Automation of IDT xGen™ DNA Library Prep Kit EZ on the Revvity Sciclone® G3 NGSx workstation

Abstract

Automated NGS library preparation reduces hands-on time and increases sample throughput, allowing researchers to generate more high-quality sequencing libraries faster. This application note walks through the process of generating whole genome sequencing libraries using the xGen DNA Library Prep Kit EZ on the Revvity Sciclone G3 NGSx workstation; and presents metrics that clearly show the quality of these libraries. The automated libraries generated expected yields, contained no detectable dimers, had high alignments rates, and resulted in expected fragment sizes.

Introduction

The IDT xGen DNA Library Prep Kit EZ is designed for easy automation of next generation sequencing (NGS) libraries. The double-stranded DNA (dsDNA) library preparation enables input amounts of 100 pg to 1 µg and contains a high efficiency ligase for adapter ligation resulting in high complexity libraries. Enzymatic fragmentation allows for a complete on-instrument library solution and is ideally suited for high-throughput research applications. To further support high-throughput capabilities, indexing via PCR is compatible with up to 1536 xGen Unique-Dual Index (UDI) indexing primers, and is also compatible with full-length xGen UDI adapters for PCR-free workflows.

The xGen DNA Library Prep Kit EZ supports the following research applications:

- Whole genome sequencing (WGS)
- Hybridization capture of targeted genomic regions (e.g., exome)
- Metagenomic sequencing
- PCR-free sequencing
- Detection of germline inherited single-nucleotide variants (SNVs) and indels
- Somatic variation detection of SNVs and indels
- Copy number variation (CNV) detection

The automation of the xGen DNA Library Preparation Kit EZ on the Revvity Sciclone® G3 NGSx workstation (Figure 1) supports the research applications listed above while reducing hands-on time; offering a standardized workflow to improve results and reduce errors; and increasing efficiency and throughput. This application note describes an automated workflow for preparing WGS libraries generated using the xGen DNA Library Prep Kit EZ with the Revvity Sciclone® G3 NGSx workstation.

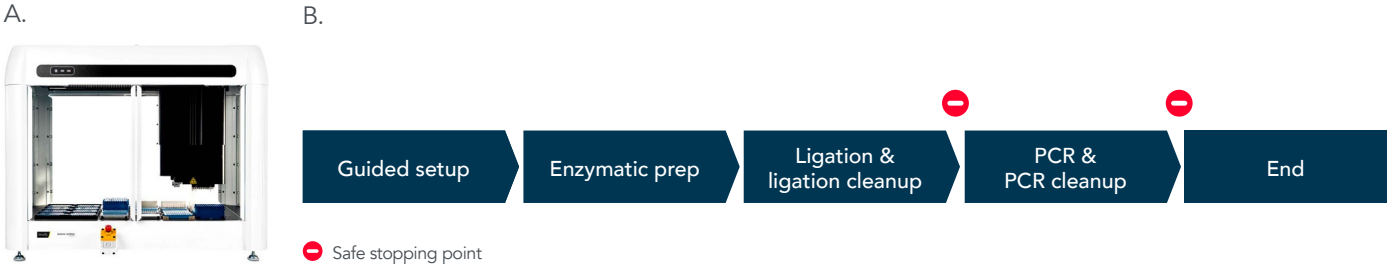


Figure 1. Combining the precision of the Revvity Sciclone® G3 NGSx workstation with reliability of the xGen DNA Library Prep Kit EZ. An image of the Revvity Sciclone® G3 NGSx workstation (**A**) and the xGen DNA Library Prep Kit EZ workflow with safe stop points noted (**B**).

Methods

Automated DNA library preparation setup

The xGen DNA Library Prep Kit EZ automated workflow requires two separate applications:

Application 1: Fragmentation through PCR setup

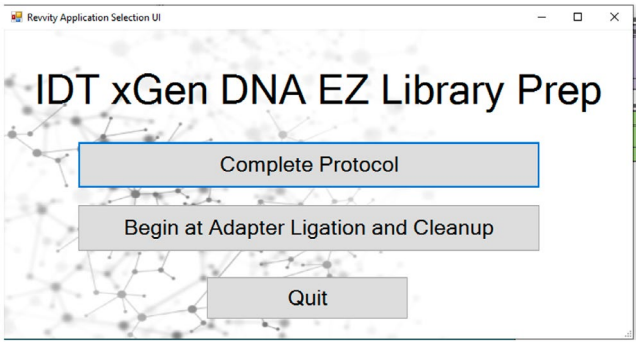
Application 2: Final PCR Cleanup

At the start of Application 1, a user prompt appears on screen for the user to decide on how to process their samples (Figure 2A).

After the user selects an option, a second user window is triggered with text fields for total number of sample columns being run, which sample column to start aspirating from for the barcode UDI Primer plate, as well as radio buttons for setting up PCR or PCR free workflows (Figure 2B). Based on the number of columns being run, the workbook for the application provides the user with master mix calculations and the volumes of reagents necessary for each consumable that is required on deck (Figure 2C). In the workbook, the user is given the option to run with or without Normalase™ UDI/CDI primers, triggering the use or lack of Reagent R7/R6 in the PCR master mix calculation.

Once the workstation is set up according to the guided user screens (Figure 2D) and workbook, the workstation proceeds with processing samples. Throughout the workflow, the master mixes are either pre-broadcasted to a clean plate and then transferred to all samples at the same time; or dispensed directly to the sample plate column-wise using a single column of the multi-channel head, changing the tips each time. After each reagent addition, reactions are tip-mixed while shaking on the thermoshaker position of the workstation.

A.



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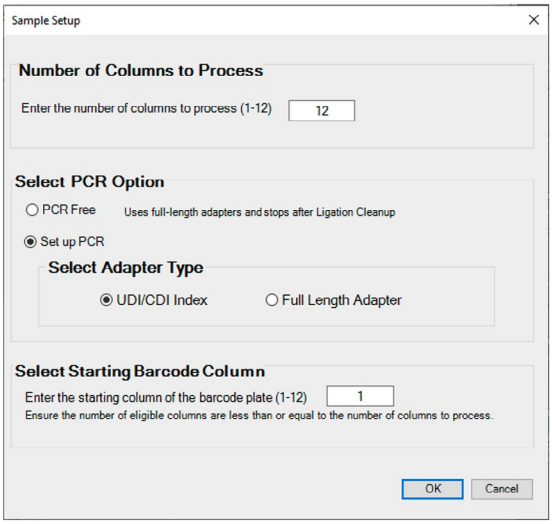

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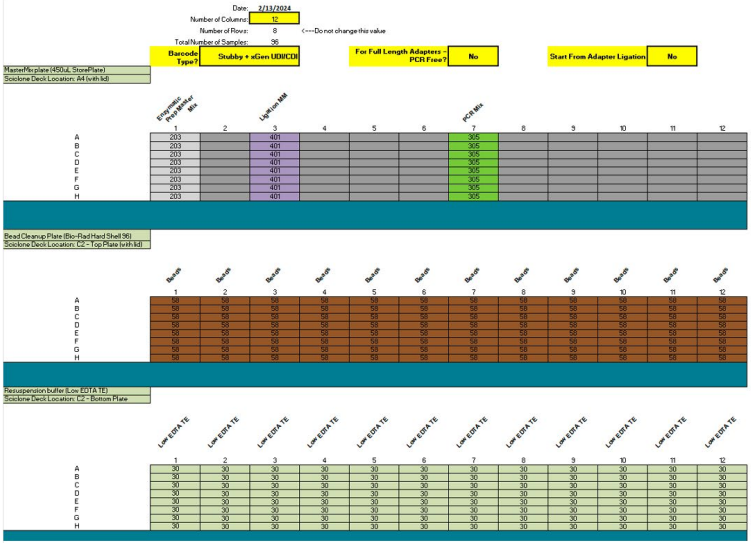

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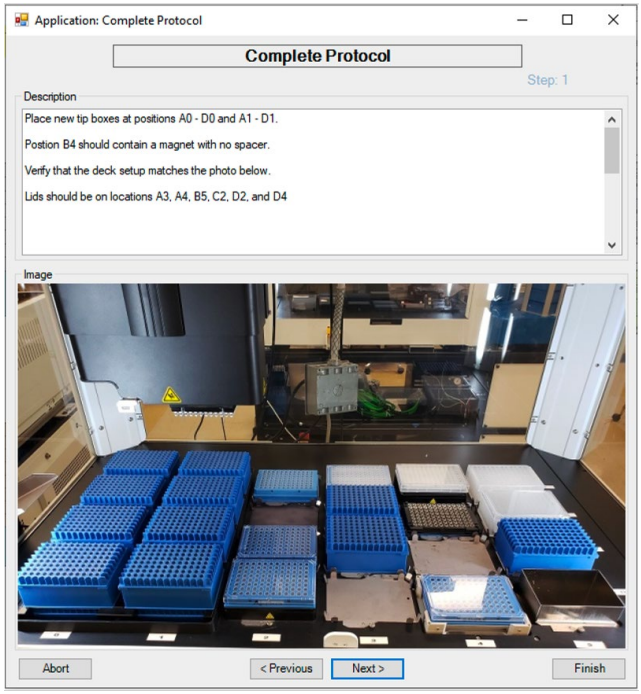

Figure 2. Setting up the automated xGen DNA Library Prep Kit EZ workflow on the Revvity Sciclone G3 NGSx workstation. xGen DNA Library Prep Kit EZ User Interface to select the application step (**A**); Sample Set up including number of sample column to process, PCR step selection and starting column for UDI Primer (**B**); workbook (**C**) and Deck Setup (**D**).

During the complete automated workflow with PCR, there are two required user interactions with the workstation—moving the sample reaction plate from the workstation to an off deck thermal cycler for incubations during enzymatic fragmentation and the PCR. Figure 3 demonstrates the complete automated workflow on Sciclone® G3 NGSx workstation along with the time required to complete each step.

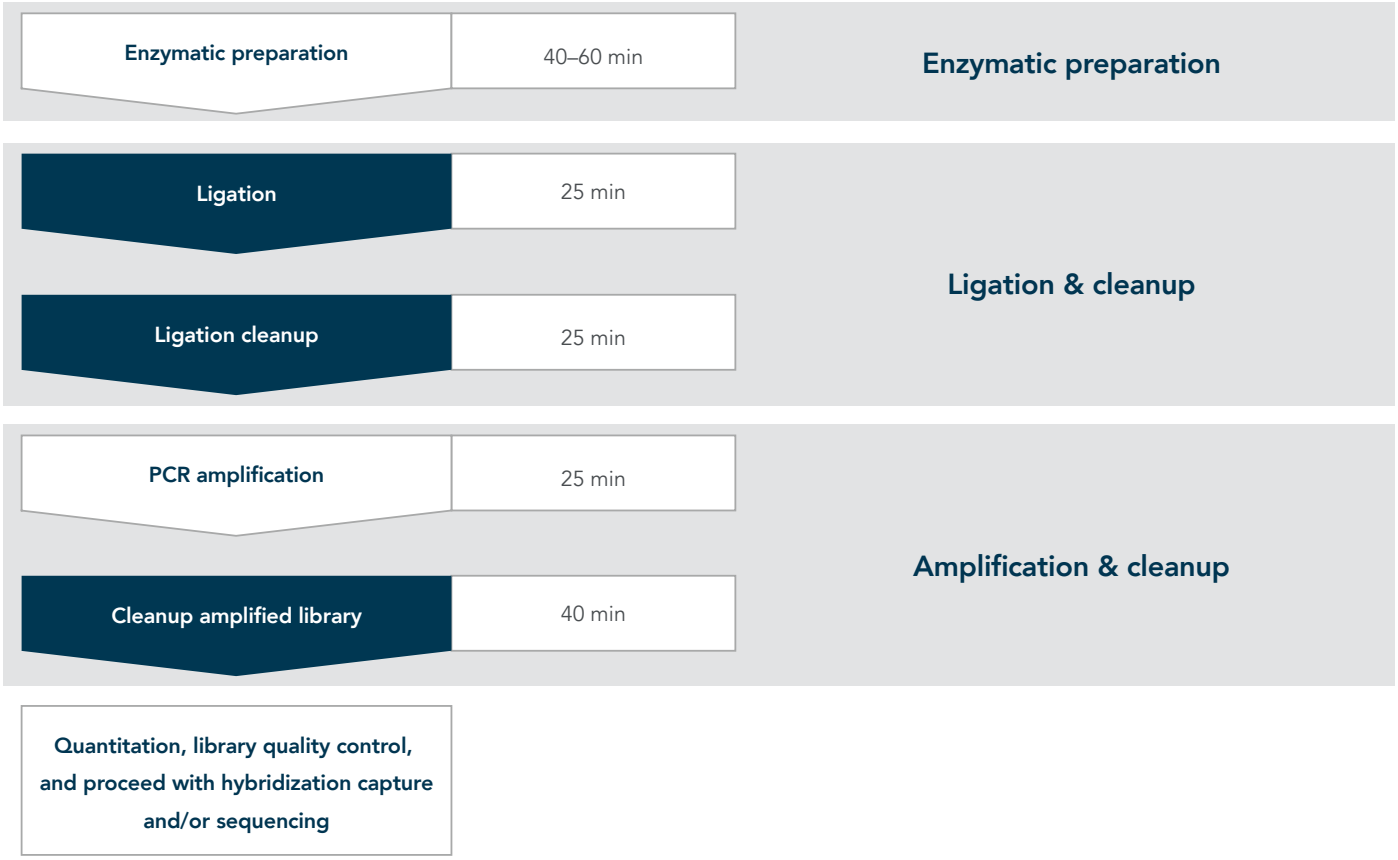

Figure 3. xGen DNA Library Prep Kit EZ automated workflow on the Sciclone® G3 NGSx workstation along with the time required to complete each step. Solid Blue blocks represents on-deck incubations and white blocks represent the steps that require off-deck thermocycler incubations after reaction setup on the Sciclone® G3 NGSx workstation.

Experimental Design

Libraries (*n* = 48) were generated using the xGen DNA Library Prep Kit EZ on the Revvity Sciclone® G3 NGSx workstation using 25 ng of Promega gDNA in Low EDTA with xGen Normalase UDI Primers and the xGen Deceleration Module following the published protocols (Table 1). The fragmentation time of 12 minutes was determined based on the specific kit CoA and sample input amount. Once the libraries were prepared, a Qubit™ 4 Fluorometer and a Qubit™ 1X dsDNA HS (High Sensitivity) Assay Kits was used for quantification. Final library size was assessed on the Labchip® GXII Touch™ HT using HT DNA X-Mark Chip and HT DNA NGS 3K Reagent Kit. All 48 libraries were pooled and sequenced on an Illumina® NextSeq 550 instrument using 2 x 150 paired-end reads. Data was subsampled to 1.2 million reads per sample and analyzed using Picard tools.

Table 1. xGen NGS chemistry components and other automation consumables.

Item	Description	Part Number	Supplier
Sterile filter tips	150 µL volume	111426	Revvity
HardShell PCR Plate	96-well, blue/clear	6008870	Revvity
StorePlate	96-well, V-bottom, 450 µL	6008290	Revvity
Polypropylene microplate	Deep-well, V-bottom, 2 mL	6008880	Revvity
Polypropylene reservoir plate	12 column, 21 mL	6008700	Revvity
Clear Universal Lid	Polystrene, robtic friendly	6000030	Revvity
xGen DNA Library Prep Kit EZ	96 rxn	10009821	IDT
xGen Deceleration Module	96 rxn	10009823	IDT
xGen Normalase UDI Primer Sets	UDI Primers Sets	10009796-10009799	IDT
Purification beads : • SPRIselect™ purification beads, or equivalent • Agencourt® AMPure® XP-PCR purification beads, or equivalent	Purification beads	B23317/B23318/B2331 A63880 or A63881	Beckman Coulter
Nuclease-Free Water	1 L 10 x 2 mL 300 mL	11-05-01-04 11-04-02-01 11-05-01-14	IDT
Absolute ethanol	200 proof	Varies	General lab supplier

Results

The 48 libraries generated using the xGen DNA Library Prep Kit EZ on the Revvity Sciclone® G3 NGSx workstation resulted in high-quality libraries of expected yields (Figure 4), with no detectable dimers (Figure 5), and high-quality sequencing results (Figure 6 and Figure 7).

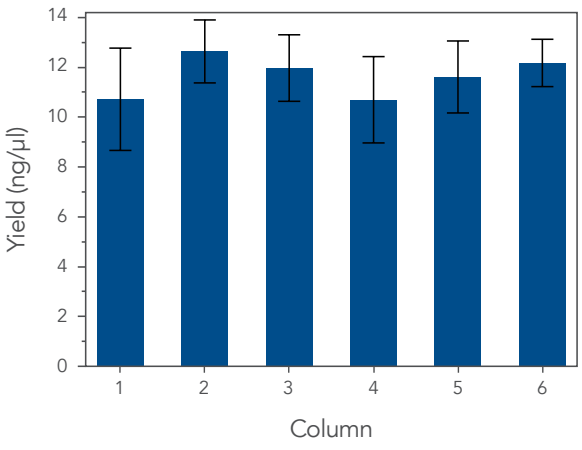

Figure 4. Automated library prep generated expected library yields across all plate columns. Column-wise quantification of 48 libraries obtained from a Qubit™ 4 Fluorometer. All library replicates prepared using the xGen DNA Library Prep Kit EZ and the Revvity Sciclone® G3 NGSx workstation generated yields > 6 ng/µl.

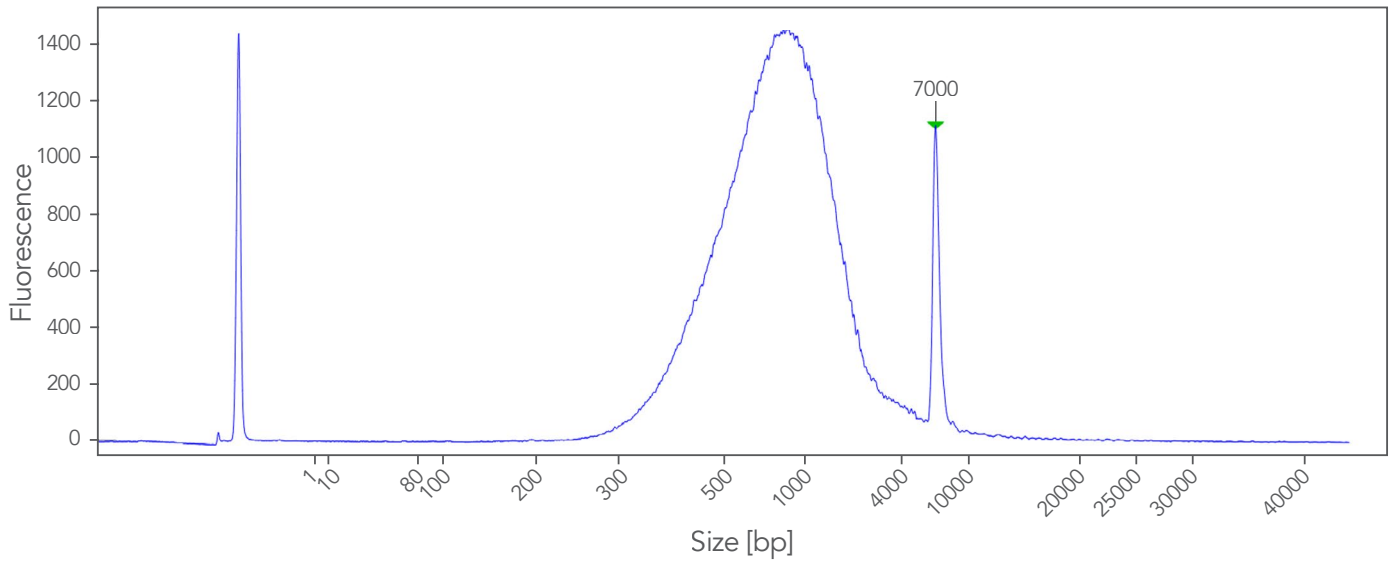

Figure 5. High-quality xGen DNA Library Prep EZ libraries are generated when automated on Revvity Sciclone. Representative LabChip trace of a single library generated on the Revvity Sciclone®. Library trace shows no detection of dimers and has a single, strong peak around the expected size (~600 bp).

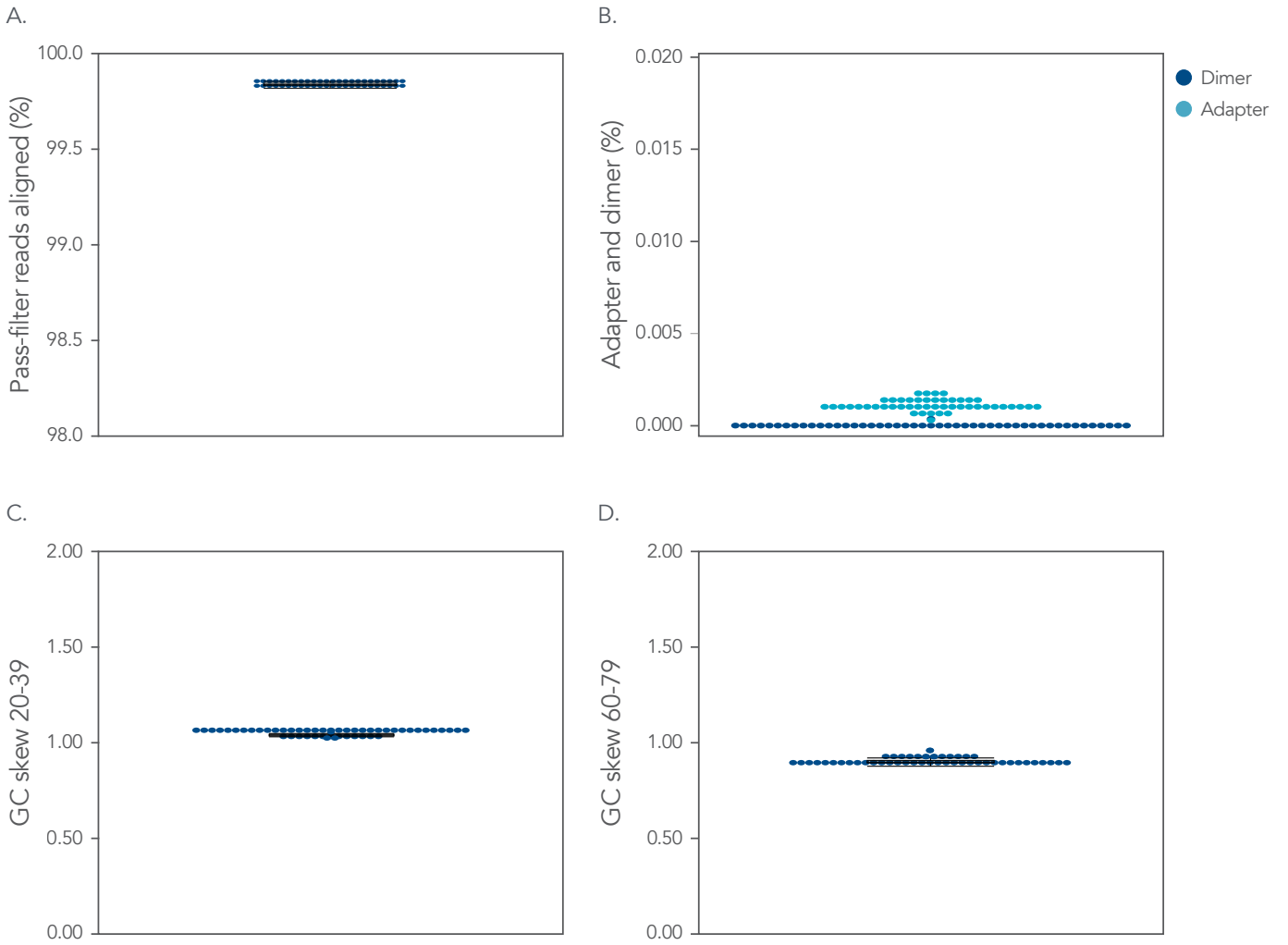

Figure 6. Sequenced libraries resulted in high alignment rates, low dimer and adapter rates, and minimal GC coverage bias. Quality sequencing data from automated libraries prepared using the xGen DNA Library Prep Kit EZ on the Revvity Sciclone® G3 NGSx workstation. Forty-eight replicate libraries were generated using 25 ng of gDNA resulting in (**A**) high reads aligned to the genome, (**B**) low dimer (dark blue) and

adapter (light blue) reads, and minimal GC bias normalized coverage over GC content ranging from 20–39 (**C**) and 60–79 (**D**) is close to 1.

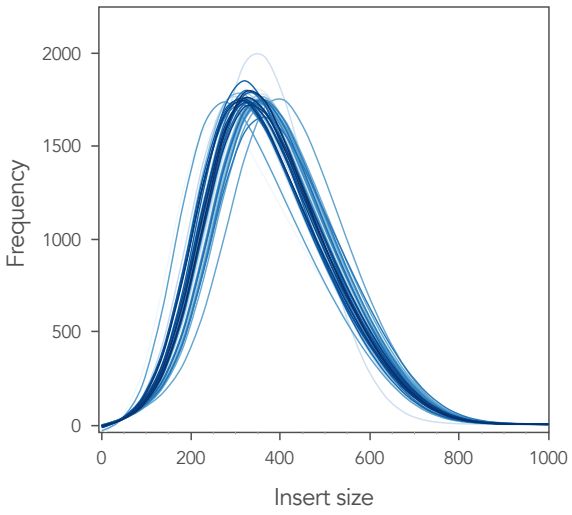

Figure 7.Enzymatic fragmentation is compatible with the Revvity Sciclone®. Histogram of insert size distribution for all 48 xGen EZ libraries generated on the Revvity Sciclone® G3 NGSx workstation. Each colored line represents a unique library. Range of mean insert size is 78 bp, with the minimum insert being 344 bp and the maximum being 422 bp.

Summary

Automated library preparation reduces hands-on time, increases efficiency and throughput, and can reduce pipetting errors. In this application note, the xGen DNA Library Prep Kit EZ workflow was automated on the Revvity Sciclone® G3 NGSx workstation. The libraries prepared via automation resulted in:

- Expected library yields across columns
- No detectable dimers
- High-quality sequencing reads with high alignment rates
- Minimal GC-sequencing bias

This automated library preparation solution offers a reliable, time and cost saving approach for generating libraries that are ready for sequencing on an Illumina® platform.

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For more information, go to: **www.idtdna.com/ContactUs**

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