

NGS Library Preparation



Sciclone® G3 NGSx Workstation

Automation of the IDT® xGen® Lockdown® Panels on the Sciclone® G3 NGS Workstation

Introduction

Integrated DNA Technologies (IDT) xGen® Lockdown® panels are predesigned

enrichment panels for targeted next generation sequencing. These inventoried panels consist of individually synthesized and quality controlled xGen® Lockdown® probes that have been internally qualified to provide the highest level of performance. The automation of the hybrid capture workflow on the Perkin Elmer® Sciclone® G3 NGS workstation ensures accuracy, consistency, and decreased variability. The automated method outlined in this document was developed by IDT and qualified with sequencing of panels of various sizes. This application note describes a step-by-step detailed guide for the workflow.

Methods

The protocol for hybridization capture with xGen® Lockdown® probes was automated and optimized for a PerkinElmer Sciclone G3 NGS workstation. Libraries were prepared using the KAPA® Hyper Prep Kit with 1 µg of input DNA sheared to an average size of 330 bp. The libraries were quantified using a fluorescence based quantification method (ThermoFisher® Qubit® fluorometer), and library DNA integrity and average fragment size were determined using an Agilent® TapeStation® instrument. PCR thermocycling was performed offline using a BioRad® CT1000™ thermal cycler. Samples were dried using a Savant® DNA 120 SpeedVac™ concentrator. All sequencing runs were performed on an Illumina® NextSeq® system, using either high output or mid output reagents and 2x150 read lengths. Five modules were developed to encompass the workflow from library normalization through post-capture PCR cleanup. The names of these scripts are:

- IDT® xGen® Pre-Capture Normalization and Pooling
- IDT® xGen® Blocking Oligos
- IDT® xGen® Hyb Setup
- IDT® Target Capture
- IDT® xGen® Post-PCR SPRI

Workflow

IDT® xGen® Pre-Capture Normalization and Pooling Module

The steps below outline how to start the protocol. The tested concentration of library for this protocol is from 50-100 ng/µl. This module will transfer 500 ng of DNA to a fresh plate and normalize the volume across the plate. The final volume is a variable and can be modified by the user. The module allows for sample multiplexing in a single capture; however, users may need to optimize the number of samples multiplexed based on panel size and experimental goals. The completion of this module takes approximately 2 minutes per sample.

Step 1: Turn on the Sciclone G3 NGS workstation and associated computer and the Inheco® controller. Open the respective module Microsoft® Excel® workbook, and fill-out the normalization spreadsheet with sample IDs, concentrations, source and destination wells. **Save the workbook.** Maestro software will read the values from the workbook directly.

Step 2: Launch the Sciclone software, open the normalization script, and press Play. The software will search for the workbook from step 1 and prompt for confirmation.

Step 3: Once the module has completed, the plate can be safely stored at this stage or you can move forward to the next module.

Before Starting

The automated protocol uses filtered 150 µl tips. The plate types used are:

- Hard-Shell PCR Plate, 96-well
- Polypropylene deep-well reservoir
- Polypropylene plate, 96 – 450 µl, v-bottom
- Polypropylene deep-well plate – 2 mL square well, v-bottom
- Universal Lid

The reagents used for with the Sciclone scripts are:

- IDT® xGen® Lockdown® Reagents kit (Cat #1072280 or #1072281)
- IDT® 4 x 1 xGen® Lockdown® Hybridization Buffer (Cat #1072987)
- ThermoFisher® Dynabeads™ M-270 Streptavidin (Cat #65305 or #65306)
- ThermoFisher® Human Cot-1 DNA™ (Cat #15279011)
- Qiagen® Vapor-Lock™ PCR encapsulation barrier (Cat #981611)
- KAPA® HiFi HotStart ReadyMix (2x) (Cat #KK2601 or #KK2602)
- Agencourt® AMPure® XP beads (Beckman Coulter®)
- IDT® xGen® Lockdown® Panel
- IDT® xGen® Blocking Oligos
- PCR primers for post-capture amplification
- Ethanol

IDT® xGen® Blocking Oligos Module

This module adds a solution of blockers to the samples. Once the module has finished, the plate will be ready to be dried down in a SpeedVac™ concentrator. The module will take approximately 1 minute per column of samples.

Step 1: Turn on the Sciclone G3 NGS workstation and associated computer and the Inheco® controller. Open the respective module Microsoft® Excel® workbook, and fill-out the spreadsheet indicating the number of columns where the mastermix will be added. **Save the workbook.** Maestro software will read the values from the workbook directly.

Blocker Master Mix	2 Samples	8 Samples
Con-2 DNA	2	16
xGen Universal Blockers	2	16
Total Volume	7	32

Figure 1. Workbook for Addition of Blockers Module

Step 2: Prepare the blocker mix based on the calculated volumes from the workbook.

Step 3: Start the module by pressing play on the script. A window will appear outlining the number of columns that will be processed, as indicated in the workbook.

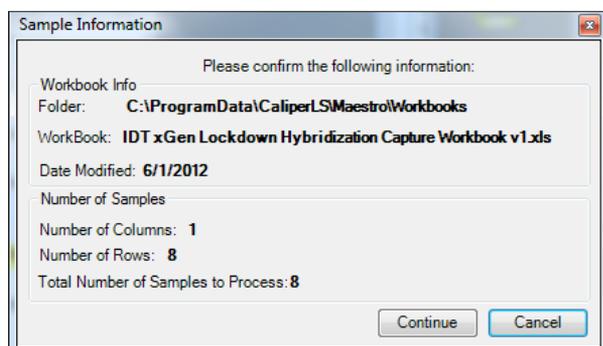


Figure 2. Sample information window outlines number of samples to be processed

Step 4: See Figure 3 for an example of a correct deck layout.

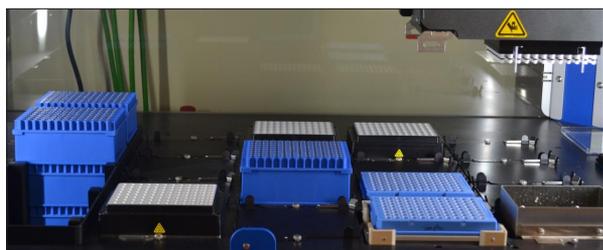


Figure 3. Deck layout for the Addition of Blockers Module

Step 5: Remove the plate from the deck and dry down the DNA/blockers. After drying down, you can move forward to the next module. You can also store the plate overnight at room temperature or in the freezer for a longer time period.

IDT® xGen® Hyb Setup

This module adds the hybridization solution, including the xGen® Lockdown® probe pool. Once the module has finished, the plate will be ready for the hybridization incubation. The module takes approximately 5 minutes to complete for 2 columns of samples.

Step 1: Turn on the Sciclone G3 NGS workstation and associated computer and the Inheco® controller. Open the respective module Microsoft® Excel® workbook, and fill-out the spreadsheet indicating the number of columns where the mastermix will be added. **Save the workbook.** Maestro software will read the values from the workbook directly.

Step 2: Prepare the hybridization mix based on the calculated volumes from the workbook (Figure 4).

Hyb Buffer and Probes	2 Samples	16 Samples
Con-2 DNA	2	16
xGen Lockdown Probe Pool	2	16
Total Volume	4	32

Figure 4. Workbook for the Hybridization Setup Module

Step 3: See Figure 5 for an example of the appropriate deck layout

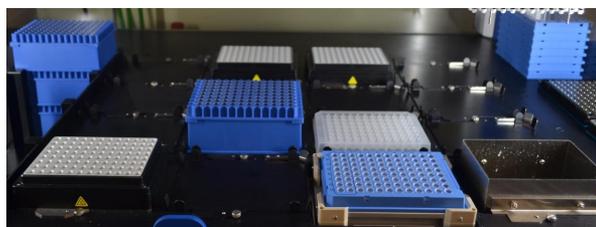


Figure 5. Deck layout for the Hybridization Setup Module

Step 4: Remove the plate from the deck. Spin down the plate. Incubate in a thermal cycler using the following program with a lid set at 100°C: 95°C – 30s, 65°C – 4hrs, 65°C – hold.

IDT® Target Capture

This module will wash streptavidin beads bind the captured xGen® Lockdown® probe-target duplexes onto the beads, and wash the beads to remove non-specific DNA. Finally, the beads are eluted in water and added to the PCR mastermix. Once the module has finalized, the plate will be ready to be loaded onto an instrument for PCR amplification. The module takes approximately 30 minutes from start to the first pause for addition of samples, 1 hour and 45 minutes from the addition of samples until the second pause for the addition of PCR mastermix, and 10 minutes from the addition of PCR mastermix until completion of the module for a plate of 96 samples.

Step 1: Turn on the Sciclone G3 NGS workstation and associated computer and the Inheco® controller. Open the respective module Microsoft® Excel® workbook, and fill-out the spreadsheet indicating the number of columns where the samples will be processed. **Save to workbook.** Maestro software will read the values from the workbook directly.

Step 2: Prepare the plates with the diluted buffers and the beads, based on the calculated volumes from the workbook. Do not prepare the PCR master mix.

Step 3: Load the plates as indicated in Figure 6. Start the protocol with 30 minutes remaining on the 65°C – 4 hour incubation.

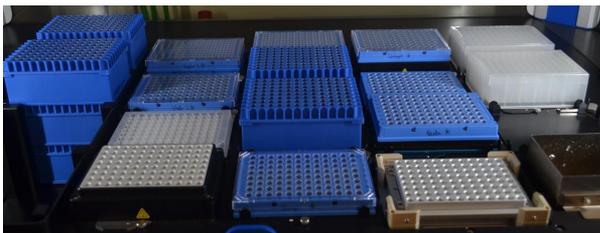


Figure 6. Deck layout for the Target Capture Module

Step 4: The first half of the module takes approximately 30 minutes. It will pause and ask the user to load the plate of samples and the plate with Vapor-Lock PCR encapsulation barrier. Ensure that the seal has been removed from the sample plate. *Note that the reagents on the deck are at a safe pausing point at this junction. If there is a delay between the completion of the bead wash and placing the plate of samples on the deck, results will not be impacted.*

Step 5: The module will bind the beads to the sample and wash the beads. Note that there will be residual beads left in the plate containing streptavidin beads and will not impact results. The script will take 1 hour and 45 minutes until the next pause point. With approximately 20 minutes remaining in the script, prepare the PCR master mix as outlined in the workbook.

Step 6: Once the beads with captured DNA have been washed and have been eluted in water, the instrument will pause with a prompt to load the plate of PCR master mix and a fresh plate. Note that the "Wash 1" buffer plate must be removed before loading the PCR master mix plate, as seen in Figure 7. Click OK to continue the protocol. *Note that the reagents on the deck are at*

a safe pausing point at this junction. If there is a delay between the completion of the target capture wash and placing the PCR master mix plate on the deck, results will not be impacted.



Figure 7. Deck layout after the second pause in the module, when the PCR mastermix is added to the deck

Step 7: At the end of the module, remove the plate from the deck. Seal and spin down the plate briefly. Begin the PCR program, as outlined in the protocol on IDT's website. *Note that the eluted beads have been transferred to the fresh plate on the deck. Residual beads may remain in the sample plate and were not found to impact results.*

IDT® xGen® Post-PCR SPRI Module

The steps described below detail the final cleanup of the PCR reaction using 1.5x AMPure® XP bead volume. Once completed, the user can store the samples or can move forward with qPCR quantification of the DNA. The module takes approximately 10 minutes to complete.

Step 1: Turn on the Sciclone G3 NGS workstation and associated computer and the Inheco® controller. Open the respective module Microsoft® Excel® workbook, and fill-out the spreadsheet indicating the number of columns where the samples will be cleaned up. **Save the workbook.** Maestro software will read the values from the workbook directly.

Step 2: Set up the deck as indicated in Figure 8. Start the Bead Cleanup Module. Upon starting, the system will aspirate the supernatant from the Dynabeads™ M-270 streptavidin from the PCR reaction. The system will pause with a prompt to remove the 96-well PCR plate. Once the plate has been removed, click OK to continue the protocol.

Step 3: Remove the plate from the deck and store.



Figure 8. Deck layout for SPRI cleanup module

Sequencing Results & Analysis

Three runs were performed on the Sciclone G3 NGS workstation with 8 samples each, and were captured using the xGen® Exome Research Panel v1.0 (IDT). xGen® Universal Blockers—TS Mix (IDT) were used, together with Human Cot-1 DNA™-Fluorometric QC (ThermoFisher). The samples were sequenced and were down-sampled to 50 million reads per exome. Illumina® NextSeq® sequencing data was aligned with BWA-MEM (H. Li) to human reference hg19. Coverage statistics were compiled with BedTools (A. Quinlan et al.).

The data shown highlights the coverage and the flanked on-target rate for 8 libraries captured in triplicate runs using the Sciclone module for xGen® Lockdown® probes. The data highlights the uniformity between samples, as well as the consistency between runs. Similar results were observed for small and mid-sized panels.

