

# Automated NGS Library Normalization using IDT xGen™ Pre-Hybridization Capture Normalase Module™ for target enrichment on Revvity Sciclone™ G3 NGSx workstation

## Abstract

Hybridization-based target enrichment allows users to increase sequencing efficiency by focusing on target of interest. Increases in sequencer output have driven demand for high-throughput workflows that generate and pool next-generation sequencing (NGS) libraries upstream of capture, while maintaining balanced sample representation. This app note details an automated workflow that integrates the xGen DNA Library Prep EZ Kit with the xGen Pre-Hybridization Capture Normalase Module to produce equimolar library pools ready for xGen Hyb Capture. Data from the target-enriched libraries show expected yields, uniform read distribution, and high-quality capture metrics.

## Introduction

The xGen Pre-Hybridization Capture Normalase Module provides a fast, automation-friendly method for generating equimolar library pools upstream of hybridization capture. Standard normalization methods require individual library quantification and pooling of variable volumes, which can significantly reduce turnaround time and sample read uniformity. The xGen Pre-Hybridization Capture Normalase Module uses enzymatic normalization and equal volume pooling, improving workflow efficiency and minimizing user error.

The xGen Pre-Hybridization Capture Normalase Module workflow integrates seamlessly with standard DNA and RNA library preparation and multiplex hybridization capture protocols, improving turnaround time and loading precision. Indexing and Normalase library conditioning can occur in a single reaction, meaning a second PCR reaction may not be required.

Previous work demonstrated the automation of IDT xGen DNA Library Prep EZ Kit on the Revvity Sciclone G3 NGSx workstation (Figure 1a). Incorporating this workflow with the xGen Pre-Hybridization Capture Normalase Module provides even greater efficiencies for applications requiring target enrichment.



**Figure 1. Combining the precision of the Revvity Sciclone G3 NGSx workstation with the efficient xGen Pre-Hybridization Capture Normalase Module and xGen DNA Library Prep EZ Kit.** An image of the Revvity Sciclone G3 NGSx workstation (A) and the xGen DNA Library Prep EZ Kit with the xGen Pre-Hybridization Capture Normalase Module workflow with safe stop points noted (B)

## Methods

### Automated DNA library preparation setup

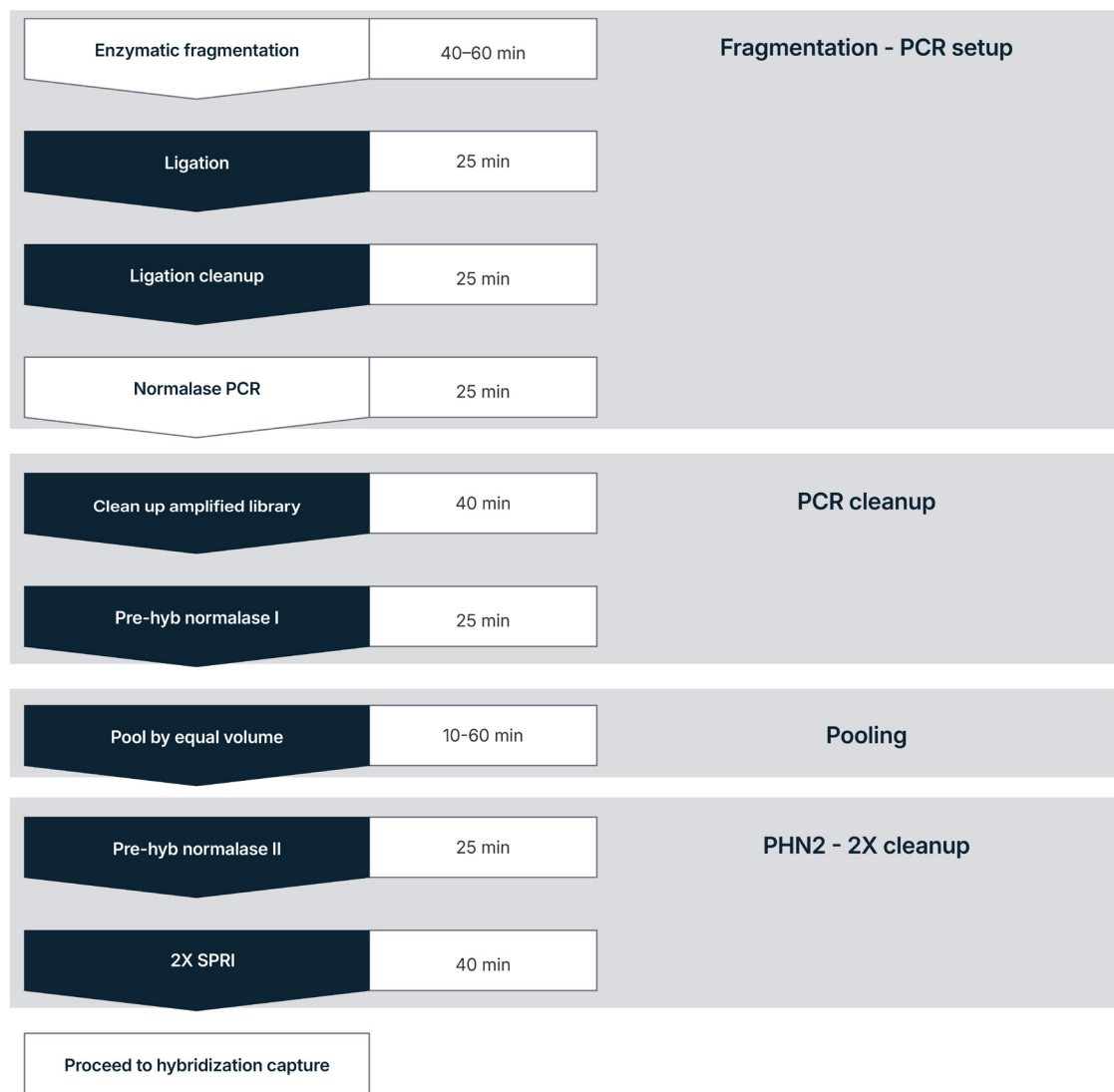
The xGen DNA Library Prep EZ Kit with the xGen Pre-Hybridization Capture Normalase Module automated workflow requires four separate applications:

Application 1: 1\_xGen DNA EZ\_Library Prep + PHN

Application 2: 2\_xGen DNA EZ\_PHN Cleanup + PHN1

Application 3: 3\_xGen\_PHN Pooling

Application 4: 4\_xGen\_PHN2 + 2X SPRI



**Figure 2. xGen EZ DNA Library Prep with xGen Pre-Hyb Normalase Module along with 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 Sciclone G3 NGSx workstation.

**Application 1: 1\_xGen DNA EZ\_Library Prep + PHN:** Performs sample fragmentation (optional), enzymatic preparation, adapter ligation and cleanup, PCR amplification

At the start of Application 1, users are prompted to select from two sample processing options (Figure 2A).

- **Complete Protocol:** Protocol starts with enzymatic fragmentation
- **Begin at Adapter Ligation and Cleanup:** Protocol starts with adapter ligation (use for pre-fragmentated samples)

After the user selects an option, a second user window will appear. User is prompted to enter the following:

1. **Number of columns:** Total number of sample columns (each column can accommodate 8 samples)
2. Which sample column to start aspirating from for the barcode plate
3. **Barcode Type:** click field to select adapter type

Based on the number of columns being run, the workbook provides Master Mix calculations and reagent volumes for each consumable on the right side of the screen (Figure 2B). Note: the PCR Master Mix calculation will include Reagent R7/R6 (primer conditioning reagent) if the user did not select an xGen Normalase barcode type. For xGen Normalase UDI/CDI primers, reagents R7/R6 will not be included in the PCR Master Mix.

Once the workstation is set up according to the guided user screens (Figure 2C) and workbook, the liquid handler proceeds with processing samples. Throughout the protocol, the Master Mixes are either pre-broadcast to a clean plate and then simultaneously added to all samples using all 12 columns of the 96-channel head, 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.

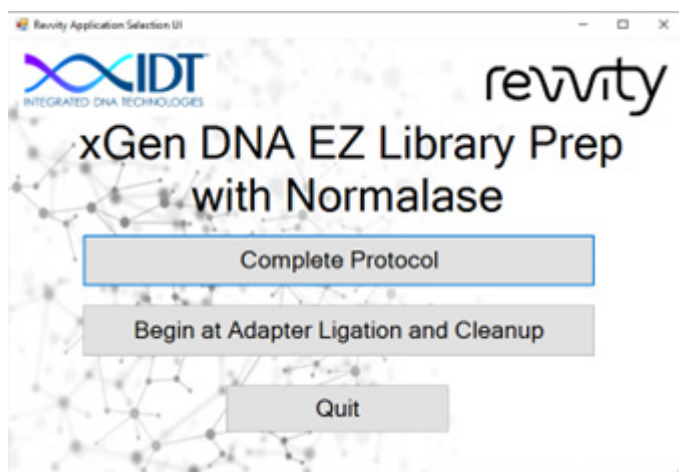


Figure 2A. User options for library prep setup.

Figure 2B. Workbook for guiding user to setup reagent plates.

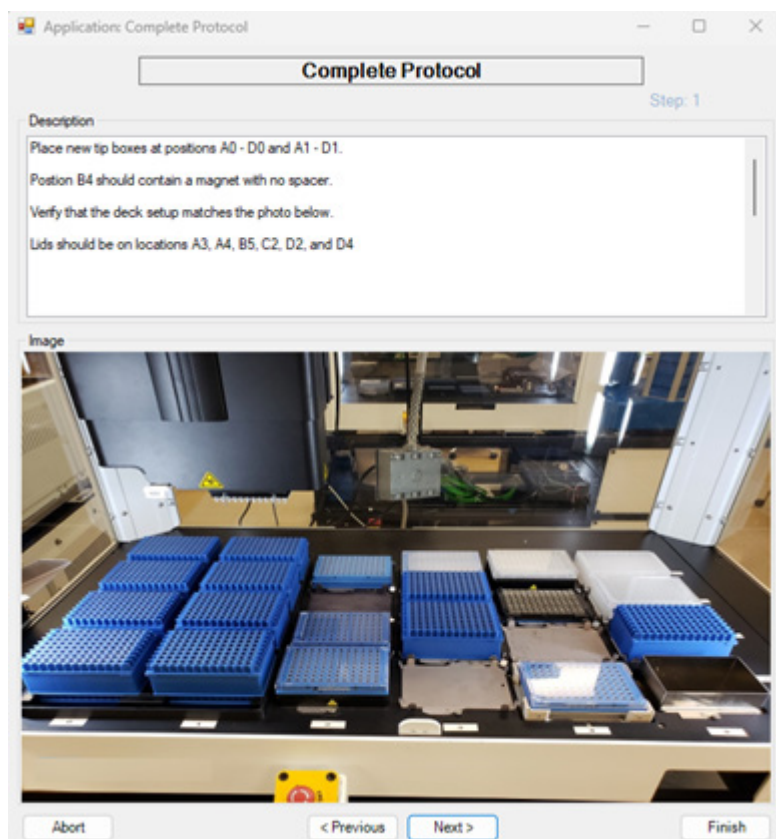


Figure 2C. Final deck layout for xGen DNA Library Prep EZ Kit with the xGen Pre-Hyb Normalase Module.

Application 1 requires two user interactions – moving the sample reaction plate from the workstation to an off deck thermal cycler for incubations during **enzymatic fragmentation** and **PCR**. At the start of Application 2, users are prompted to select from two sample processing options (Figure 3).

- **PCR Cleanup with PHN1 option:** Performs post-PCR cleanup followed by PHN1 option (includes option to pause after cleanup if a sample QC check is required prior to PHN1 step)
- **Stand-alone Pre-Hyb Normalase I:** Standalone PHN step 1 without cleanup

During Pre-Hyb Normalase I, PHN1 Master Mix is added and the 15 minute incubation is completed on deck while the plate is lidded and held at CPAC set to 30° C.

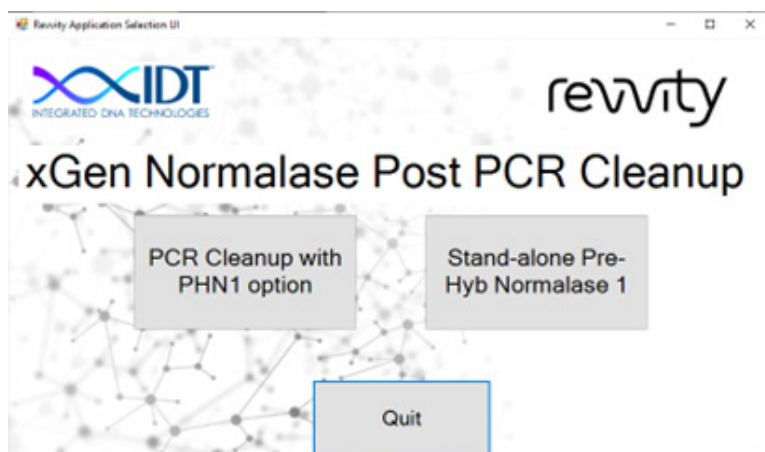


Figure 3. User starting options for Normalase PCR cleanup and Pre-Hyb Normalase I step.

**Application 3:** 3\_xGen\_PHN Pooling - Equal volume library pooling

In the workbook, specify the location of each starting library (source well) and desired pool destination (destination well) in the yellow columns (Figure 4). The saved workbook creates a worklist for the Maestro pooling application to read volumes and wells for each pre-hyb pool. Libraries are pooled to wells of a 96 well plate PCR plate so they can be further processed by the Sciclone NGSx workstation in the fourth application. Please note that each pool should contain no more than 12 libraries, and the final library pool volume should not exceed 180 µL.

Figure 4. In the pooling tab of the workbook, the user should adjust the areas highlighted in yellow specific to each setup on the Sciclone NGSx.

**Application 4:** 4\_xGen\_PHN2 + 2X SPRI – Pre-Hyb Normalase step II and 2x SPRI cleanup

Enter the total number of pools, and the number of libraries per pool (Figure 5).

After the on-deck pre-hyb II reaction incubation, Cot-1 DNA is added to the samples prior to 2X bead cleanup. Once the cleanup is complete, the samples are ready for xGen Universal Blocker and dry down prior to xGen Hybridization Capture.

Figure 5. User starting options for Pre-Hyb Normalase II and final cleanup step.

## Experimental Design

Normalized pools (n = 5) were generated using the xGen DNA Library Prep EZ Kit with the xGen Pre-Hybridization Capture Normalase Module automated workflow on the Sciclone G3 NGSx workstation using 25 ng of Promega human gDNA in Low EDTA following the published protocols (Table 1). Selection was for 100 ng input into manual hybridization capture with the xGen Acute Myeloid Leukemia (AML) Cancer Hybridization Panel (1.19 Mb panel). All libraries were sequenced on an Illumina® MiSeq™ sequencer using 2×150 paired-end reads. Data was subsampled to 4 million reads per sample and analyzed using Picard tools.

The following normalization condition was followed:

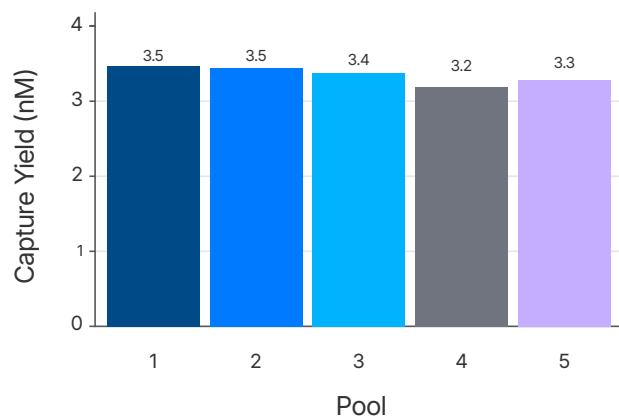
- 5 normalized pools, 12 plex per pool, using the xGen Pre-Hybridization Capture Normalase Module script on the Revvity Sciclone G3 NGSx workstation. Selection with Y2 was for 100 ng input into hybridization capture.

Table 1. xGen NGS chemistry components and other automation consumables

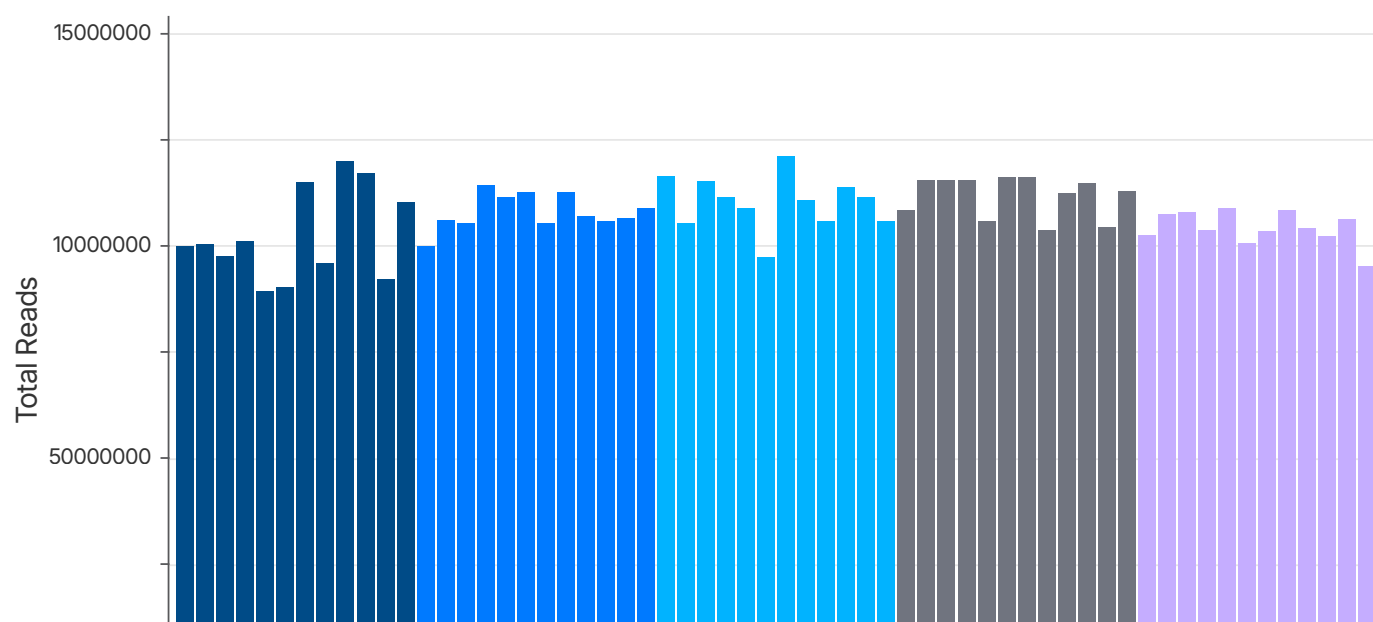
Item	Description	Part Number	Supplier
Pre-sterilized 150 µL filter tips	150 µL	6070441	Revvity
Pre-sterilized 80 µL filter tips	80 µL	6070341	Revvity
HardShell PCR Plate	96-well blue/clear	6008870	Revvity
StorePlate	96-well, V-bottom, 450 µL	6008290	Revvity
Polypropylene deep-well V-bottom 96-well storage plate	96-well, Deep-well, V-bottom, 2 mL	6008880	Revvity
Polypropylene-12 column reservoir plate	12-column, Deep-well, 21 mL	6008700	Revvity
Clear Universal Lid	Polystyrene, robotic friendly	6000030	Revvity
xGen DNA Library Prep EZ Kit	96 rxn	10009821	IDT
xGen Pre-Hybridization Normalase Module	96 rxn	10017913	IDT
Purification beads: • SPRIselect purification beads or equivalent • AMPure XP PCR purification beads, or equivalent	Purification beads	B23317/B23318/B2331 A63880 or A63881	Beckman Coulter Life Sciences
Nuclease-Free Water	1 L	11-05-01-04	IDT
	10 × 2 mL	11-04-02-01	
	300 mL	11-05-01-14	
Absolute ethanol	200 proof	Varies	General lab supplier

## Results

The 5 pools of 12 normalized libraries generated using the xGen DNA Library Prep EZ Kit with the xGen Pre-Hybridization Capture Normalase Module automated workflow on the Sciclone G3 NGSx workstation resulted in high-quality libraries of expected yields (Figure 7), with balanced libraries and high-quality sequencing results (Figure 8).



**Figure 7. Post-capture library concentrations (nM).** Each capture pool contained 12 samples and reflected the expected yield.



**Figure 8. Sequencing results for individual libraries show balanced sample distribution and high-quality capture performance.** Per-sample sequencing reads show balanced sample representation (A) and consistent flanked on-target rates demonstrate uniform capture performance (B).

## Summary

Automation of the xGen DNA Library Prep EZ Kit with the xGen Pre-Hybridization Capture Normalase Module reduces hands-on time, increases efficiency and throughput, and produces balanced libraries for hybridization capture. The libraries normalized via automation resulted in:

- Expected yields
- Balanced libraries
- High quality sequencing reads with high alignment rates

This automated normalization solution delivers a rapid, cost-effective method for generating normalized libraries for hybridization capture and Illumina sequencing.

# Automation of IDT xGen™ Pre-Hybridization Capture Normalase™ Module on the Revvity Sciclone™ G3 NGSx workstation

For more information, go to: [idtdna.com/ContactUs](https://idtdna.com/ContactUs)

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