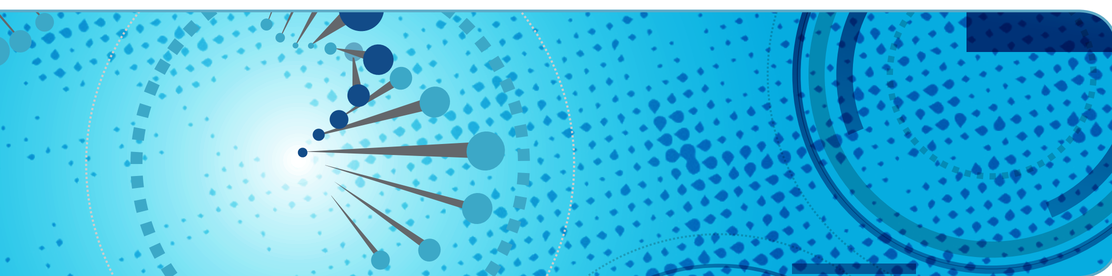
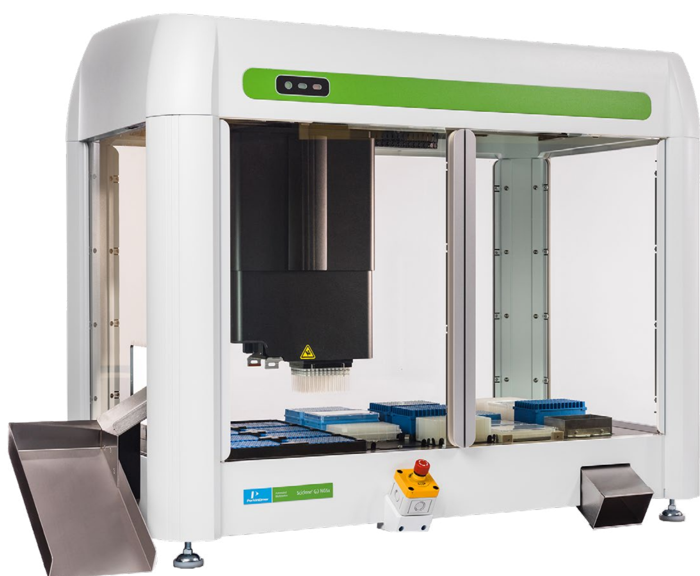
# Automation of xGen hybridization capture on the Sciclone® G3 NGS Workstation





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# Revision history

| Document version | Date released | Description of changes |
| --- | --- | --- |
| 2 | March 2019 | Added Appendix to describe combining panels |
| 1 | March 2018 | Initial release |

# Table of contents

Revision history 2

Introduction 4

Methods 4

Before you start 5

Workflow 6

Automation protocol 7

IDT xGen Normalization and Pooling module 7

IDT xGen Blocking Setup module 8

IDT xGen Hybridization Setup module 10

IDT xGen Target Capture module 11

IDT xGen Post-Capture PCR SPRI module 14

Sequencing results and analysis 15

References 16

Appendix 17

Combining xGen Lockdown Panels and Probes 17

# Introduction

IDT's predesigned and custom enrichment panels for targeted next generation sequencing (NGS) consist of individually synthesized and quality controlled xGen Lockdown Probes that have been internally qualified to provide the highest level of performance. The xGen Hybridization and Wash Kit comprises buffers, Cot DNA, and Streptavidin beads required for hybridization capture of DNA. The automation of the hybrid capture workflow on the Sciclone G3 NGS Workstation (Perkin Elmer) ensures accuracy, consistency, and decreased variability. The automated method outlined in this document was developed by IDT and qualified using sequencing panels of various sizes.

## Methods

This protocol describes hybridization capture using IDT xGen Lockdown Probes and the xGen Hybridization and Wash Kit that has been 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 (Qubit® fluorometer; Thermo Fisher). Library DNA integrity and average fragment size were determined using an TapeStation® instrument (Agilent). PCR thermocycling was performed offline using a CT1000™ thermal cycler (BioRad). Samples were dried using a DNA 120 SpeedVac™ concentrator (Savant). All sequencing runs were performed on a NextSeq® system (Illumina), using either high-output or mid-output reagents and 2 x 150 read lengths. Five modules were developed to encompass the workflow from library normalization through post-capture PCR cleanup. The modules include:

- IDT xGen Normalization and Pooling
- IDT xGen Blocking Setup
- IDT xGen Hybridization Setup
- IDT xGen Target Capture
- IDT xGen Post-Capture PCR SPRI

## Before you start

At the start of each module, ensure the Sciclone G3 NGS workstation, associated computer, and the Inheco® controller are turned on. All modules are associated with Excel® workbooks (Microsoft) that integrate directly with the Sciclone G3 NGS workstation.

Because samples are processed by column, the scripts support sample numbers that are multiples of 8. The maximum number of samples that can be processed in a run is 96.

Use filtered 150 µL tips during this protocol. Use the following plate types:

- 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

Reagents to use with the Sciclone scripts include:

- xGen Hybridization and Wash Kit (IDT; cat # 1080577 or 1080584)
- Human Cot DNA (IDT; cat # 1080768 or 1080769)
- Vapor-Lock™ PCR encapsulation barrier (QIAGEN; cat # 981611)
- KAPA® HiFi HotStart ReadyMix (2X) (Kapa Biosystems; cat # KK2601 or KK 2602)
- Agencourt® AMPure® XP beads (Beckman Coulter; cat # A63880)
- xGen Lockdown Probe Pools (IDT)
- xGen Blocking Oligos (IDT)
- xGen Library Amplification Primer Mix (IDT; cat # 1077675, 1077676, or 1077677)
- Ethanol

# Workflow

| Step | Module | Time |
|---|---|---|
| 1 | IDT xGen Normalization and Pooling | Total time: 2 min per sample |
| 2 | IDT xGen Blocking Setup | Total time: ~1 min per column of sample |
| 3 | IDT xGen Hybridization Setup | Total time: ~5 min for every 2 columns of sample |
| 4 | IDT xGen Target Capture | Total time*: ~2 hrs 25 min<br>Breakdown:<br>– From start of module to first pause for addition of sample: ~30 min<br>– From addition of samples to second pause for addition of PCR Master Mix: ~1 hr 45 min<br>– From addition of PCR Master Mix until module completes: 10 min |
| 5 | IDT xGen Post-Capture PCR SPRI | Total time: ~10 min |

* Total time for target capture is based on using a 96-well plate.

# Automation protocol

## IDT xGen Normalization and Pooling module

This module uses 500 ng of each sample library and normalizes with IDTE to yield even volumes for optimal dry down. Begin this module with a library concentration of 50–100 ng/μL.

**Note:** Libraries of higher or lower concentration can be normalized and pooled with this module; however, other library concentrations were not tested during our protocol qualification. Therefore, manual normalization and pooling of libraries outside of the indicated range is strongly recommended.

### Duration

2 min per sample

### Procedure

1. Complete the Normalization and Pooling spreadsheet by entering your sample IDs, concentrations, source, final normalization sample volume, and destination wells.
2. Save the workbook.

   **Important!** When saving the workbook, never change the name of the workbook, or the destination folder. The Maestro software script reads the values from the workbook automatically using the preprogrammed workbook names.
3. Launch the Sciclone software, open the normalization script, then press **Play**.

   The software will prompt for confirmation that it is referencing the correct workbook.
4. Follow the on-screen instructions for preparation of the deck layout. The deck placement of the library plate, and clean PCR plate, used for normalization is indicated. Ensure a supply of clean tips is available.
5. After the Normalization and Pooling script completes, remove the original library plate from the deck, then seal and freeze for storage.
6. Remove the normalized library sample plate from the deck, then proceed to the IDT xGEN Blocking Setup module.

   **Safe Stop:** At the end of this module, the plate can safely be stored, or continue to the next module in the protocol.

# IDT xGen Blocking Setup module

This module adds a solution of blockers to the samples. At the end of this module, the plate will be ready to dry down in the SpeedVac concentrator.

## Duration

~1 min per column of samples

## Procedure

1. Complete the Blocking Setup spreadsheet by indicating the number of columns where the Blocker Master Mix will be added.

2. Save the workbook.

| | A | D | F |
|---|---|---|---|
| 1 | Date | Experiment | |
| 2 | Number of Columns | 1 | |
| 3 | Number of Rows | 8 | Do not change this value |
| 4 | Total Number of Samples | 8 | |
| 6 | Blocker Mix Plate (Bio-Rad HSP-96 PCR Plate) | | |
| 7 | Sciclone Deck Location: D4 | | |

| | A | Blocker Master Mix (column 1) | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | A | 12 | | | | | | | | | | | |
| 11 | B | 12 | | | | | | | | | | | |
| 12 | C | 12 | | | | | | | | | | | |
| 13 | D | 12 | | | | | | | | | | | |
| 14 | E | 12 | | | | | | | | | | | |
| 15 | F | 12 | | | | | | | | | | | |
| 16 | G | 12 | | | | | | | | | | | |
| 17 | H | 12 | | | | | | | | | | | |

| Blocker Master Mix | 1 Sample | 8 Samples |
|---|---|---|
| Cot-1 DNA | 5 | 75.4 |
| xGen Blockers | 2 | 30.2 |
| Total Volume | 7 | 105.6 |

Normalization with Pooling | Blocking Setup | Hybridization Setup | Target Capture | Post-Capture PCR SPRI | Outl ...

**Figure 1. Workbook for the Blocking Setup module**

3. Prepare the Blocker Master Mix based on the calculated volumes from the workbook.

4. Press **Play** on the script to start the module.

A window displays the number of columns that will be processed, as directed in the workbook.

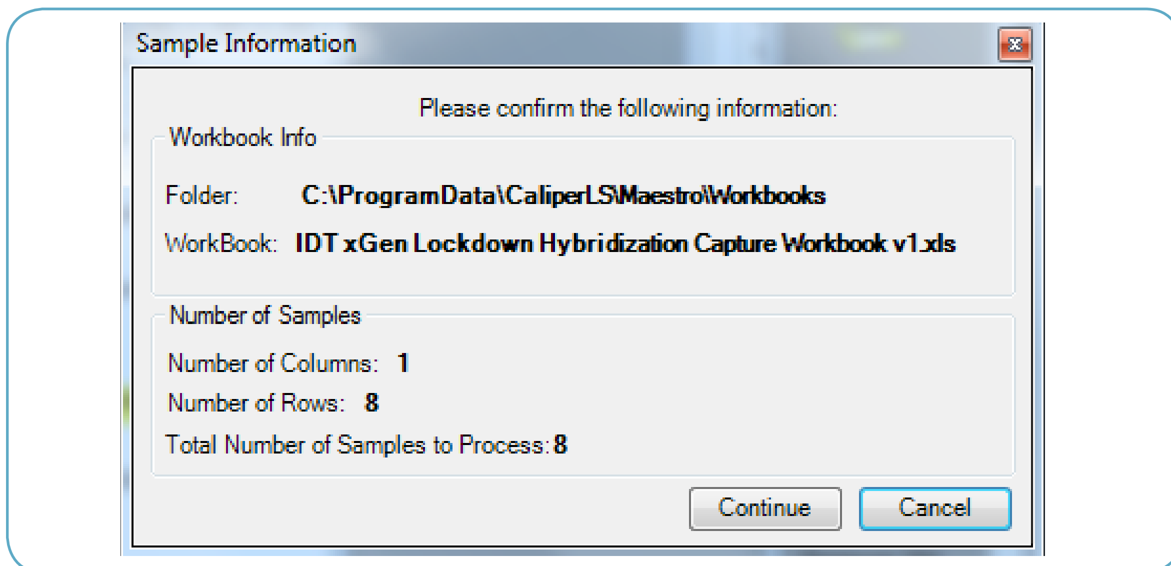



**Figure 2. Sample information confirmation window**

5. Follow the on-screen instructions to set up the deck, as shown in Figure 3.

**Figure 3. This is the correct deck layout.**

6. Remove the sample plate from the deck, then transfer the plate with nominalized libraries and added blockers to the SpeedVac concentrator for dry down.

   When the dry down is complete, proceed to the xGen Hybridization Setup module.

**Safe Stop:** If you are stopping here, seal and store the plate overnight at room temperature, or in the freezer for longer. If you are not stopping, continue immediately to the next module.

# IDT xGen Hybridization Setup module

This module adds the hybridization solution, including the xGen Lockdown probe pool. At the end of this module, the plate is ready for the hybridization incubation.

## Duration

~ 5 min, based on 2 columns of samples

## Procedure

1. If you are combining xGen Lockdown Panels and Probes, follow the recommendations as described in the Appendix.

**Tip:** Update the table shown in Figure 4 to allow the Maestro software to obtain the values directly from this workbook.

2. Complete the Hybridization Setup spreadsheet by indicating the number of columns where the Hybridization Master Mix is added.

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | Date | | | Experiment | | |
| 2 | Number of Columns | | | 2 | | |
| 3 | Number of Rows | | | 8 | | Do not change this value |
| 4 | Total Number of Samples | | | 16 | | |
| 5 | | | | | | |
| 6 | Hybridization Buffer and Probes Plate (PE StorPlate) | | | | | |
| 7 | Sciclone Deck Location: B4 | | | | | |
| 8 | | Hyb Buffer and Probes | | | | |

| | A | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | A | 39 | | | | | | | | | | | |
| 11 | B | 39 | | | | | | | | | | | |
| 12 | C | 39 | | | | | | | | | | | |
| 13 | D | 39 | | | | | | | | | | | |
| 14 | E | 39 | | | | | | | | | | | |
| 15 | F | 39 | | | | | | | | | | | |
| 16 | G | 39 | | | | | | | | | | | |
| 17 | H | 39 | | | | | | | | | | | |

| *Hyb Buffer and Probes* | *1 Sample* | *16 Samples* |
|---|---|---|
| xGen 2X Hyb Buffer | 8.5 | 160.7 |
| xGen Hyb Buffer Enhancer | 2.72 | 51.4 |
| Nuclease-Free Water | 1.78 | 33.6 |
| xGen Lockdown Probe Pool | 4 | 75.6 |
| Total | 17 | 321.4 |

Normalization with Pooling | Blocking Setup | Hybridization Setup | Target Capture | Post-Capture PCR SPRI | Outl ...

**Figure 4. Workbook for the Hybridization Setup**

3. Save the workbook.

4. Prepare the Hybridization Master Mix based on the calculated volumes from the workbook (Figure 4).

5. Follow the on-screen instructions to set up the deck, as shown in Figure 5.

**Figure 5. Deck layout for the Hybridization Setup**

6. Remove the sample plate from the deck and seal the plate with a Microseal B seal.
7. Briefly centrifuge the plate.
8. Incubate the plate in a thermal cycler using the following program with a lid set at 100°C:

| Temperature | Time |
|---|---|
| 95°C | 30 sec |
| 65°C | 4 hr |
| 65°C | Hold |

## IDT xGen Target Capture module

This module will wash the Streptavidin beads, bind the captured target duplexes onto the beads, then wash the beads again to remove any non-specific DNA. As a final step, the PCR Master Mix is added to the resuspended beads. At the end of this module, the plate is ready to load onto a thermal cycler for PCR amplification.

### Duration

Based on a 96-well sample plate:

- From start of module to the first pause for addition of samples: ~30 min
- From addition of samples to the second pause for addition of PCR Master Mix: 1 hr and 45 min
- From the addition of PCR Master Mix until completion of the module: 10 min

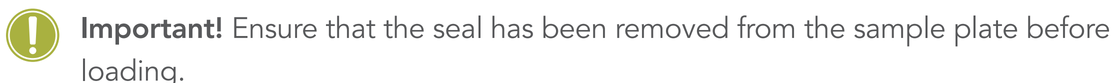
### Procedure

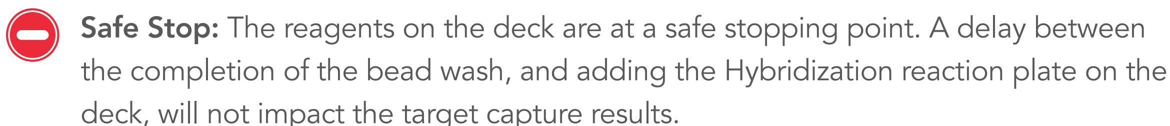
1. Complete the Target Capture spreadsheet by indicating the number of columns that contain samples to be processed.
2. Save the workbook.
3. Prepare the plates with the diluted buffers and the beads, based on the calculated volumes from the workbook. Do not prepare the PCR Master Mix.
4. Follow the on-screen instructions to set up the deck, as shown in Figure 6.

**Figure 6. Deck layout for the Target Capture**

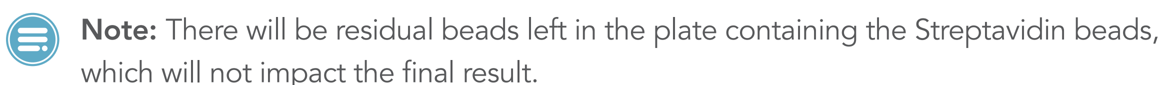
5. Start the protocol with 30 min remaining on the 65°C, 4 hr incubation.

6. During the first half of the module, the software will pause and direct you to load the plate of samples and the plate containing the Vapor-Lock PCR encapsulation barrier.


**Important!** Ensure that the seal has been removed from the sample plate before loading.


**Safe Stop:** The reagents on the deck are at a safe stopping point. A delay between the completion of the bead wash, and adding the Hybridization reaction plate on the deck, will not impact the target capture results.

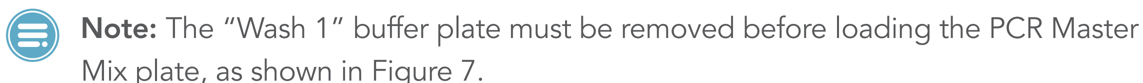
7. The module next binds the beads to the sample and washes the beads.


**Note:** There will be residual beads left in the plate containing the Streptavidin beads, which will not impact the final result.

**Tip:**The script takes 1 hr and 45 min until the next pause point.

8. With approximately 20 min remaining in the script, prepare the PCR Master Mix as outlined in the workbook.

9. After the beads with captured DNA have been washed and resuspended in water, the instrument will pause to prompt you to load the plate of PCR Master Mix and a fresh plate.


**Note:** The "Wash 1" buffer plate must be removed before loading the PCR Master Mix plate, as shown in Figure 7.

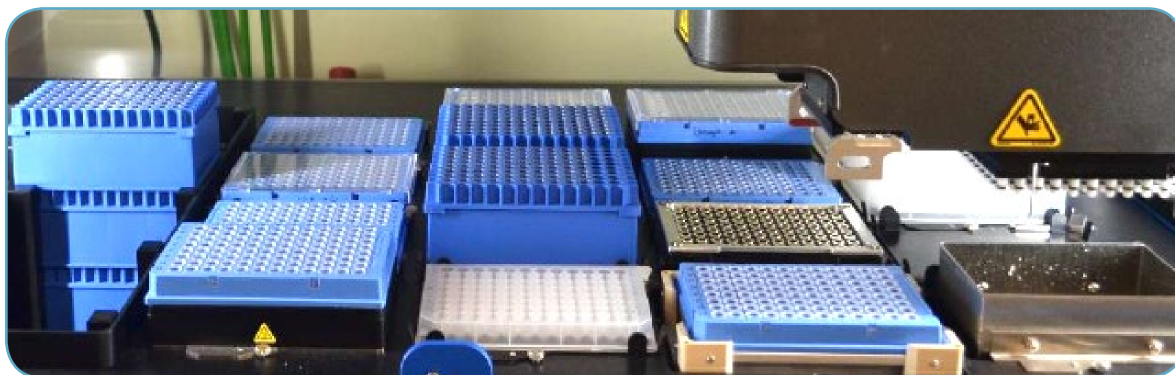
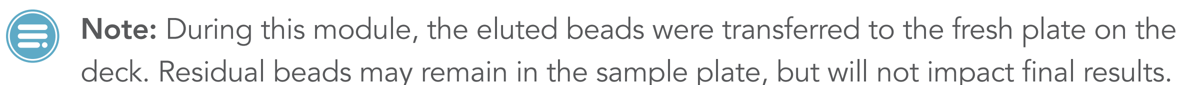


**Figure 7. Deck layout when the PCR Master Mix is added to the deck.**

10. Click **OK** to continue the protocol.

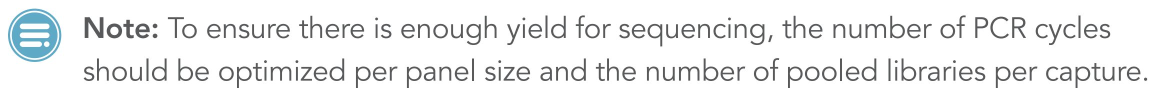

**Note:** During this module, the eluted beads were transferred to the fresh plate on the deck. Residual beads may remain in the sample plate, but will not impact final results.

11. At the end of the module, remove the sample plate that contains the PCR Master Mix from the deck.

12. Seal the plate, then briefly centrifuge.

13. Place the plate in a thermal cycler, and run the following program with the heated lid set to 105°C:

| Step | Number of cycles | Temperature (°C) | Time |
|---|---|---|---|
| Polymerase activation | 1 | 98 | 45 sec |
| Amplification | | | |
| Denaturation | Variable—refer to the Panel size table below. | 98 | 15 sec |
| Annealing | | 60 | 30 sec |
| Extension | | 72 | 30 sec |
| Final extension | 1 | 72 | 1 min |
| Hold | 1 | 4 | Hold |



**Note:** To ensure there is enough yield for sequencing, the number of PCR cycles should be optimized per panel size and the number of pooled libraries per capture.

We recommend starting optimization with the following:

| Panel size | 1-plex | 4-plex | 8-plex | 12-plex |
|---|---|---|---|---|
| >100,000 probes (xGen Exome) | 10 cycles | 8 cycles | 7 cycles | 6 cycles |
| 10,001–100,000 probes | 12 cycles | 10 cycles | 9 cycles | 8 cycles |
| 501–10,000 probes | 13 cycles | 11 cycles | 10 cycles | 10 cycles |
| 1–500 probes | 14 cycles | 12 cycles | 11 cycles | 11 cycles |

# IDT xGen Post-Capture PCR SPRI module

This module performs the final cleanup of the PCR reaction using 1.5X AMPure XP bead volume. At the end of this module, you can store the samples, or continue with qPCR quantification of the DNA.

## Duration

~10 min

## Procedure

1. Complete the Post-Capture PCR SPRI (Bead Cleanup) spreadsheet by indicating the number of columns that contain samples for the cleanup.

2. Save the workbook.

3. Follow the on-screen instructions to set up the deck, as shown in Figure 8.

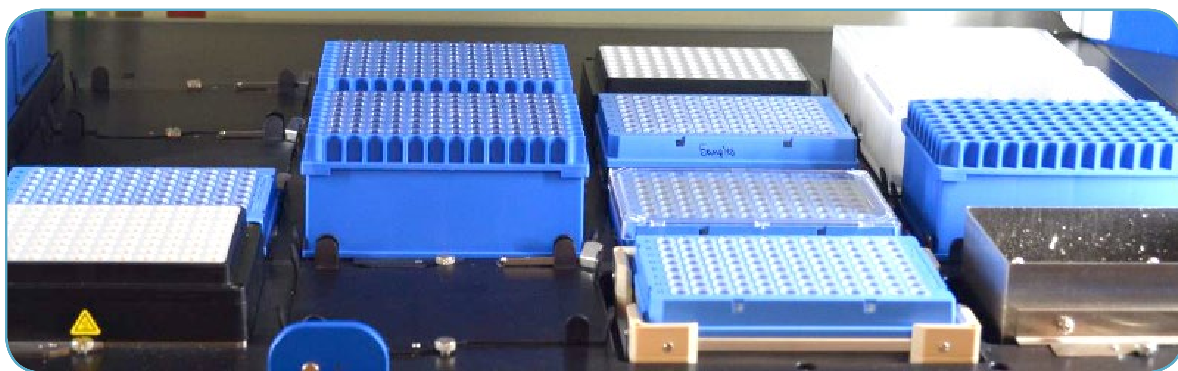


**Figure 8. Deck layout for the Post-Capture PCR SPRI cleanup**

4. Start the Bead Cleanup module.

   When initiated, the system will aspirate the supernatant of the PCR from the Dynabeads M-270 Streptavidin.

5. The software will pause to prompt you to remove the 96-well PCR plate. After you have removed the plate, click OK to continue the protocol.

6. After this module completes, remove the sample plate from the deck and seal for storage, or proceed immediately to library quantification and sequencing.

# Sequencing results and analysis

Three runs were performed on the Sciclone G3 NGS workstation with 8 samples each. The samples were captured using the xGen Exome Research Panel v1.0 and xGen Universal Blockers—TS Mix, together with Human Cot DNA. The samples were sequenced and down-sampled to 50 million reads per exome. Sequencing data were aligned with BWA-MEM [1] to human reference hg19. Coverage statistics were compiled with BedTools [2].

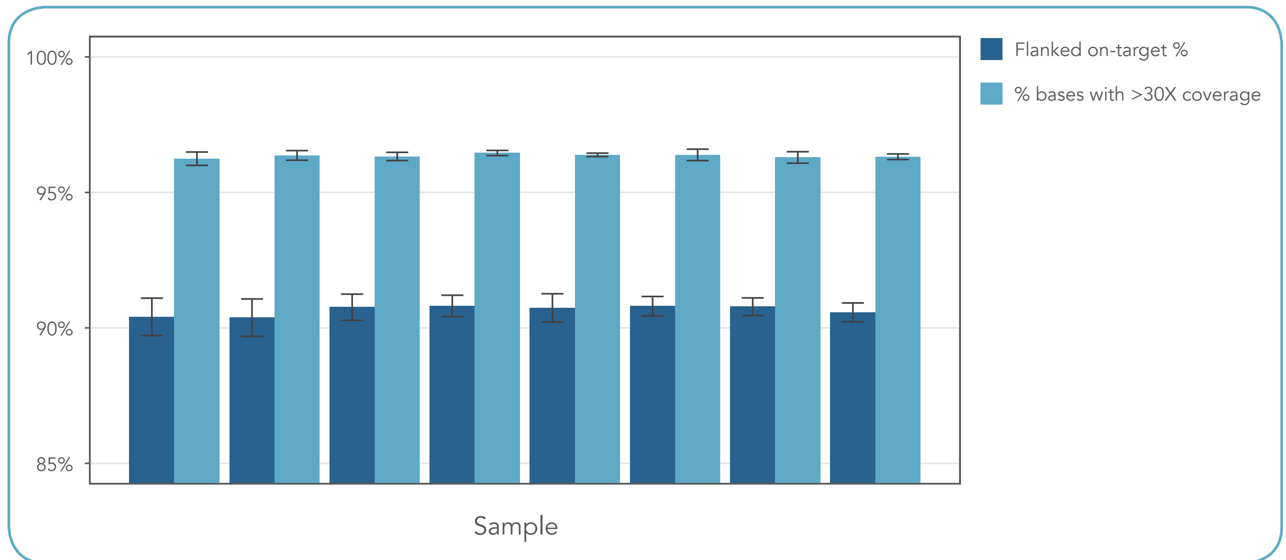



**Figure 9. IDT Target Capture sample statistics**

# References


1. Li H. (2013) Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv:1303.3997v1 [q-bio.GN].
2. Quinlan AR, Hall IM. (2010) BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics, 26(6):841–842.

# Appendix

## Combining xGen Lockdown Panels and Probes

Combine panels at equimolar amounts to achieve uniform coverage. When combining panels (during the IDT xGen Hybridization Setup module), prepare the Hybridization Master Mix based on the recommendations in **Table 1**. Multiply by the number of samples, then add a 10% overfill (See **Table 2**).

**Tip:** For more information or assistance with your specific experimental design, contact our technical support group at applicationsupport@idtdna.com.

**Table 1. Spike-in volumes when combining two panels**

| | | Spike-in panel types | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Gene Capture Pools | | Lockdown Probe Panel ≥4000 probes | | Lockdown Probe Panel <4000 probes | | xGen Spike-in panel* | |
| | | Volume | | Volume | | Volume | | Volume | |
| | | Panel | Spike-in | Panel | Spike-in | Panel | Spike-in | Panel | Spike-in |
| Main panel types | Gene Capture Pools | 3 µL | 3 µL | 4 µL | 2 µL | 4 µL | 1 µL | 4 µL | 2 µL |
| | Lockdown Probe Panel ≥4000 probes | N/A | N/A | 3 µL | 3 µL | 4 µL | 2 µL | 4 µL | 2 µL |
| | Lockdown Probe Panel <4000 probes | N/A | N/A | N/A | N/A | 3 µL | 3 µL | 4 µL | 2 µL |

* Inventoried spike-in panels (e.g., xGen CNV Backbone Panel—Tech Access, Human mtDNA Research Panel, or Human ID Panel)

**Table 2. Volumes per component of the Hybridization Master Mix**

| Hybridization Master Mix components | Volume per reaction (µL) |
|---|---|
| xGen 2X Hybridization Buffer | 8.5 |
| xGen Hybridization Buffer Enhancer | 2.7 |
| Main panel | See **Table 1** |
| Spike-in panel | See **Table 1** |
| Nuclease-Free Water | 0–0.8 (only if needed*) |
| **Total volume*** | **17–17.2 µL** |

* Depending on the spike-in panel used, your volume could be less than 17 µL; if so, add a small amount of water to reach the total recommended volume (17–17.2 µL).

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