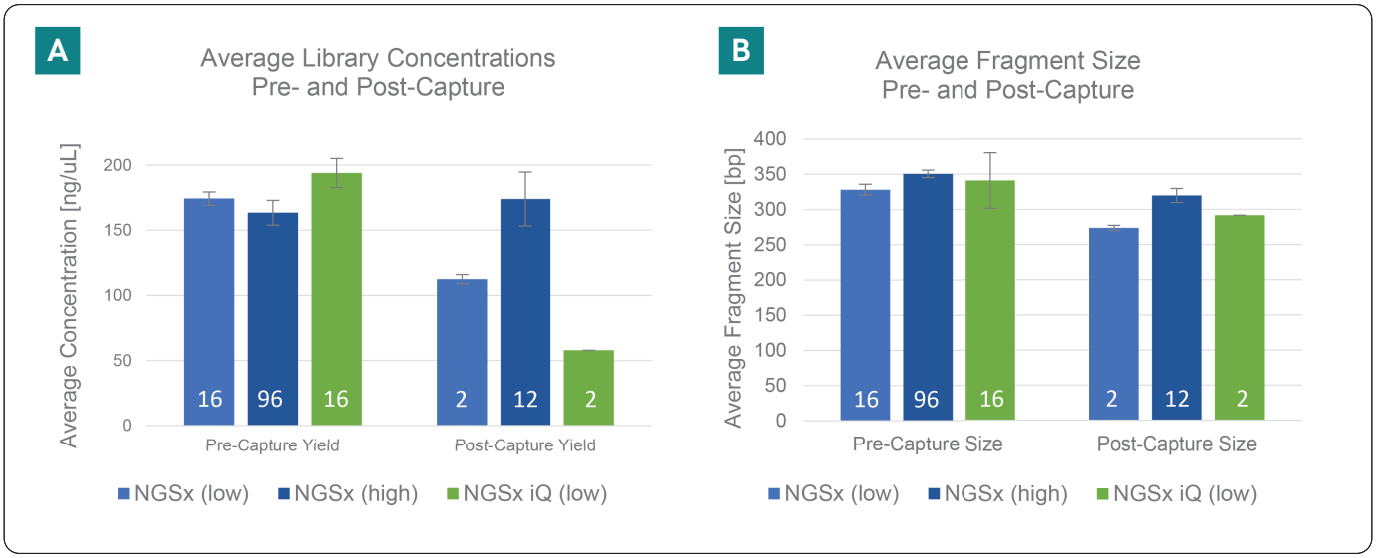

Figure 5: Comparison of average library yields (A) and average fragment sizes (B) between low-throughput and high-throughput conditions between the Sciclone G3 NGSx and NGSx iQ workstations. The numbers in each column of the charts (white) identifies the number of replicate samples (pre-capture) or pools (post-capture) measured. Error bars represent standard deviation across averaged data.

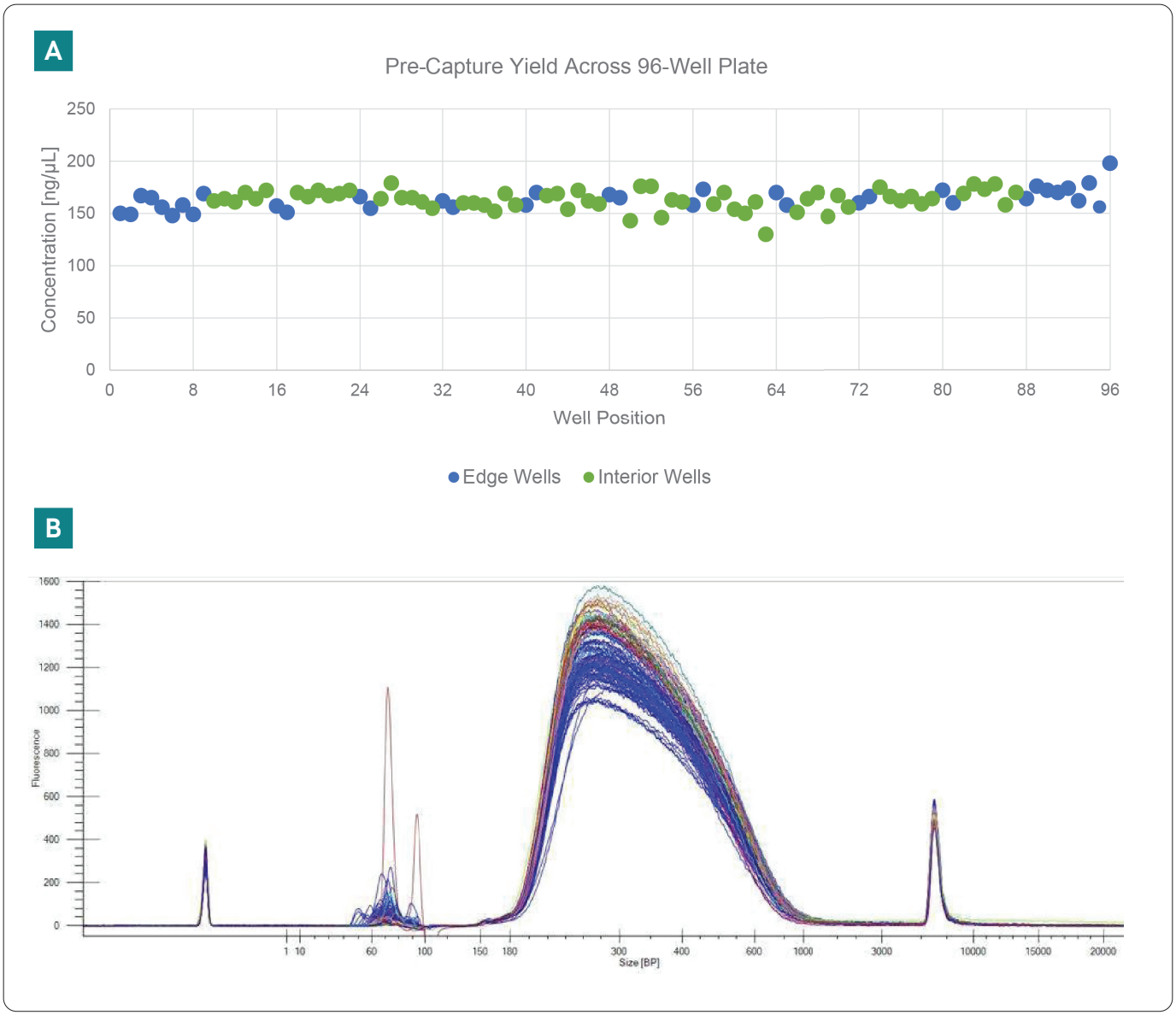

Figure 6: Pre-capture library concentrations [ng/µL] by well position in a 96-well plate (A). Libraries from edge wells are blue, and those from interior wells are green. An overlay of the fragment distribution of 96 pre-captured libraries (B) obtained from the LabChip GXII Touch HT Nucleic Acid Analyzer.

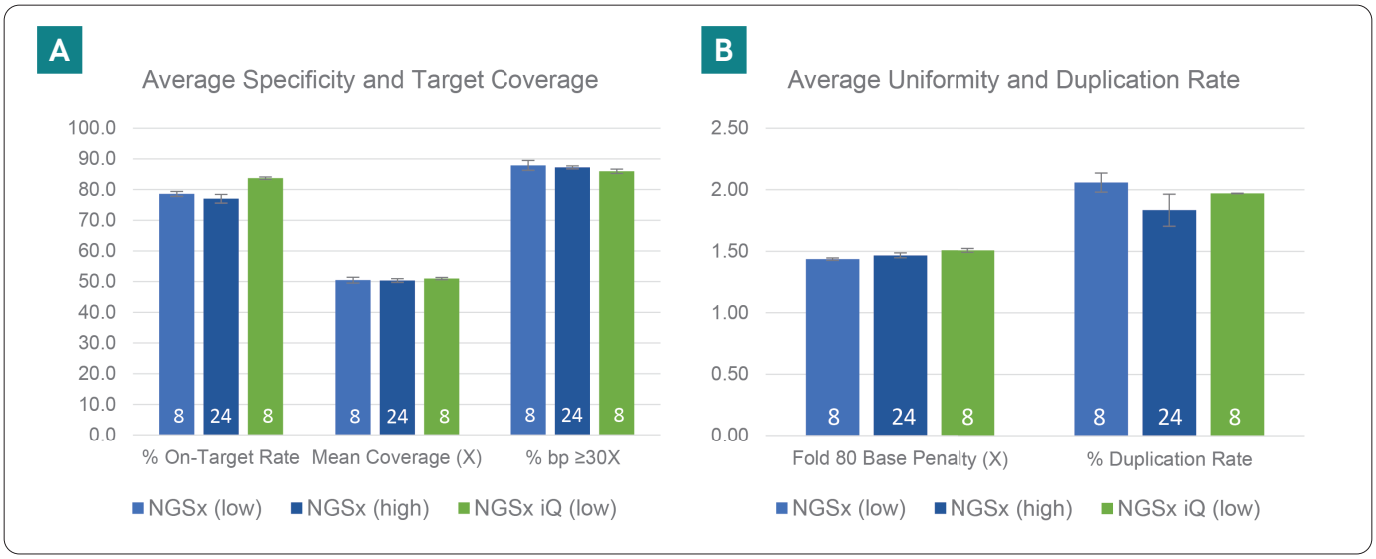

Figure 7: Sequencing analysis of a subset of enriched libraries show the proportion of the libraries mapped to targeted regions (% On-Target Rate), along with the depth (Mean Coverage) and evenness (% bp ≥30X) of coverage across the mapped reads (A). The Fold 80 Base Penalty indicates uniformity of read distribution across targeted regions, and the Duplication Rate identifies how much of the data is composed of PCR duplicates (B). The numbers in each column of the charts (white) identifies the number of libraries sequenced. Error bars represent standard deviation across averaged data.

Conclusion

The KAPA HyperCap Workflow v3 was automated on the Sciclone G3 NGSx and NGSx iQ workstations. Sequencing libraries were generated from replicate samples in a low-throughput format on both workstations, and a high-throughput condition was performed on the Sciclone G3 NGSx workstation. High library yields and consistent fragment distribution were reproducible with no discernable plate effects. Sequencing data showed successful capture and even coverage of targeted regions with minimal duplicate reads due to amplification. This automated solution generates high-quality, enriched sequencing libraries in both low-and high-throughput conditions on both the Sciclone G3 NGSx and NGSx iQ workstations.

Automate Your Library Prep

For more information about Roche NGS solutions visit: https://sequencing.roche.com/en-us.html

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