Automation of xGen™ cfDNA & FFPE DNA Library Prep v2 MC Kit on Revvity Sciclone® G3 NGSx workstation

Abstract

Automated NGS library preparation can be a high-throughput, time and cost saving alternative to manual library preparation. This application note provides an overview of the automated workflow of the xGen cfDNA & FFPE DNA Library Prep v2 MC Kit on the Revvity Sciclone® G3 NGSx workstation. To show the value of this approach when generating sequencing libraries or preparing samples for hybridization capture, the quality of the automated NGS libraries was measured using key metrics and compared to DNA libraries generated manually. The automated libraries were as high-quality as the manually prepared libraries and generated total yields fit for downstream hybridization capture workflows.

Introduction

xGen cfDNA & FFPE DNA Library Prep v2 MC Kit produces Illumina® compatible next generation sequencing (NGS) libraries from degraded samples such as input from extracted cell-free DNA (cfDNA) and formalin fixed paraffin embedded (FFPE).The Revvity Sciclone® G3 NGSx liquid handling workstation is designed for high-throughput, rapid, and reliable NGS library construction that reduces overall operational cost, hands-on time, error rate, and sample variability thereby reducing the standard deviation and variance. A high conversion from degraded or damaged samples to sequencing ready libraries was achieved by automating the xGen cfDNA & FFPE DNA Library Prep v2 MC Kit workflow on the Sciclone® G3 NGSx workstation using 10 ng of input.

The automation of the xGen cfDNA & FFPE DNA Library Prep v2 MC Kit workflow reduces hand-on time and produces high-quality sequencing libraries (**Figures 1**, **2**). Combining the xGen cfDNA & FFPE DNA Library Prep v2 MC Kit with the high-throughput Sciclone® G3 NGSx workstation, enables users to load up to 96 DNA samples to prepare, amplify, and purify NGS libraries. This application note walks through the steps needed for automation of the xGen cfDNA & FFPE DNA Library Prep v2 MC Kit on the Sciclone® G3 NGSx workstation, and showcases the utility of this application when generating NGS libraries from cfDNA.

Fragmented input DNA

End repair

Input DNA blunting

Ligation 1

Single-stranded ligation of
Ligation 1 Adapter to 3′ ends of insert

Ligation 2

Ligation 2 Adapter primes gap filling across
the UMI followed by 5′ ligation

PCR

Amplification with xGen™ Unique Dual Index (UDI)
Primer Pairs

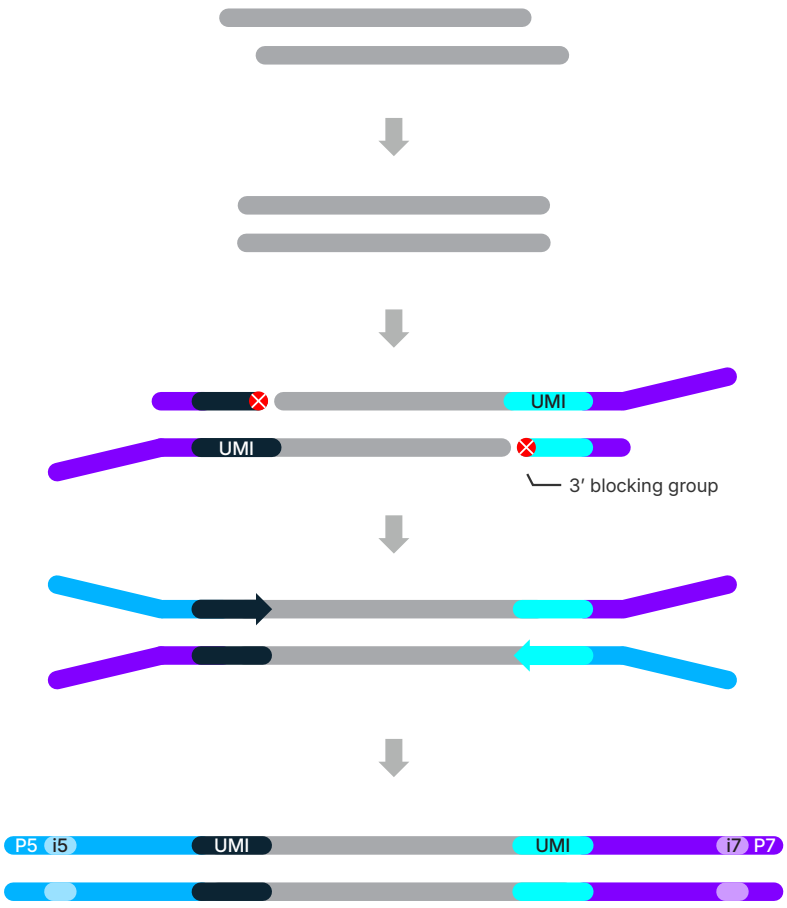


Figure 1. Chemistry workflow for the xGen cfDNA & FFPE DNA Library Prep v2 MC Kit.

Methods

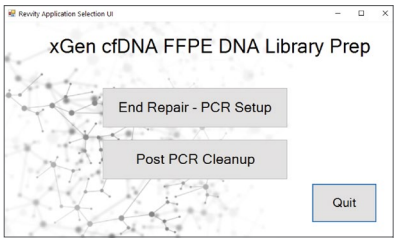
Automated DNA library preparation setup

Automated DNA library preparation when using the xGen cfDNA & FFPE DNA Library Prep v2 MC Kit with the Sciclone® G3 NGSx workstation allows for an input of up to 96 samples. Many of the steps for preparing a library using the xGen cfDNA & FFPE DNA Library Prep v2 MC Kit can be completed directly on the Sciclone® G3 NGSx workstation deck, minimizing the amount of hands-on time needed to generate an NGS library (**Figure 2**).

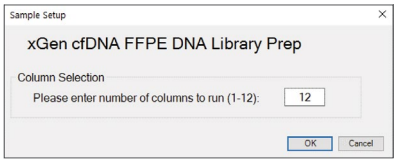
Figure 2. The time required to complete xGen cfDNA & FFPE DNA Library Prep v2 MC Kit workflow on the Sciclone® G3 NGSx workstation. Solid blue blocks represent on-deck incubations and white blocks represent the steps that require off-deck thermocycler incubations on the Sciclone® G3 NGSx workstation.

At the start of a run on the Sciclone® G3 NGSx workstation, the Sciclone® application prompts the user to select which step they want to start from; either End Repair – PCR Setup or Post PCR Cleanup (**Figure 3A**). A second window is triggered for the user to enter the total number of sample columns being run on the plate (**Figure 3B**). For the End Repair – PCR Setup selection, the user can select which column of the PCR Primer plate to start from (**Figure 3C**). At completion of the PCR-Setup application, the user is reminded with a pop window stating to seal and store the remainder of the PCR Primer plate.

A.



B.



C.

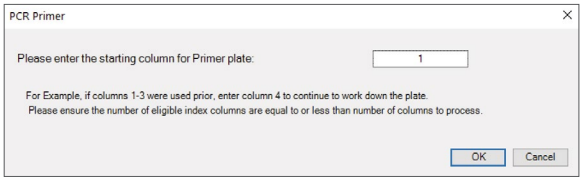


Figure 3. The Revvity Sciclone® G3 NGSx user interface. Select a (**A**) protocol step, (**B**) indicate the number of samples being used, and (**C**) choose a starting column for the PCR primer plate.

The Sciclone® application guides the user with images and text to ensure that the Sciclone® G3 NGSx workstation deck is properly setup at the start of a run (**Figure 4**). 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 5**). Once the workstation is set up according to instructions, the liquid handler proceeds with processing samples. Depending on the step in the protocol, the master mixes are either pre-broadcast to a clean plate and then aspirated and dispensed to the samples all at once or master mix is broadcasted directly to the sample plate. To ensure thorough mixing, samples are tip-mixed while shaking on thermoshaker position of the workstation. Incubations for End-repair and Ligation 1 remain on the on-deck CPAC location holding at the appropriate temperature. An off-deck thermocycler is required for Ligation 2 and PCR amplification (**Figure 2**).

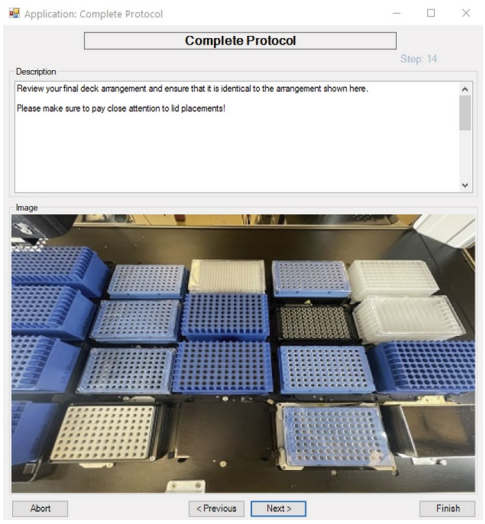


Figure 4. Deck layout to start the xGen cfDNA & FFPE DNA Library Prep v2 MC Kit application on Sciclone® G3 NGSx workstation.

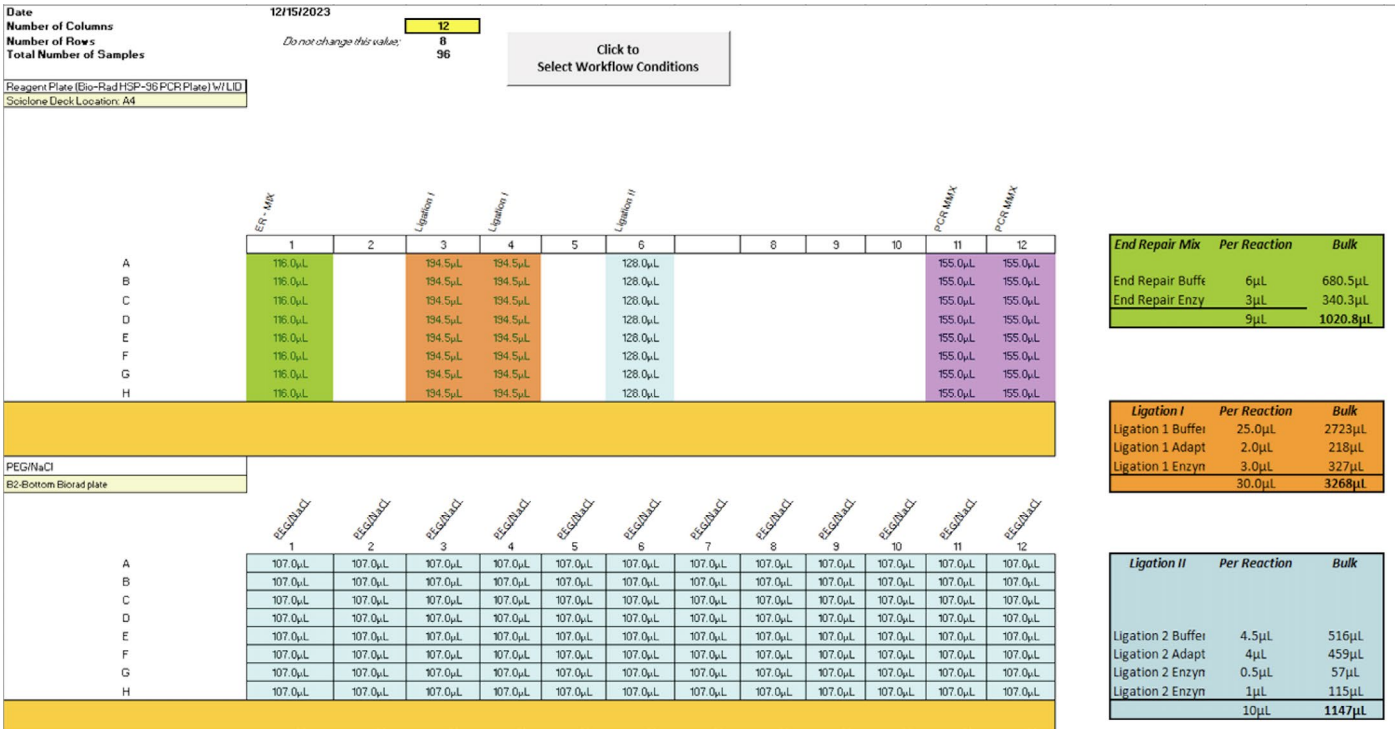


Figure 5. The Excel workbook for setting up the xGen cfDNA & FFPE DNA Library Prep v2 MC Kit application on Sciclone® G3 NGSx workstation.

Automation of the xGen cfDNA & FFPE DNA Library Prep v2 MC Kit workflow

A full plate (*n* = 96) was processed during one automation run using the xGen cfDNA & FFPE DNA Library Prep v2 MC Kit on the Sciclone® G3 NGSx workstation. All consumables used are outlined in **Table 1**. On the 96-well plate, a checkered pattern was used for sample input versus a no library template control (NTC) (**Table 2**). NTC wells (*n* = 52) used low EDTA TE buffer with no DNA template. Ten nanograms of DNA from Horizon FFPE 100% Multiplex I wild type (WT) was used as input for the xGen cfDNA & FFPE DNA Library Prep v2 MC Kit (*n* = 44). Twelve manually prepared NGS libraries were also generated using 10 ng of NA12878 Coriell to determine the reliability of the library prepared using the Sciclone® G3 NGSx workstation.

After library preparation, quantification was performed with the Qubit™ 4 Fluorometer (Thermo Fisher Scientific) and a 1X High Sense DNA kit (Thermo Fisher Scientific). Final library size was assessed using LabChip GXII Touch HT (Revvity) using HT DNA X-Mark chip and HT DNA NGS 3K Reagent Kit (Revvity). Libraries were sequenced on NextSeq 550 (Illumina) using 2 × 151 bp reads.

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

Item	Description	Catalog number	Supplier
xGen cfDNA & FFPE DNA Library Prep v2 MC Kit	xGen cfDNA & FFPE DNA Library Prep v2 MC, 16 rxn xGen cfDNA & FFPE DNA Library Prep v2 MC, 96 rxn	10010206 10010207	IDT
xGen UDI Primers	xGen UDI Primers, 16 rxn xGen UDI Primers Plate 1, 8nt	10005975 10005922	IDT
Sterile filter tips	150 µL		Revvity
HardShell PCR Plate	96-well, blue/clear		Revvity
Polypropylene low-volume microplate	384-well, 35 µL		Revvity
StorPlate-96V	Deep-well, V-bottom, 2 mL		Revvity
Polypropylene 12 column reservoir plate	21 mL		Revvity
Clear Universal Lid	Polystyrene, robotic friendly		Revvity
IDTE pH 8.0	1X TE Solution, 300 mL		IDT
Nuclease Free Water	1 L 10 × 2 mL 300 mL	11-05-01-04 11-04-02-01 11-05-01-14	IDT
Buffer EB	10 mM Tris-HCL, pH 8.5, 250 mL	19086	QIAGEN
Absolute Ethanol	200 proof	Varies	General lab supplier
Agencourt AMPure XP	PCR purification beads 5 mL 60 mL	 A63880 A63881	Beckman Coulter
High Sensitivity DNA Kit	Tapestation 4200 Reagents Tapestation 4200 D1000 ScreenTape	5067-4626 5067-5584	Agilent
Qubit dsDNA HS Assay Kit	Qubit dsDNA HS Assay Kit	032851 032854	Thermo Fisher Scientific

Table 2. Plate map of the xGen cfDNA & FFPE DNA Library Prep v2 MC Kit workflow completed on Revvity Sciclone® G3 NGSx workstation.

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

Results

The 44 libraries generated using the xGen cfDNA & FFPE DNA Library Prep v2 MC Kit on the Sciclone® G3 NGSx workstation had nearly zero dimers (**Figure 6** & **7**), generated high total yields (**Figure 8**), and resulted in NGS quality metrics that were on par with the manually generated NGS libraries (**Figure 7**).

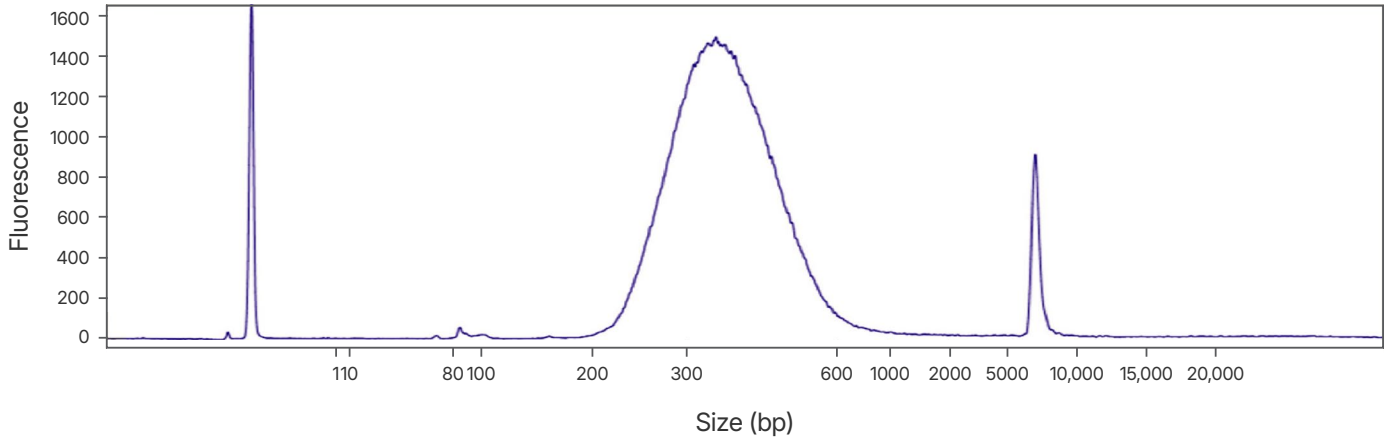


Figure 6. Automated libraries have nearly zero dimers present. A representative quantitation trace from well E2, showing the lack of dimers present after library preparation. All wells were analyzed on a LabChip GXII Touch HT instrument (Revvity).

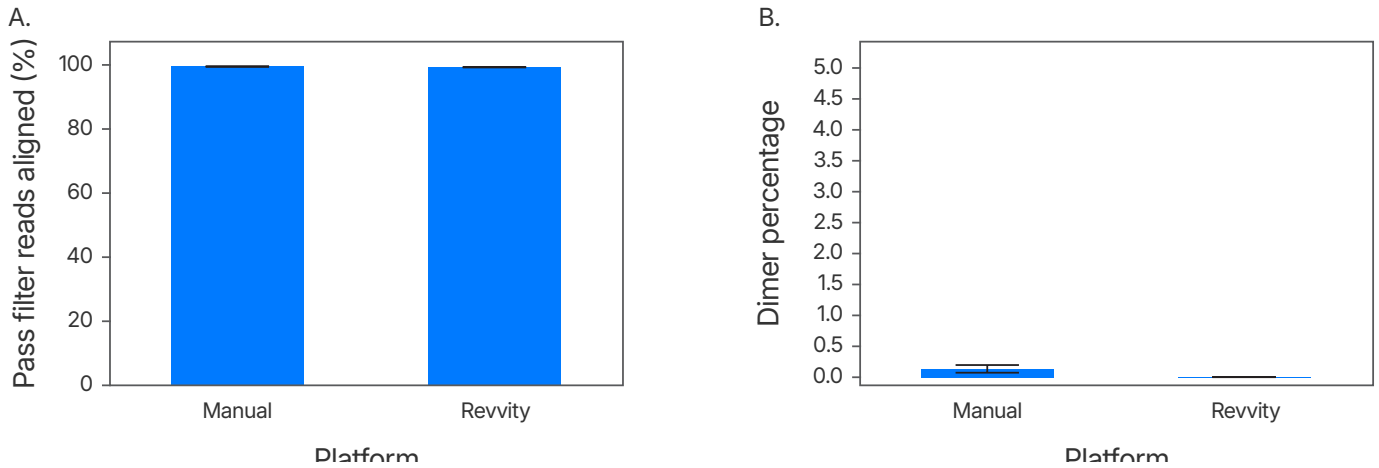


Figure 7. Automated libraries resulted in high-quality NGS sequencing libraries. Libraries prepared on the Sciclone® G3 NGSx workstation from 10 ng of DNA from Horizon FFPE 100% Multiplex I WT input resulted in sequencing data of similar quality to libraries prepared manually from 10 ng of NA12878 Coriell (*n* = 12). Automated libraries resulted in equivalent NGS metrics for (**A**) percent passing filter reads aligned and (**B**) dimer percentage. Picard Alignment Summary Metrics were used for analysis (Broad Institute).

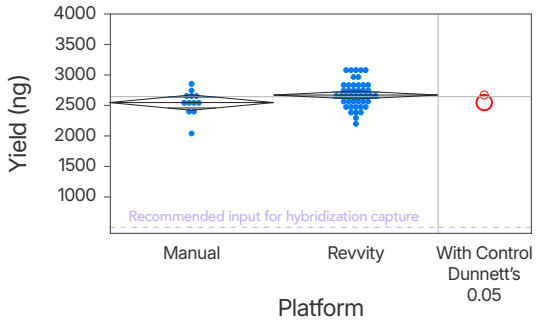


Figure 8. Automated libraries result in high yields for input into a hybridization capture workflow. All libraries (*n* = 44) generated on the Sciclone® G3 NGSx workstation using the xGen cfDNA & FFPE DNA Library Prep v2 MC Kit yield well above the recommended input for hybridization capture (500 ng), and similar library yields of the manually prepared libraries (*n* = 12).

Conclusion

These results illustrate the utility of automating the xGen cfDNA & FFPE DNA Library Prep v2 MC Kit on the Sciclone® G3 NGSx liquid handling workstation to generate high-quality NGS sequencing libraries or input for hybridization capture. The automation of cfDNA library preparation resulted in high-quality libraries with nearly zero dimers. When compared to manually prepared DNA libraries, the automated libraries were of the same quality but were prepared with minimal hands-on time and in a high-throughput fashion. Additionally, this workflow generated total yields high enough for hybridization capture input. This automation workflow offers a reliable, time and cost saving approach for generating high-quality NGS libraries in a high-throughput manner.

Automation of xGen™ cfDNA & FFPE Library Prep v2 MC Kit on Revvity Sciclone® G3 NGSx workstation

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