# xGen™ Adapters & Indexing Primers

## From basic indexing to custom adapters

**Our NGS quality control minimizes risk of misassignment**

**UDI is the standard for data accuracy and confidence**

**Pre-configured and ready-to-ship**

## xGen NGS indexing options

The xGen line of high-quality indexing solutions is compatible with a wide variety of NGS workflows and offers you a vast array of options to fit your needs. Indexes are available in 8 and 10 nt formats for the IDT Library Prep Kits and are also compatible with other commercial library kits. The IDT proprietary Normalase™ technology provides a simple enzymatic reaction for normalizing libraries before sequencing. Special indexing primers compatible with the Normalase reaction are available in both 8 and 10 nt indexing options.

- xGen Combinatorial Dual Index (CDI) primer barcoding strategies for sequencing experiments with basic library demultiplexing needs
- xGen Unique Dual Index (UDI) primers for reduction of index hopping issues
- xGen UDI-UMI Adapters utilize molecular barcodes that provide resolution of low-level mutations in PCR-free workflows (**Table 1**)
- Barcode designs have an edit distance of ≥3
- Compatible with 2- and 4- color Illumina® sequencing platforms

| **Stubby adapters and UDI primers** | **Stand-alone indexing primers** | **Full-length adapters (UDI-UMI)** |
|---|---|---|
| Use for TA-ligation in construction of TruSeq™ compatible libraries. | Pair with the IDT xGen Library Prep Kits that include stubby adapters to complete the indexing by PCR reaction. | Complete indexing/adapter solution for IDT Library Prep kits with "UNI" in the name. Can also be used for constructing Tru-Seq™ compatible libraries via TA-ligation. |

## xGen NGS adapter ligation options

A. xGen UDI-UMI Adapters

B. xGen Stubby Adapter and UDI primers



**Figure 1. Adapter ligation options.** Adapters can be attached to library fragments by either direct ligation of duplex full-length adapters (Figure 1A) or by ligation of stubby adapters followed by indexing PCR (Figure 1B). For RNA-seq, methyl-seq, the xGen cfDNA & FFPE DNA Library Prep Kit, and the xGen ssDNA & Low-Input DNA Library Prep Kit, the IDT proprietary Adaptase™ chemistry is designed to anneal a single-stranded stubby adapter directly to a single-stranded library fragment, which enhances the library complexity.

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**Table 1. Choosing the best indexing/adapter for your application**.

| Application | Best choice | Alternatives | Rationale |
|---|---|---|---|
| PCR-free whole genome sequencing (WGS) | xGen UDI-UMI Adapters | N/A | Full-length adapters are required for PCR-free workflows. |
| PCR-free metagenomics | | | |
| PCR-free sequencing (high-input) | | | |
| Low-level mutation detection (~1% VAF) | xGen UDI-UMI Adapters | N/A | Increased sensitivity possible with UMI. |
| RNA-seq | xGen Normalase UDI Primers | xGen UDI Primers, xGen Normalase CDI Primers, xGen CDI Primers | Enable higher sensitivity using UDI primers or for basic counting applications use CDI. |
| Methyl-seq | xGen Normalase UDI Primers | xGen UDI Primers, xGen Normalase CDI Primers, xGen CDI Primers | Since the xGen Methyl-Seq Library Prep Kit enables post bisulfite-conversion library construction, ANY xGen indexing primer may be used without costly methylation modifications. |
| WGS | xGen Normalase UDI Primers | xGen UDI Primers, xGen Normalase CDI Primers, xGen CDI Primers | Simpler workflow—most xGen Library Prep Kits are designed for utilizing indexing by PCR. |
| Metagenomics (1-250 ng input) | | | |
| Whole exome sequencing (WES) | | | |
| Targeted germline sequencing (SNP, indel) | | | |

**Table 2. Choosing the best indexing/adapter strategy for your xGen Library Prep Kits**.

| xGen Library Prep Kit | Full-length adapters | Indexing primer options | | | | |
|---|---|---|---|---|---|---|
| | UDI + UMI | UDI | Stubby + UDI | CDI | Normalase - UDI | Normalase - CDI |
| xGen Broad-Range RNA Library Prep Kit | | • | | • | • | • |
| xGen RNA Library Prep Kit | | • | | • | • | • |
| xGen DNA Library Prep (MC or EZ) Kit | | • | | • | • | • |
| xGen DNA Library Prep (MC or EZ) UNI Kit | • | | • | | | |
| xGen ssDNA & Low-input DNA Library Prep Kit | | • | | • | • | • |
| xGen Methyl-Seq Library Prep Kit | | • | | • | • | • |
| xGen cfDNA & FFPE DNA Prep Kit | | • | | | | |

# Easy multiplex library normalization with xGen Normalase Technology

Proprietary Normalase technology allows for enzymatic selection of library molecules to reduce hands-on-time required for manual quantification and library normalization.

- Easily integrated into xGen Library Prep Kit workflows.
- Replaces either terminal or indexing primers used in regular library amplification steps.
- No need for individual library quantification, calculations, and manual equimolar pooling.
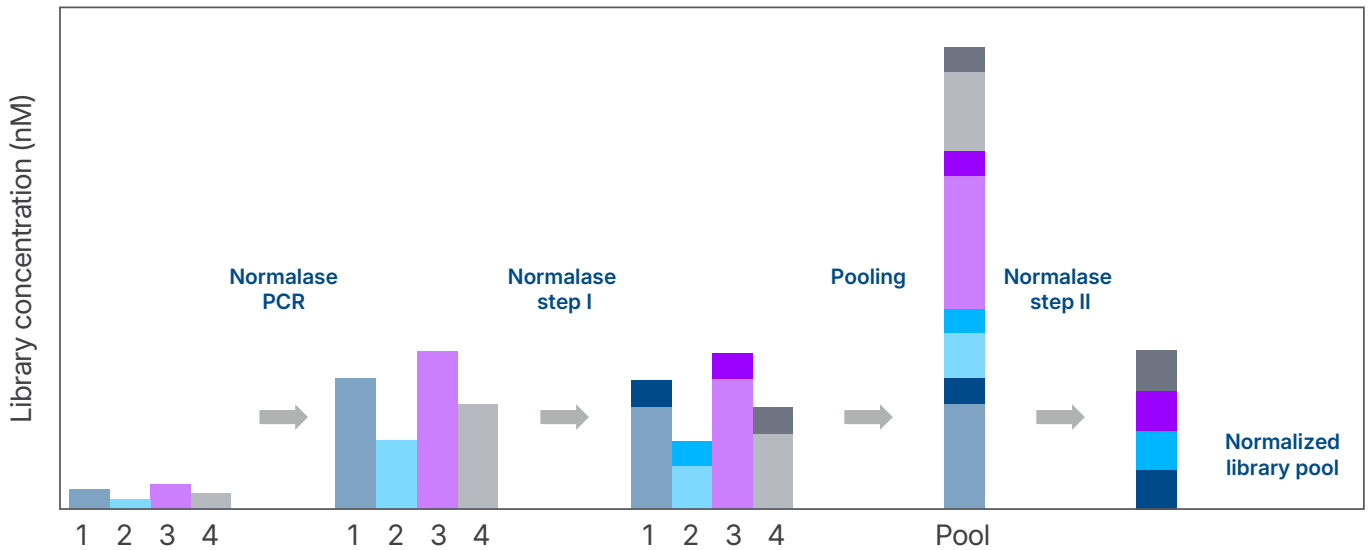- Easy adaptation to high-throughput research laboratories.

**Figure 2. The xGen Normalase Module generates an equimolar library pool for multiplexing.** Normalized libraries are produced in four main steps. First, Normalase PCR increases the initial library concentrations. Second, Normalase I treatment selects a 4 nM library fraction. Third, the samples are pooled in equal amounts for multiplexed sequencing. Finally, the Normalase II step generates the final pool of libraries. Four libraries following adapter ligation are shown in the first through third step. Pooling and normalizing the four separate libraries are shown in the last two steps.

## Consistent DNA library normalization and reliable results for a variety of insert sizes

**Table 3. The IDT xGen Normalase Module provides consistent cluster density generation from library pools normalized to 4 nM.**

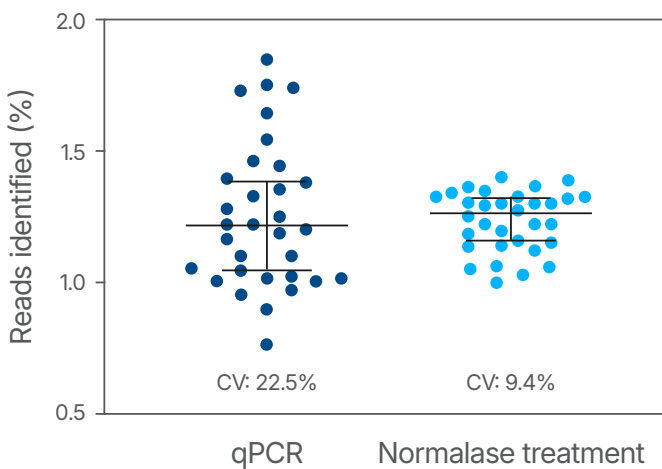| Insert size | # of libraries | Loading (pM) on MiSeq® V2 chemistry | Cluster density (K/mm$^2$) | Library balance (CV%) |
|---|---|---|---|---|
| 150 | 6 | 12 | 1370 | 9.7 |
| 200 | 16 | 12 | 1043 | 8.2 |
| 350 | 6 | 12 | 1157 | 5.4 |
| 600 | 5 | 12 | 1070 | 3.7 |



**Figure 3. The xGen Normalase technology provides less variation in sequencing reads in multiplex pools.** Thirty-two xGen DNA libraries were generated from 1–250 ng of NA12878 genomic DNA using full-length indexed adapters (*n*=32; 16 per user). Post-Normalase PCR libraries were quantified using qPCR and normalized and pooled manually (left) or were pooled and normalized using the xGen Normalase Module. After sequencing on the MiSeq V2 50 cycle Nano, the coefficient of variation (CV) was determined based on the number of reads obtained for each library. The CV for qPCR was 22.5% whereas the CV for Normalase was 9.4%, showing that each library had more equal representation in the Normalase generated pool.

# Complete workflow for hybridization capture experiments

xGen DNA Library Prep Kits paired with xGen Adapters create libraries that are compatible with xGen hybridization capture reagents for targeted next generation sequencing (**Figure 4**). For custom options, use our Custom Adapter **Configurator tool.**

### Prepare libraries

IDT library prep kit and xGen adapters

### Perform hybridization capture

xGen hybridization capture probes and reagents
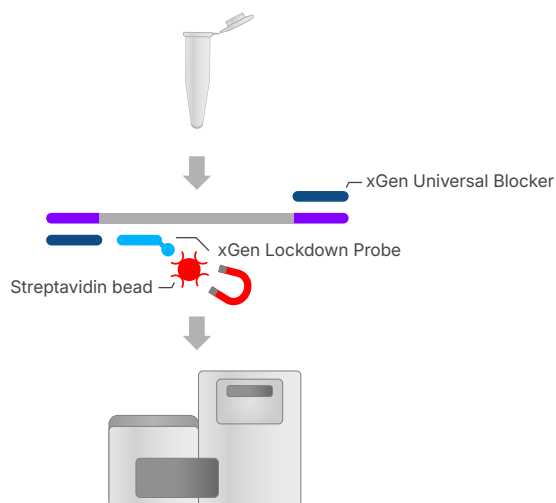
### Sequence libraries and analyze data

Illumina platforms

**Figure 4. Overview of hybridization capture sequencing workflow.**

## Ordering information

| Indexing strategy | Product name | Catalog number |
|---|---|---|
| UDI + UMI | xGen UDI-UMI Adapters, 16 rxn | 10006914 |
| UDI + UMI | xGen UDI-UMI Adapters, 96 rxn | 10005903 |
| UDI | xGen UDI Primers, 16 rxn | 10005975 |
| UDI | xGen UDI Primers Plate 1, 8nt | 10005922 |
| UDI | xGen UDI Primers Plate 2, 8nt | 10009816 |
| UDI | xGen Stubby Adapter-UDI Primers, 16rxn | 10005976 |
| UDI | xGen Stubby Adapter-UDI Primers, 96rxn | 10005921 |
| UDI | xGen UDI 10nt Primer Plates 1-4 | 10008052 |
| UDI | xGen UDI 10nt Primer Plates 1-8 | 10008053 |
| UDI | xGen UDI 10nt Primer Plates 1-16 | 10008054 |
| UDI | xGen UDI 10nt Primer Plates 1-4, 2 nmol | 10008055 |
| UDI | xGen UDI 10nt Primer Plates 1-8, 2 nmol | 10008056 |
| UDI | xGen UDI 10nt Primer Plates 1-16, 2 nmol | 10008057 |
| CDI | xGen CDI Primers, 96 rxn | 10009815 |
| Normalase – CDI | xGen Normalase CDI Primers | 10009794 |
| Normalase - UDI | xGen Normalase UDI Primers Plate 1 | 10009796 |
| Normalase - UDI | xGen Normalase UDI Primers Plate 2 | 10009797 |
| Normalase - UDI | xGen Normalase UDI Primers Plate 3 | 10009798 |
| Normalase - UDI | xGen Normalase UDI Primers Plate 4 | 10009799 |
| Normalase - UDI | xGen Normalase UDI Primers Set 1 | 10009795 |
| Normalase - UDI | xGen Normalase UDI Primers Set 2 | 10009800 |
| Normalase - UDI | xGen Normalase UDI Primers Set 3 | 10009811 |
| Normalase - UDI | xGen Normalase UDI Primers Set 4 | 10009812 |

## For more information, visit idtdna.com/NGS-Adapters

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