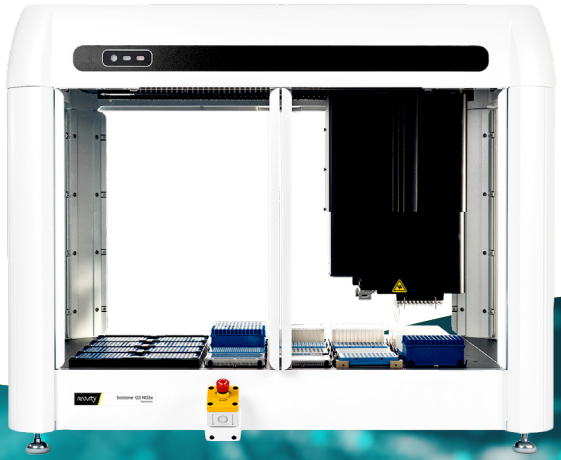
APPLICATION NOTE

Illumina DNA Prep with Exome 2.5 Enrichment on the Revvity Sciclone NGSx and iQ workstations.

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

Sciclone® G3 NGSx workstation



Introduction

Illumina DNA Prep with Exome 2.5 Enrichment Kit automation on Revvity's Sciclone® NGSx and iQ™ automation workstations has minimized the human input by reducing hands-on time and increasing walkaway time. The workflow automated on the Sciclone NGSx workstation has few touch points for the off-deck thermocycler program, while the Sciclone NGSx iQ workstation offers complete walkaway, allowing user to focus on intellectual contributions.

Illumina DNA Prep with Exome 2.5 Enrichment protocol is compatible with high-quality, double-stranded human genomic DNA (gDNA) inputs of 10-1000ng (minimum 50ng is recommended for optimal performance), fresh whole blood, and saliva samples. The kit enables the construction of 96 libraries followed by the enrichment of those libraries in eight hybridization reactions, each containing 12 libraries (12-plex enrichment) for a total output of 96 exome enriched, human libraries.

The Sciclone NGSx and iQ liquid handling workstations are designed for high throughput, rapid, and reliable NGS library construction that reduces overall operational cost, error rate, and sample variability thereby reducing the standard deviation and variance. These automated liquid handlers combined with Illumina DNA Prep with Exome 2.5 Enrichment workflow use on-bead tagmentation, an enzymatic reaction, to both fragment the input DNA and add adapter sequences in only 15 minutes. This eliminates the step of library quantitation prior to hybridization due to innovative sample normalization at inputs ≥50 ng.

The high-throughput system enables users to load up to 96 DNA samples onto the Sciclone NGSx or iQ workstation. These workstations perform all the needed liquid handling steps including tagmentation, amplification, purification, hybridization (12-plex), and enrichment, producing libraries for sequencing that is both highly reproducible and reliable.

Experimental setup

A set of 24 human NA12878 DNA samples across 12 columns of a plate (rows A and H) were processed on the Sciclone iQ version and 24 samples in columns 1-3 on the Sciclone NGSx version following the workflow as described in the Illumina DNA Prep with Exome 2.5 Enrichment reference guide. The total starting input for each DNA sample was 60ng. The input DNA and purified libraries were quantified using Qubit® dsDNA HS Assay Kit respectively on Qubit® 4 fluorometer. The fragment distribution was analyzed using the LabChip® GXII Touch™ HT instrument.

Methods

Illumina DNA Prep with Exome 2.5 Enrichment workflow consists of nine steps that make up a total of four individual automated applications for both workstations:

APPLICATION 1

1. Tagment Genomic DNA
2. Post Tagmentation Clean Up
3. Amplify Tagmented DNA
4. Clean Up Libraries

MANUAL STEP

5. Pool Libraries

APPLICATION 2

6. Hybridize Probes

APPLICATION 3

7. Capture Hybridized Probes
8. Amplify Enriched Library

APPLICATION 4

9. Clean Up Amplified Enriched Library

Workflow

Figure 1 demonstrates the workflow and the time required to complete each step. The application features a user-friendly interface, starting with a prompt for the user to select the protocol step and to enter the number of columns to process (Figure 2). The Sciclone NGSx deck was set up as shown in Figure 3. The workbook (Figure 4) calculates the total volumes of reagents and master mixes needed based on the number of sample columns needing to be processed. The reagents and master mixes required for a setup were pipetted into the specific consumable called out in the workbook and setup screens. At the end of each run, a prompt reminded the user to place the index plate at 4°C for future use. All the master mixes were maintained at 4°C on CPAC throughout the experiment. The master mixes were either pre-broadcasted to a new plate or broadcasted directly to the sample plate to save time and eliminate manual pipetting errors. Samples were mixed on the on-deck shaker. Incubations were completed on the on-deck CPAC locations.

For the Sciclone NGSx process, an off-deck thermocycler was used for incubations at Tagment DNA, Amplify Tagmented DNA, Hybridize Probes, and Amplify Enriched libraries. The workflow was completely walkaway for the Sciclone NGSx iQ process except for pooling, which was completed manually for both system workflows. For library QC, the Qubit 4 Fluorometer was used to quantify the libraries after cleanup (Pre-Enriched and Exome-Enriched). The final library size was assessed using the LabChip GXII Touch HT followed by sequencing on the NextSeq™ 500 using 2x74 bp reads.

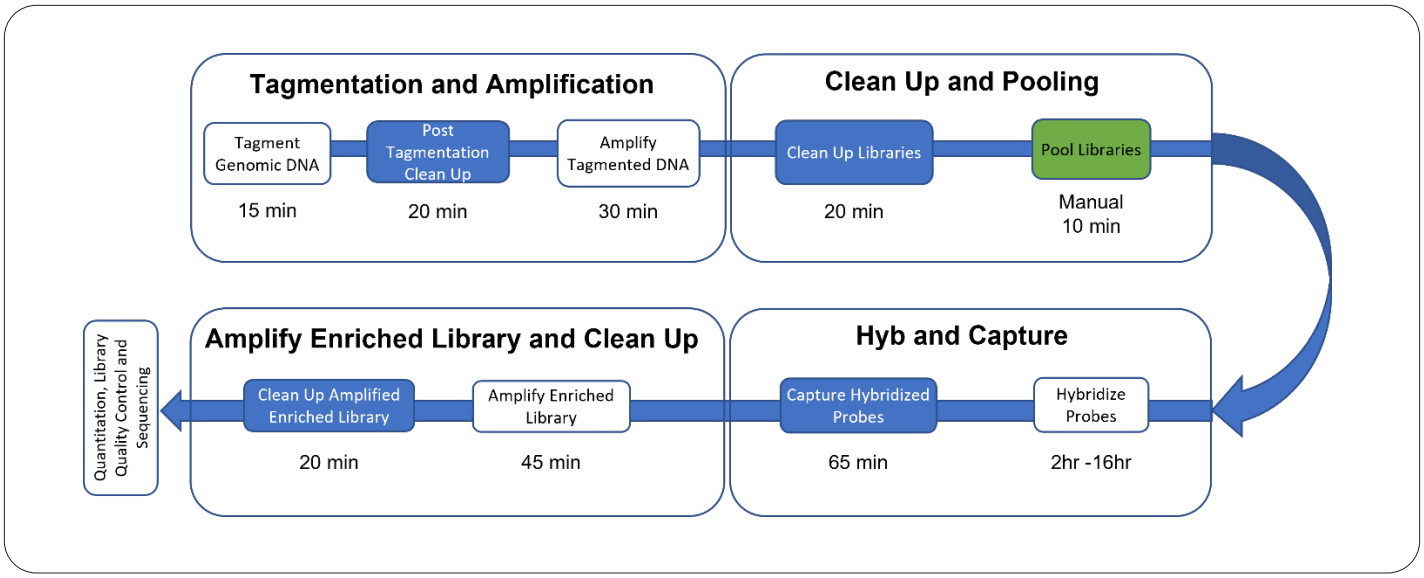


Figure 1: Illumina® DNA Prep with Exome 2.5 Enrichment Workflow and the time required to complete each step. Solid blue block represents on-deck incubations and white blocks represent the steps that require off-deck thermocycler incubations on the Sciclone NGSx workstation. All the incubations are performed on-deck on the Sciclone NGSx iQ workstation. Pooling was done manually (represented in solid green block).

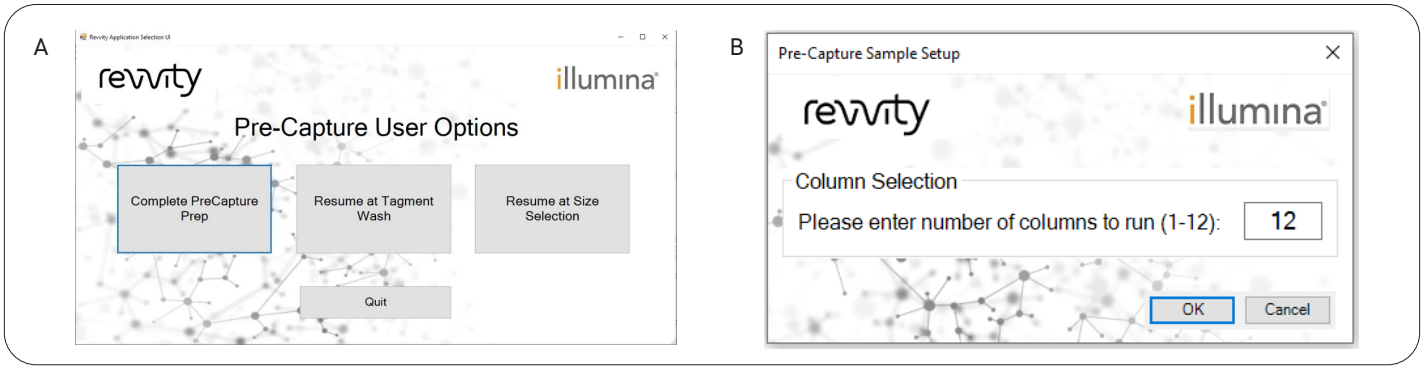


Figure 2. Example user interface from Pre-capture application to select a step (A) selection menu and (B) number of columns to run.

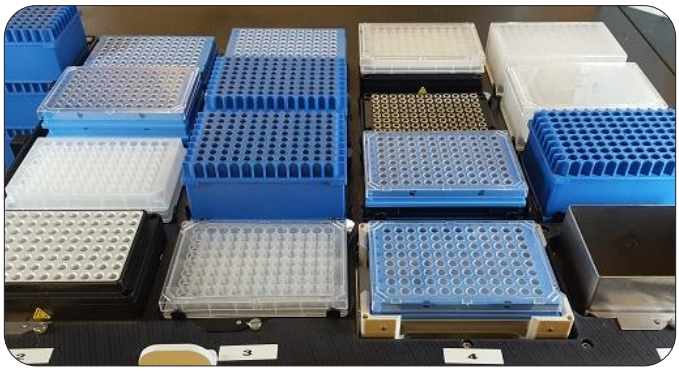


Figure 3. Deck layout to start the Illumina DNA Prep with Exome 2.5 Enrichment application on the Sciclone NGSx workstation.

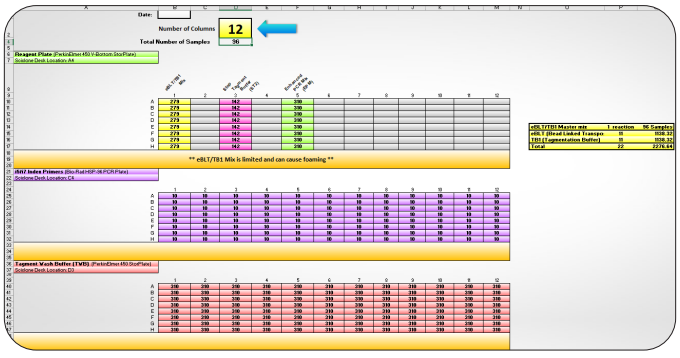


Figure 4. The Excel workbook for setting up the Illumina DNA Prep with Exome 2.5 Enrichment application on the Sciclone NGSx workstation.

Results

The Sciclone NGSx and iQ workstations used to prepare 24 libraries using 60 ng input DNA, produced pre-enriched libraries averaging 60 ng/µL with 287 nM concentration (Figure 5A and 5B) and 32.9 ng/µL with 142 nM concentration respectively (Figure 5C and 5D). A pool of 12-plex produced exome-enriched libraries averaging 24ng/µL with 103 nM concentration and 16.4ng/µL with 70.9 nM concentration, on Sciclone NGSx and IQ workstations respectively (Figures 6A and 6B). No cross-contamination was observed. The gel image and LabChip trace of pre-enriched and exome-enriched libraries are shown in Figure 7 and Figure 8, respectively. Two 12-plex pools of enriched libraries from the Sciclone NGSx and Sciclone NGSx iQ systems respectively were sequenced on NextSeq™ 500 using 2x74 bp reads. Some of the key sequencing metrics are shown in Figure 9. The sequencing data obtained from the exome-enriched libraries prepared on the Sciclone NGSx and iQ workstations was within expected ranges.

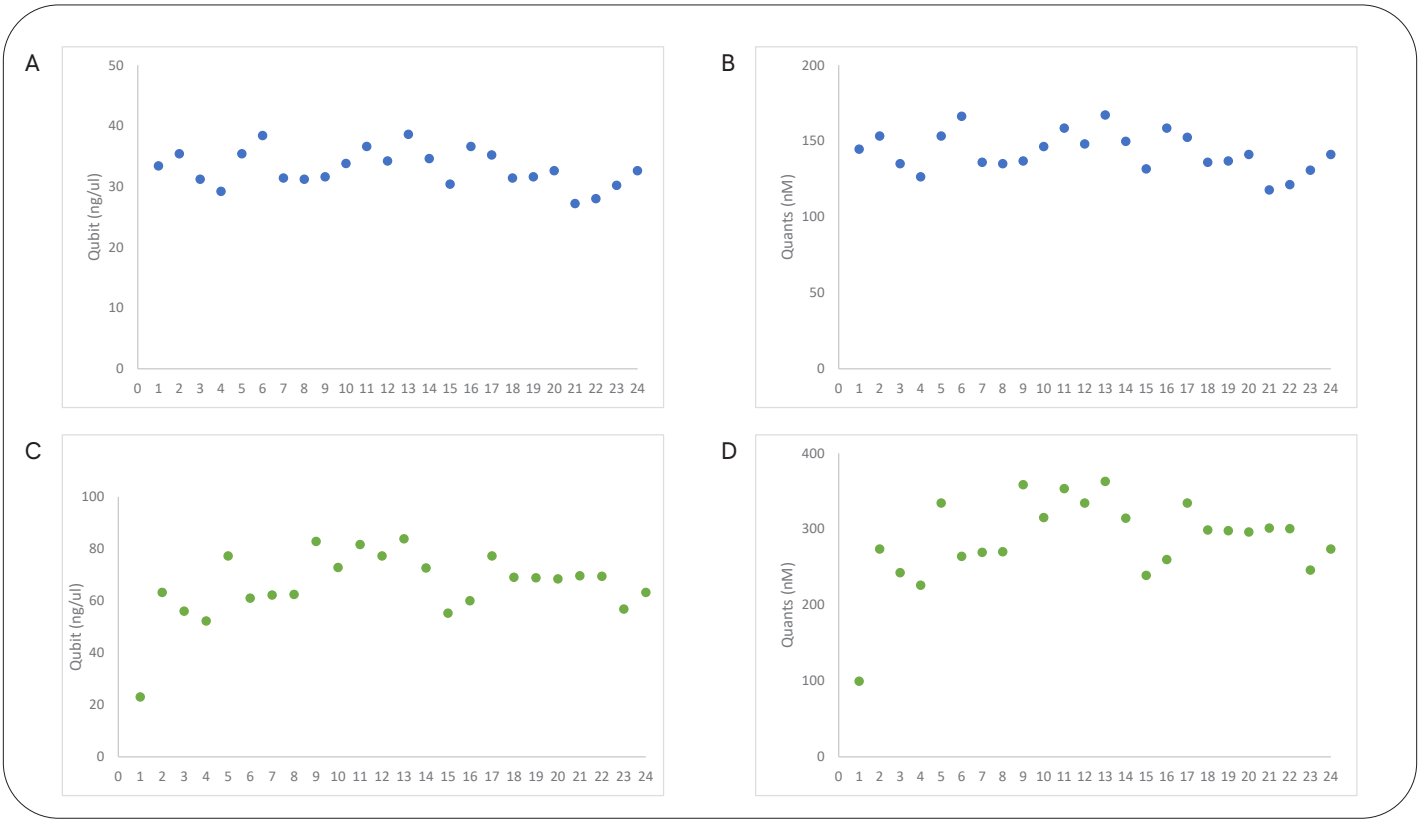


Figure 5. (A and B) Quantification of 24 libraries prepared on the Sciclone NGSx IQ workstation - (C and D) 24 libraries prepared on the Sciclone NGSx workstation using Qubit.

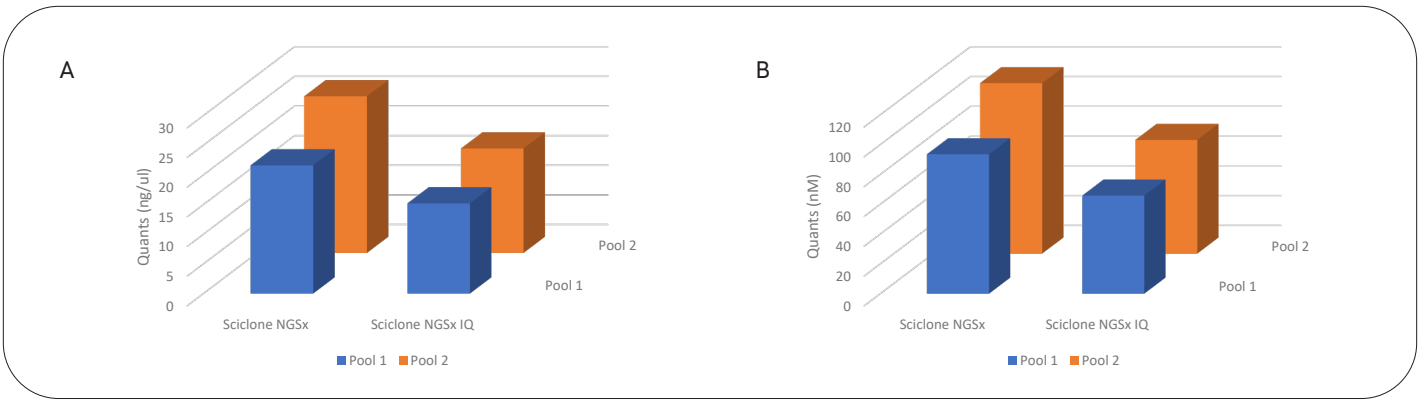


Figure 6. Quantification of Pooled Enriched Libraries (12-plex) prepared on the Sciclone NGSx and Sciclone NGSx IQ workstations.

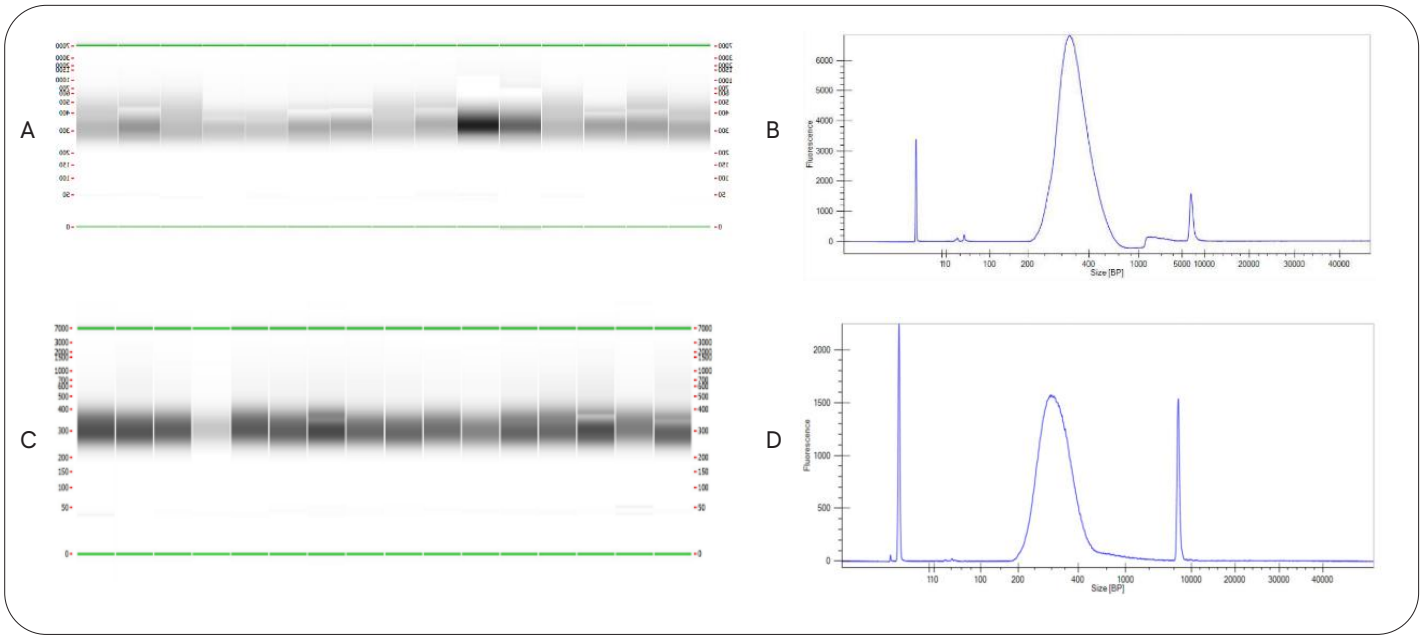


Figure 7. (A) Gel image and (B) Labchip trace of Pre-Capture Library prepared on the Sciclone NGSx IQ workstation; (C) Gel image, (D) Labchip trace of Pre-Capture Library prepared on the Sciclone NGSx workstation obtained from LabChip GXII Touch HT.

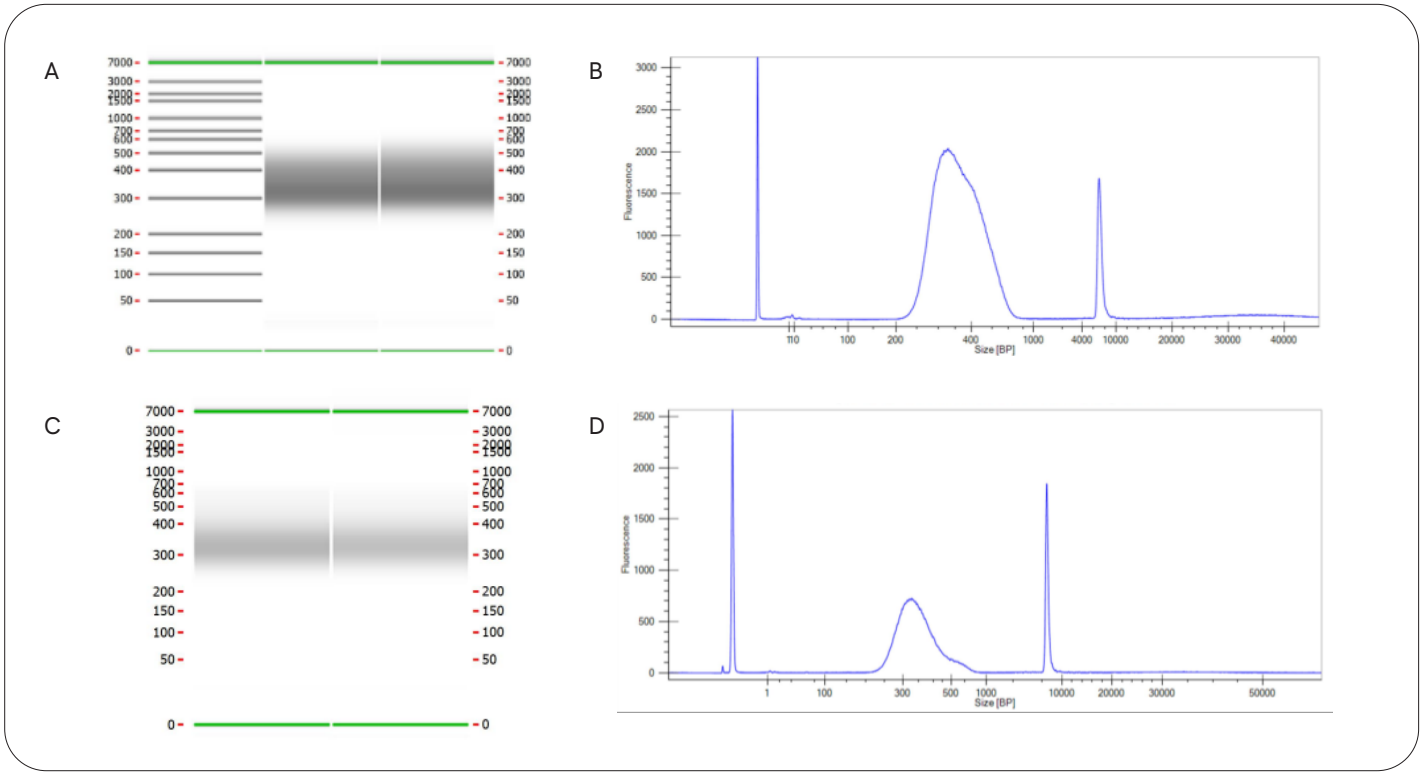


Figure 8. (A) Gel image and (B) Labchip trace of Post-Capture (12-plex) Library prepared on the Sciclone NGSx IQ workstation; (C) Gel image, (D) Labchip trace of Post-Capture (12-plex) Library prepared on the Sciclone NGSx workstation obtained from LabChip GXII Touch HT.

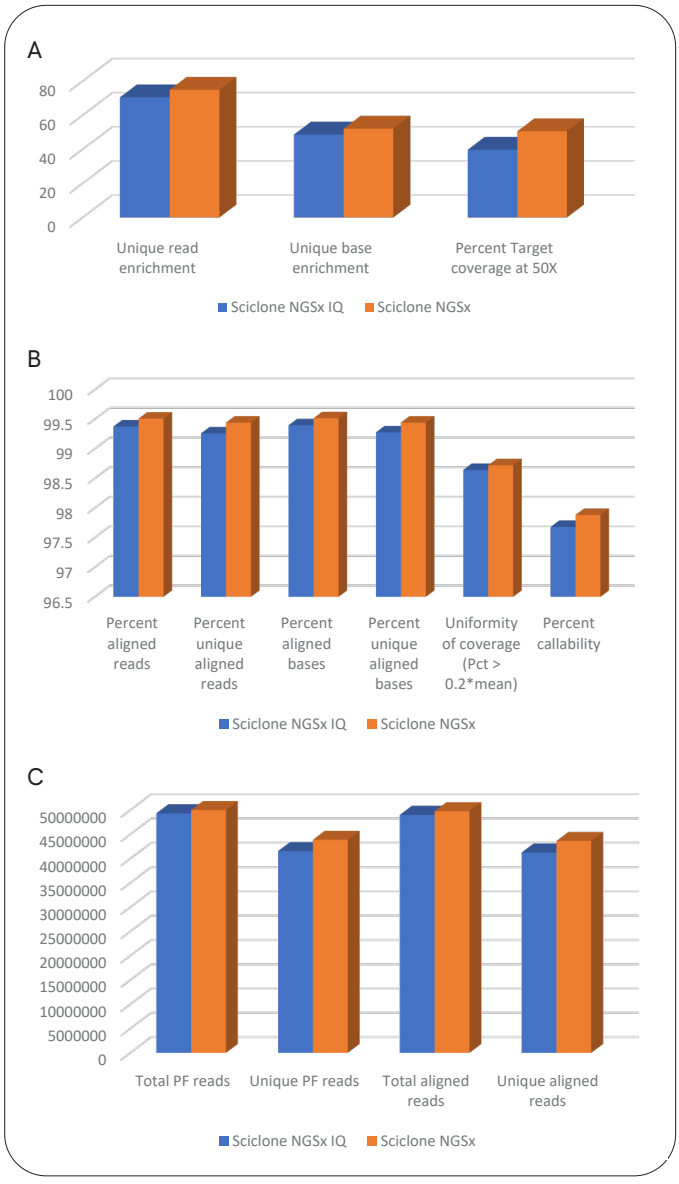


Figure 9. Key sequencing metrics obtained from NextSeq™ 500 from the exome-enriched libraries on the Sciclone NGSx and iQ workstations.

Conclusion

The Illumina DNA Prep with Exome 2.5 Enrichment library construction is automatable on Revvity's Sciclone NGSx and iQ automated liquid handlers. In partnership with Illumina, the final data were analyzed and determined acceptable when compared with Illumina's quality metrics and standards. The library yields and sizes were within the expected range as per the DNA Prep with Exome 2.5 Enrichment workflow protocol. No cross-contamination was observed between the wells. The data obtained from the Sciclone NGSx and Sciclone NGSx iQ workstations was comparable.

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