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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NGS • CRISPR • Functional Genomics • Synthetic Biology • PCR & qPCR • Custom Oligos • Contract Manufacturing

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

Application	Best choice	Alternatives	Rationale
PCR-free whole genome sequencing (WGS)			
PCR-free metagenomics	xGen UDI-UMI Adapters	N/A	Full-length adapters are required for PCR-free workflows.
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			
Metagenomics (1-250 ng input)	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.
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.

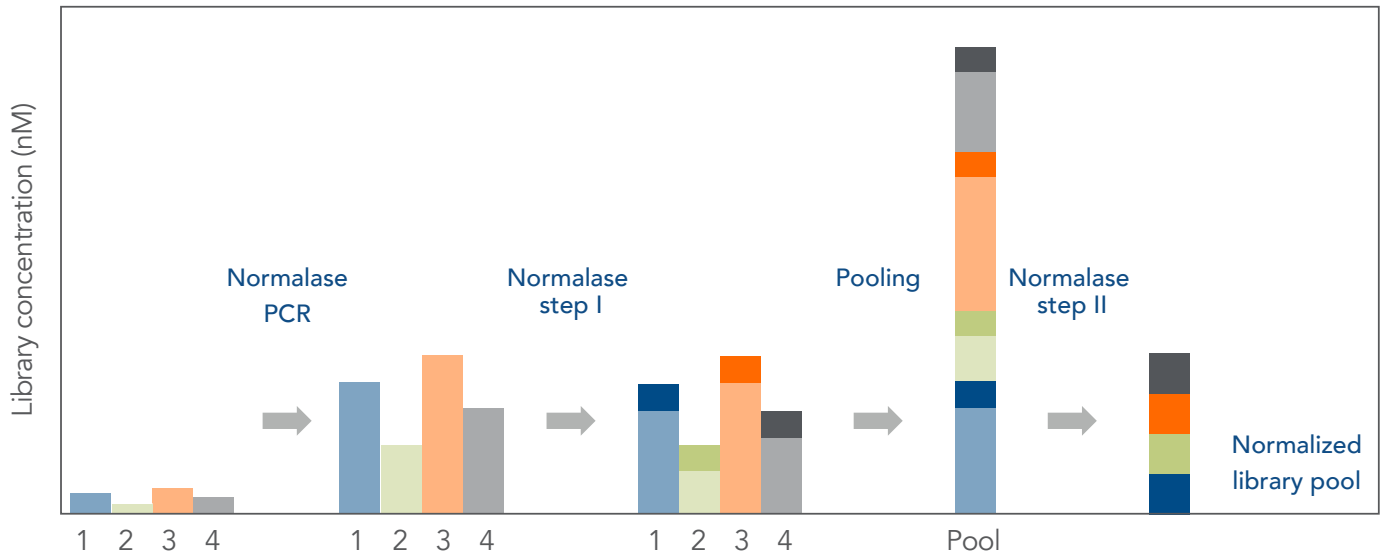- Easy adaptation to high-throughput research laboratories.

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.

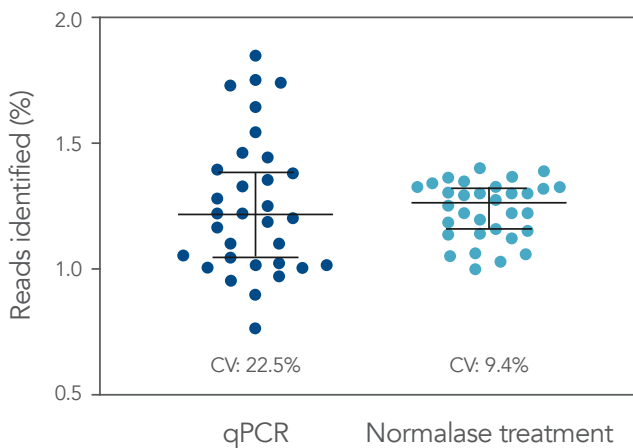| Insert size	# of libraries	Loading (pM) on MiSeq® V2 chemistry	Cluster density (K/mm^2)	Library balance (CV%)
150	6	12	1370	9.7
200	16	12	1043	8.2
350	6	12	1157	5.4
600	5	12	1070	3.7

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

xGen Universal Blocker

xGen Lockdown Probe

Streptavidin bead

Sequence libraries and analyze data
Illumina platforms
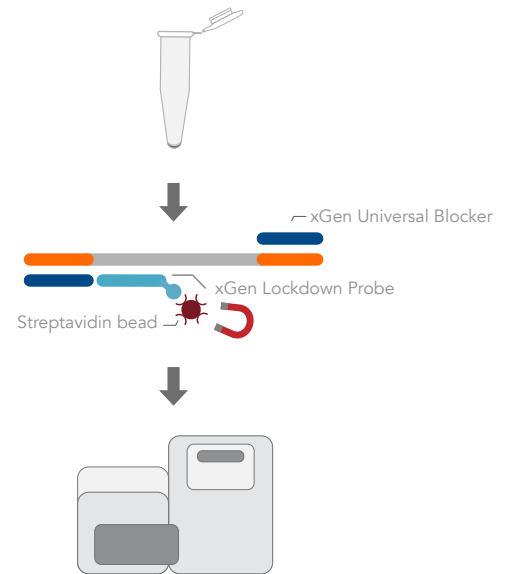
Figure 4. Overview of hybridization capture sequencing workflow.

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

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