



# xGen™ Amplicon Panels for oncology research, inherited disease research, and sample tracking

Protocol for the following xGen products:

- xGen Amplicon Core
- xGen Oncology & Inherited Diseases Amplicon Panels
  - Lung Amplicon Panel
  - Colorectal Amplicon Panel
  - Lynch Syndrome Amplicon Panel
  - Myeloid Amplicon Panel
  - 57G Pan-Cancer Amplicon Panel
  - 56G Oncology Amplicon Panel v2
  - EGFR Pathway Amplicon Panel
  - BRCA1, BRCA2 Amplicon Panel
  - BRCA1, BRCA2, PALB2 Amplicon Panel
  - TP53 Amplicon Panel
  - CFTR Amplicon Panel ACE2 Amplicon Panel
- xGen Human Sample ID Amplicon Panel
- xGen Custom Amplicon Panels

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# REVISION HISTORY

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Version	Release date	Description of changes
3.1	December 2022	Update multiplex PCR program table
3	October 2022	Revision of Appendix B
2	June 2022	Adjustment to product size offerings
1	December 2021	Initial release

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# OVERVIEW

xGen™ Amplicon Panels offer a comprehensive next generation sequencing (NGS) workflow that provides optimal coverage and NGS data quality on Illumina® sequencing platforms for research. These kits leverage patented, multiplex PCR technology, enabling library construction from a variety of sample types, including formalin-fixed, paraffin-embedded (FFPE) and circulating, cell-free DNA (cfDNA), using tiled, overlapping primer pairs within a single multiplexed pool.

See [Appendix A](#) for panel-specific information on panel content and size as well as multiplexed sequencing recommendations. See individual panel data sheets for details regarding target design and coverage.

Product feature	Specification
Recommended input material	10–25 ng DNA
Time	2 hours: DNA to library 3 hours: DNA to normalized library pool
Components provided	Target-specific multiplex primer pool* PCR and library prep reagents Normalase™ reagents (optional, included) Indexing primers*
	Note: Kits do not include magnetic beads
Multiplexing capability	Up to 96 combinatorial dual index (CDI) or 1536 unique dual index (UDI)
Recommended depth	500X coverage (germline variant detection); 5000X coverage (somatic variant detection down to 1% allele frequency)
Specifications†	>90% on target >90% coverage uniformity (% bases at >20% of the mean)

\* Customizable kit components available. For more information, go to [www.idtdna.com/ContactUs](http://www.idtdna.com/ContactUs).

† Panel specific

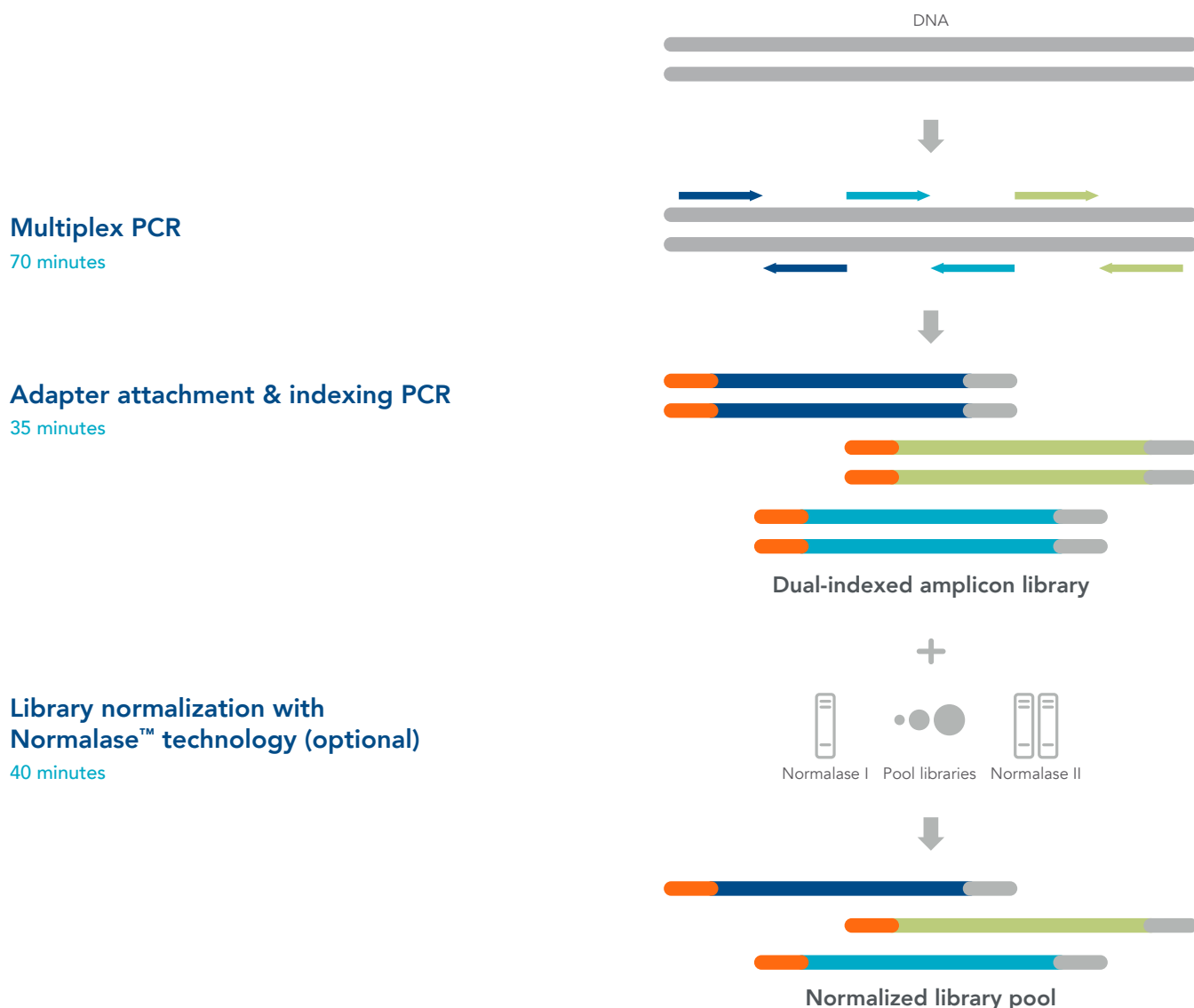
## Supported applications and sample types

- **Applications:** Variant discovery research, disease disposition research, oncology research, sample tracking research
- **Sample types including, but not limited to:** genomic DNA (gDNA) from fresh frozen samples, HMW, FFPE, cell-free DNA (cfDNA), circulating tumor DNA (ctDNA), blood

## xGen Amplicon workflow

xGen Amplicon Panels utilize multiple overlapping amplicons in a single tube, using a rapid, 2-hour workflow to prepare ready-to-sequence libraries for research studies. The PCR1+PCR2 workflow generates comprehensive libraries, even from low input quantities. The libraries may be quantified with conventional methods such as Qubit™ (Thermo Fisher Scientific) or Bioanalyzer® (Agilent) and normalized by manual pooling, or normalized enzymatically with the included Normalase reagents.

This protocol includes instructions for the multiplex PCR step to enrich target sequences, an indexing PCR step to amplify and add combinatorial or unique dual indexed adapter sequences, and an optional, downstream Normalase step to produce an equimolar library pool.



**Figure 1. xGen Amplicon workflow.** A dual-indexed, amplicon library is prepared from DNA in three main steps: 1) multiplex PCR; 2) adapter attachment with indexing PCR; 3) an optional Normalase treatment step to produce equimolar library pools.

# CONSUMABLES AND EQUIPMENT

These kits contain sufficient reagents for the preparation of 96 libraries (10% excess volume provided).

## Consumables from IDT—Kit contents

Workflow stage	Component	96 rxn (μL)	Storage
Pre-PCR	• Reagent G2	317	–20°C
	• Enzyme G3	1584	–20°C
	• Pre-PCR TE	6000	Room temperature
Post-PCR	• Reagent I1	348	–20°C
	• Enzyme I2	53	–20°C
	• Enzyme I3	15	–20°C
	• Enzyme I4	2640	–20°C
	• Post-PCR TE	6000	Room temperature
	PEG NaCl	6000	Room temperature
Normalase	• Buffer S1	454	–20°C
	• Reagent S2	21	–20°C
	• Enzyme S3	53	–20°C
	• Buffer N1	101	–20°C
	• Enzyme N2	10	–20°C
	• Reagent X1	21	–20°C

## IDT—Customizable kit contents

Workflow stage	Component*	96 rxn (μL)	Storage
Pre-PCR	• Reagent G1	211	–20°C
Post-PCR	• xGen Amplicon CDI D50X	26 each	–20°C
	• xGen Amplicon CDI S7XX	15 each	–20°C
	• xGen Amplicon UDI (pre-mixed pairs)	12 per well	–20°C

\* See options for ordering customizable kit components below; either CDI or UDI primers are required.

## Consumables from IDT—Reagents

Workflow component	Product name	Index number	Reaction size (rxn)	Catalog number
xGen Core Reagents	xGen Amplicon Core	N/A	96	10009827
xGen G1 Specific Panel Primers*	xGen Lung Amplicon Panel	N/A	96	10009833
	xGen Colorectal Amplicon Panel	N/A	96	10009834
	xGen Lynch Syndrome Amplicon Panel	N/A	96	10009835
	xGen Myeloid Amplicon Panel	N/A	96	10009836
	xGen 57G Pan-Cancer Amplicon Panel	N/A	96	10009837
	xGen 56G Oncology Amplicon Panel v2	N/A	96	10009838
	xGen HS EGFR Pathway Amplicon Panel	N/A	96	10009839
	xGen BRCA1 BRCA2 Amplicon Panel	N/A	96	10009840
	xGen BRCA1 BRCA2 PALB2 Amplicon Panel	N/A	96	10009841
	xGen TP53 Amplicon Panel	N/A	96	10009842
	xGen Sample_ID Amplicon Panel	N/A	96	10009843
	xGen CFTR Amplicon Panel	N/A	96	10009844
	xGen ACE2 Amplicon Panel	N/A	96	10009830
xGen Amplicon CDI Primers*	xGen Amplicon CDI Primers	D501N-D508N/ S701N-S712N	96	10009845
xGen Amplicon UDI Primer Plates*	xGen Amplicon UDI Primer Plate 1	SU001-SU096	96	10009847
	xGen Amplicon UDI Primer Plate 2	SU097-SU192	96	10009848
	xGen Amplicon UDI Primer Plate 3	SU193-SU288	96	10009849
	xGen Amplicon UDI Primer Plate 4	SU289-SU384	96	10009850
	xGen Amplicon UDI Primer Set 1	SU001-SU384	4x96	10009846
	xGen Amplicon UDI Primer Set 2	SU385-SU768	4x96	10009851
	xGen Amplicon UDI Primer Set 3	SU769-SU1152	4x96	10009852
	xGen Amplicon UDI Primer Set 4	SU1153-SU1536	4x96	10009853

\* Customizable kit components; select one option for the xGen G1 specific panel primers, and one option of the single-use indexing primers (CDI or UDI). Each UDI primer set contains four 96-well plates. Each plate contains 96 pre-mixed primer pairs. This kit is recommended for research purposes only.



## Consumables from other suppliers

Item	Supplier	Catalog number
SPRIselect® or AMPure® XP beads	Beckman Coulter	B23317/B23318/B23319 or A63880/A63881/A63882
Aerosol-resistant pipette tips ranging from 1 to 1000 µL	Various suppliers	Varies
0.2 mL PCR tubes or 96-well plates	Various suppliers	Varies
1.5 mL microcentrifuge tubes	Various suppliers	Varies
200 proof (absolute) ethanol (molecular biology grade)	Various suppliers	Varies
Nuclease-free water (molecular biology grade)	Various suppliers	Varies
Reagents for qPCR-, electrophoretic-, or fluorometric-based library quantification assay for Illumina libraries	Various suppliers	Varies

## Equipment

Item	Supplier	Catalog number
Permagen® Magnetic Separator, or similar	Permagen	MSR812, MSP750
Instrument for qPCR-, electrophoretic-, or fluorometric-based library quantification assay for Illumina libraries	Various suppliers	Varies
Microcentrifuge	Various suppliers	Varies
Vortex	Various suppliers	Varies
Programmable thermal cycler*	Various suppliers	Varies
Pipettes ranging from 1 to 1000 µL capacity	Various suppliers	Varies

\*All xGen Amplicon Panel libraries have been created with a Bio-Rad® T100 thermal cycler in our internal testing.

# GUIDELINES

## Reagent handling

- Upon receipt, store the xGen Amplicon Library Kit products at  $-25$  to  $-15^{\circ}\text{C}$  with the exception of the PEG solution and TE, which are stored at room temperature.
- Separate the multiplex Pre-PCR Reagents (keep in pre-PCR area) from the Indexing and Normalase Reagents (keep in post-PCR area).
- To maximize use of enzyme reagents when ready to use, remove enzyme tubes from  $-20^{\circ}\text{C}$  storage and place on ice for 10 minutes prior to pipetting. Attempting to pipette enzymes at  $-20^{\circ}\text{C}$  may result in shortage of enzyme reagents.
- After thawing reagents on ice, briefly vortex (except enzymes) to mix well, then pulse spin to collect contents before proceeding. Enzyme G3 is the only enzyme that may be vortexed.
- Always add reagents to the master mix in the specified order, as stated throughout the [Protocol](#). The indexing primers (xGen Amplicon CD and UD Indexes) are the only reagents that are added individually to each sample.
- Assemble all reagent master mixes and reactions ON ICE and scale volumes as appropriate, using 5% excess volume to compensate for pipetting loss. Neglecting to store master mixes and reagents on ice prior to incubations reduces yields and function of this product.

## DNA input considerations

The starting material should be quantified with the appropriate assay: qPCR-based for FFPE and cfDNA to determine usable DNA content and sample integrity, and Qubit for high-quality DNA from whole blood or fresh frozen or cultured cells. Many panels are designed with amplicon size of  $<150$  bp to ensure compatibility with both FFPE and cfDNA samples.

For qPCR-based determination of sample quantity and integrity, xGen Input DNA Quant Primers (Alu primers) are available (Catalog No. 10009856). See the [Input DNA quantification protocol](#) for more information.

The optimal coverage uniformity of this technology is achieved with input amounts in the 10–25 ng range. Between 25–100 ng, coverage uniformity may be mildly reduced. Using less than 10 ng may reduce library yields and variant calling for low frequency alleles. Consider the following example allele frequencies and detection limits. As shown below, using at least 10 ng input for 1% allele detection limit ensures sufficient copy number of the allele of interest. If less than 10 ng is available, the expected result would be a lower detection limit. For example, at 1 ng input, a 10% limit of detection is acceptable. When using damaged samples, make sure that the usable, amplifiable input is qualified by a qPCR assay, as mentioned above.

Sample quantity (ng)	Human genome equivalents (total copies)	Example allele frequency	Example allele copies	Likelihood of calling variant
10	3000	10%	300	✓
10	3000	1%	30	✓
1	300	10%	30	✓
1	300	1%	3	X

## Avoid cross-contamination

To reduce the risk of DNA and library contamination, physically separate the laboratory space, equipment, and supplies where pre-PCR and post-PCR processes are performed, including appropriate reagent boxes for pre-PCR (multiplex) and post-PCR (Indexing and Normalase) reagents. Move samples to post-PCR area before opening tubes. This workflow, like any amplicon enrichment technology, poses a risk of contamination of surfaces and other samples following the amplification step. To help minimize this concern, use caution when opening your sample tubes after the multiplex PCR step. Follow these guidelines to avoid cross-contamination:


- Clean lab areas using 0.5% sodium hypochlorite (10% bleach).
- Use barrier pipette tips to avoid exposure to potential contaminants.
- Always change pipette tips between each sample.
- Perform pre-PCR reactions in a separate location from the post-PCR area, ideally in a PCR workstation.
- Separate the multiplex pre-PCR Reagents (keep in pre-PCR area) from the Indexing and Normalase reagents (keep in post-PCR area).

## Size selection during cleanups

SPRIselect beads from Beckman Coulter (B23317/B23318/B23319) are recommended with this protocol, however, they can be substituted with Agencourt® AMPure XP beads (Beckman Coulter, A63880/A63881/A63882). Make sure that the beads and samples are at room temperature. At no time should the “with bead” samples be stored on ice, as this affects binding to magnetic beads. Briefly vortex beads to homogenize before use. Make sure that the beads and samples never completely dry during processing.

## Automation

This protocol is readily automatable. A 10% overage volume of reagents is supplied to accommodate automation. Go to [www.idtdna.com/ContactUs](http://www.idtdna.com/ContactUs) if you require additional reagent overage volume, or would like to learn about our custom packaging options.

 **Tip:** While IDT does not supply automated liquid handling instruments or consumables, our Automation Team collaborates with automation solution providers and customers to develop and qualify optimized, automated scripts for use of our kits with liquid handling platforms routinely used in NGS library preparation. Go to [www.idtdna.com/ContactUs](http://www.idtdna.com/ContactUs) to discuss any specifics with automating the xGen Amplicon Kits with your automated liquid handling system.

# PROTOCOL

## Prepare Multiplex PCR

1. Load the Multiplex PCR program and allow the block to reach 98°C before loading samples (confirm lid heating is turned ON and has reached 105°C).

### Pre-program thermal cycler

**!** **Important:** Temperature at this step is panel-specific. Use the panel-specific annealing and extension temperatures specified in the table below:

Reagents	Lid heating ON	
Multiplex PCR thermal cycler program	30 sec	98°C
	10 sec	98°C
	5 min	* °C
	1 min	* °C
	10 sec	98°C
	1 min	64°C
	1 min	65°C
	Hold	4°C

Panel	Annealing temperature (5 min)	Extension temperature (1 min)
xGen 56G Oncology Amplicon Panel v2	63°C	65°C
xGen BRCA1 BRCA2 Amplicon Panel		
xGen BRCA1 BRCA2 PALB2 Amplicon Panel		
xGen CFTR Amplicon Panel		
xGen TP53 Amplicon Panel		
xGen Sample_ID Amplicon Panel		
xGen EGFR Pathway Amplicon Panel	65°C	65°C
xGen ACE2 Amplicon Panel		
xGen 57G Pan-Cancer Amplicon Panel		
xGen Lung Amplicon Panel		
xGen Myeloid Amplicon Panel		
xGen Colorectal Amplicon Panel		
xGen Lynch Syndrome Amplicon Panel	66°C	66°C

2. Gently rock Enzyme G3 at room temperature for 5 minutes, or until any solutes appear to be in solution. Place back on ice for remainder of use.
3. Load 10 µL of DNA sample directly into each PCR tube.
4. Keep all tubes on ice during assembly of the master mix and the reaction, until placed in thermal cycler.

## Prepare panel-specific Multiplex PCR Master Mix

Before mixing, calculate the total volume of the Master Mix based on the number of reactions required, with appropriate overage for pipetting. Vortex components G1 and G2, and pulse-spin tubes to collect contents. Make the Multiplex PCR Master Mix. Keep the prepared Master Mix on ice until ready to use.

Component	Volume (1 rxn, $\mu$ L)
• Reagent G1*	2
• Reagent G2	3
• Enzyme G3	15
<b>Total volume</b>	<b>20</b>

\* Reagent G1 is the panel-specific set of multiplex primers.

1. Combine the PCR Master Mix well and then add 20  $\mu$ L of the multiplex PCR reaction mix to each 10  $\mu$ L input DNA sample on ice. Mix well, then place in the thermal cycler and run the Multiplex PCR thermal cycler program.



**Important:** Move samples to post-PCR area before opening tubes.

2. Near the completion of the thermal cycler program, prepare the indexing reaction mix (below) in the post-PCR area. Assemble the Indexing PCR Master Mix on ice and keep cold until you add it to samples in the indexing step. All components except indexes should be master-mixed when running multiple samples in parallel.

## Prepare Indexing PCR Master Mix

Before mixing, calculate the total volume of the Indexing PCR Master Mix based on the number of reactions with appropriate overage for pipetting. To maintain a volume of Enzyme I3 that can be accurately pipetted, prepare at least 10 reactions at a time. Keep the prepared master mix on ice.

Component	Volume (1 rxn, $\mu$ L)
• Reagent I1	3.3
• Enzyme I2	0.5
• Enzyme I3	0.1
• Enzyme I4	25
<b>Total volume</b>	<b>28.9</b>

Keep prepared master mix on ice during Post-multiplex PCR size selection and cleanup.

## Perform post-multiplex PCR size selection and cleanup

1. Ensure beads and samples are at room temperature. Briefly vortex beads to homogenize before use.
2. Add 30  $\mu\text{L}$  (ratio: 1.0) of magnetic beads to each 30  $\mu\text{L}$  sample. Mix by vortexing. Pulse-spin the samples in a microcentrifuge. Ensure no bead-sample suspension droplets are left on the sides of the tube.
3. Incubate the samples for 5 minutes at room temperature, off the magnet.
4. Place the sample tubes on a magnetic rack until the solution clears and a pellet is formed (<5 minutes).
5. While sample is on the magnet, remove and discard the supernatant without disturbing the pellet (approximately 5  $\mu\text{L}$  may be left behind). Leave tubes on the magnet.
6. Carefully add 180  $\mu\text{L}$  of freshly prepared 80% ethanol solution to the pellet while it is still on the magnet. Do not disturb the pellet. Incubate for 30 seconds, and then slowly remove the ethanol solution.
7. Repeat for a second wash with the ethanol solution.
8. Pulse-spin the samples in a microcentrifuge, place back onto the magnet and remove any residual ethanol solution from the bottom of the tube with a small volume tip.
9. Resuspend each bead pellet in 17.4  $\mu\text{L}$  Post-PCR TE Buffer. Proceed to the Indexing PCR step. If an off-bead PCR is preferred, place the tubes back on the magnet and transfer the 17.4  $\mu\text{L}$  of eluate to a fresh tube.



**Important:** Continue working in the post-PCR area. Keep samples at room temperature. At no time should the “with bead” samples be stored on ice, as this affects binding to magnetic beads.

## Perform Indexing PCR


Set up the Indexing PCR thermal cycler program as follows:

Reagents	Lid heating ON (105°C)	
Indexing PCR thermal cycler program	20 min	37°C
	30 sec	98°C
	10 sec	98°C
	30 sec	60°C
	1 min	66°C
	Hold	4°C
		5 cycles*

\* The PCR cycle number can be increased for samples that may give low yields.

1. Load the Indexing PCR program and allow the block to reach 37°C before loading samples (confirm lid heating is turned ON and has reached 105°C).  
Add a unique combination of 2  $\mu\text{L}$  xGen Amplicon CD Index D50X + 1.7  $\mu\text{L}$  xGen Amplicon CD Index S7XX to each sample, OR add 3.7  $\mu\text{L}$  of a premixed xGen Amplicon UD indexing primer pair to each sample, if you are using the single use plates (See [Appendix F](#) for UDI plate usage guidelines).
2. Add 28.9  $\mu\text{L}$  of the cold Indexing PCR Reaction Mix to each sample and mix thoroughly (total volume 50  $\mu\text{L}$ ).
3. Place in the thermal cycler and run the Indexing PCR program.

## Perform post-indexing PCR cleanup


 **Note:** Make sure the PEG NaCl solution is at room temperature before starting this section.

1. Briefly vortex the PEG NaCl solution to homogenize before use.
2. Add 42.5  $\mu$ L (ratio: 0.85X) of PEG NaCl solution to each 50  $\mu$ L sample. Mix by vortexing. Make sure that there are no bead-sample suspension droplets left on the sides of the tube. If performing an "off bead" PCR, use a 42.5  $\mu$ L (ratio: 0.85X) of fresh magnetic beads.
3. Incubate the samples for 5 minutes at room temperature, off the magnet.
4. Pulse-spin the samples in a microcentrifuge. Place the sample tubes on a magnetic rack until the solution clears and a pellet is formed (<5 minutes).
5. While leaving your sample on the magnet, remove and discard the supernatant without disturbing the pellet (approximately 5  $\mu$ L may be left behind). Leave tubes on the magnet.
6. Carefully add 180  $\mu$ L of freshly prepared, 80% ethanol solution to the pellet while it is still on the magnet. Do not disturb the pellet. Incubate for 30 seconds, then carefully remove the ethanol solution.
7. Repeat for a second wash with the 80% ethanol solution.
8. Pulse-spin the samples in a microcentrifuge, place back onto the magnet, and remove any residual ethanol solution from the bottom of the tube with a small-volume tip.
9. Proceed immediately to add 20  $\mu$ L of post-PCR TE buffer and resuspend the pellet, mixing well by pipetting up and down until homogenous. Incubate at room temperature for 2 minutes off the magnet, then place the sample back on the magnet and transfer the clear 20  $\mu$ L library eluate to a fresh tube. Make sure that the eluate does not contain magnetic beads (indicated by brown coloration in eluate). If magnetic beads are present, place back on magnet, and transfer eluate again.

 **Safe Stop:** Store freshly prepared libraries at  $-20^{\circ}\text{C}$ .

## Perform NGS library quantification

Accurate library quantification is essential to load the sequencing instrument properly. Libraries can be quantified using fluorometric-, electrophoretic-, or qPCR-based methods and normalized manually. Alternatively, libraries can be enzymatically normalized following the Normalase protocol below.

 **Note:** For optimal normalization using Normalase reagents, a minimum of 12 nM yield is needed per sample. If library yields are below 12 nM, increase the number of PCR cycles to pass the 12 nM threshold, or switch to the 6 nM threshold Normalase protocol described below.

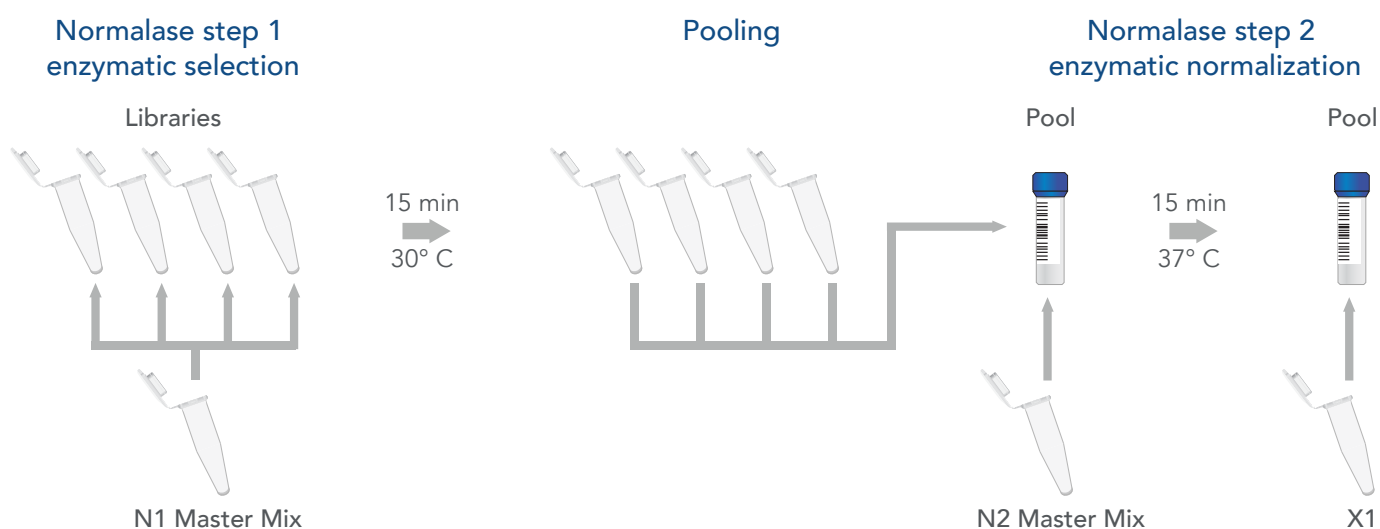
## Introduction to Normalase™ Treatment

This guide provides instructions for optional enzymatic normalization of multiplexed xGen Amplicon libraries for equimolar pooling and balanced sample representation in sequencing. The protocol is designed for xGen Amplicon libraries that produce consistent amplified library yields of  $\geq 12$  nM following indexing PCR. Most samples processed with this protocol produce amplified library yields of 12 nM or greater; however, if there is concern that not all samples will reach 12 nM, adjusting Normalase chemistry to require a minimum of only 6 nM can alternatively be performed.

Use this simple [calculator](#) for converting between ng/ $\mu$ L and nM. Finish with a library size of 285 bp, or a size appropriate to the panel (for use in the Base Pair Length column).

The workflow consists of three steps for libraries amplified to a minimum yield of 12 nM during the adapter attachment and indexing PCR step:

1. **Normalase I** to enzymatically select a 4 nM (or 2 nM if using  $\geq 6$  nM option) library fraction
2. **Equal volume library pooling** of samples for multiplexed sequencing
3. **Normalase II** to enzymatically generate an equimolar library pool



**Figure 2. Workflow schematic.** Normalase I Master Mix is added to samples and incubated at 30°C for 15 minutes. Sample pooling is performed, and then the Normalase II Master Mix is added to the pool and incubated at 37°C for 15 minutes. Reagent X1 inactivates the reaction and a final, equimolar pool is produced.

## Normalase specification

The Normalase product specification is defined by cluster density of the Normalase pool when loaded on a MiSeq® v2 flow cell at 12 pM to achieve a 1000–1200 K/mm<sup>2</sup> cluster density and CV  $\leq 15\%$  within a pool. Across Illumina platforms, library types, and insert sizes, optimization of loading concentration may be required to achieve the optimal number of reads supported by the flow cell of choice.



## Perform the Normalase I reaction

If you are concerned that the 12 nM threshold has not been met for each library after indexing PCR:

- Spot check library yields using any fluorometric method (e.g., Qubit fluorometer) or electrophoretic method (e.g., Bioanalyzer machine).
- A Normalase workflow modification can be performed that requires a 6 nM threshold to obtain a 2 nM Normalase pool (see below).
  1. Preset a thermal cycler program as listed below.

### Thermal cycler program

15 min at 30°C with open lid or lid heating OFF

2. Prepare the Normalase I Master Mix, as listed in the table below. The mix can be prepared at room temperature and stored on ice until use if prepared in advance. Ensure that it is thoroughly mixed by moderate vortexing followed by a pulse spin to collect contents prior to use. For libraries with lower yields >6 nM, or for a final pool of 2 nM (instead of 4 nM), use only half the specified volume of Reagent S2 with an equal volume of TE, reducing the concentration by two-fold.

Reagent	Per library (µL)	16 libraries (µL)	96 libraries (µL)
• Buffer S1	4.3	68.8	412.8
• Reagent S2	0.2	3.2	19.2
• Enzyme S3	0.5	8.0	48
<b>Total volume</b>	<b>5</b>	<b>80</b>	<b>480</b>







**Important:** The Normalase I Master Mix should be built for a minimum of 10 reactions to ensure pipetting accuracy.

3. With a calibrated P10 pipette, add 5 µL of Normalase I Master Mix into each 20 µL library eluate at room temperature, then thoroughly mix by moderate vortexing for 5 seconds.
4. Spin down the sample tube in a microcentrifuge. Place in the thermal cycler and run the program described in step 1.

## Perform equal volume library pooling

Sufficient Normalase II reagents are supplied so that this step can be repeated to enable various repooling combinations, as only 5 µL of post-Normalase I library (out of a 25 µL volume) is used for pooling. Also note that stability of normalized pools (after Normalase II) is limited, with a storage time of four weeks, since the resulting normalized pools contain single-stranded DNA. Therefore, if re-sequencing is required after four weeks, re-pool the Normalase I libraries and repeat Normalase II and inactivation.

-  **Note:** If pooling <5 libraries, see the [Appendix A: Sequencing recommendations](#) for low-plex pooling recommendations.
  -  **Note:** If pooling 5 µL per sample does not generate a normalized pool of sufficient volume for instrument loading, see the [Appendix A: Sequencing recommendations](#) for high sample volume pooling recommendations.
  -  **Important:** Consider your desired number of reads for each sample and pool only those samples together that have the same required depth. For example, samples each requiring 50,000 reads can be pooled together, whereas samples requiring 1 million reads should be combined in a separate pool. Thus, you can adjust your ratio of pools when loading the instrument to achieve the desired sequence depth for each pool.
- Following the Normalase I incubation, generate a library pool (or pools) by placing 5 µL of each individual library into a single 0.2 mL PCR tube if pooling 30 libraries or less (achieves up to a final volume of 186 µL). Alternatively, use a 1.5 mL microcentrifuge tube, particularly when pooling greater than 30 libraries, as the volume will exceed the PCR tube maximum volume.
-  **Tip:** To ensure even pooling, use a calibrated P10 pipette.
- Thoroughly mix samples, spin the library pools in a microcentrifuge, and proceed to [Perform the Normalase II reaction](#).

## Perform the Normalase II reaction

- Preset a thermal cycler program as shown below. Alternatively, if using a 1.5 mL microcentrifuge tube, set a heat block at 37°C.

Thermal cycler program	Heat block (1.5 mL microcentrifuge tube)
15 min at 37°C with open lid or lid heating OFF	15 min at 37°C

- Pre-mix Normalase II Master Mix (listed in the table below). The master mix can be stored on ice until use and then added to pools at room temperature.

Reagent	Per library (µL)	24 libraries (µL)	96 libraries (µL)
• Buffer N1	0.96	23.04	92.16
• Enzyme N2	0.04	0.96	3.84
<b>Total volume</b>	<b>1</b>	<b>24</b>	<b>96</b>

\* It is recommended to prepare Normalase II Master Mix for a minimum of 16 samples, even if you are processing less than 16 samples, to avoid pipetting extremely low volumes. For best results, use a calibrated, P2 pipette to add Enzyme N2. Although sufficient reagents are supplied for up to 5 repeated Normalase II reactions per sample, repeatedly processing a lower number of samples will result in significant loss of Normalase II reagents.

- Add 1 µL of Normalase II Master Mix, multiplied by the total number of libraries in each prepared pool.
- Mix well by vortexing for 5 seconds, and spin down the library pools in a microcentrifuge.
- Place the library pools in the thermal cycler and run the program or place the 1.5 mL microcentrifuge tubes into the 37°C heat block.

## Perform Normalase inactivation

- Following the Normalase II reaction, preset a thermal cycler program as shown below. Alternatively, if using a 1.5 mL microcentrifuge tube, set a heat block at 95°C.

Thermal cycler program	Heat block (1.5 mL microcentrifuge tube)
Hold at 95°C	
2 min at 95°C with lid kept at 95°C	2 min at 95°C
Hold at 4°C	

- Add 0.2 µL of Reagent X1, multiplied by the total number of libraries in each prepared pool. See examples below:

Reagent	Per library	24-plex pool	96-plex pool
• Reagent X1	0.2 µL	4.8 µL	19.2 µL

- Place the library pools in the thermal cycler and advance the program, or place the 1.5 mL microcentrifuge tubes into the heat block. If using a 1.5 mL microcentrifuge tube, set a heat block at 95°C to incubate your library pools, being careful not to incubate the samples longer than 2 minutes.
- Your final multiplexed library pools are now equimolar. Proceed to qPCR quantification of your Normalase pool and sequencing. It is not necessary to perform an additional purification step.

## Perform calibration of Normalase pools

For better sequencing results, perform a qPCR quantification on your final Normalase pool(s). Final library pools are ssDNA and cannot be quantified by dsDNA-based fluorometric methods or electrophoretic fragment analysis. If you do not have a qPCR assay, use a commercially available kit by calibrating your qPCR results and sequencer loading concentrations for optimal clustering before proceeding (KAPA Library Quantification Kit, Cat. No. KK4828).

Calibration of Normalase output to your qPCR assay, sequencer loading procedure, and clustering output is required during testing due to variation across different qPCR assays and laboratory practices. Use a qPCR assay that predicts an optimal number of reads on your sequencing instrument, as this is important to achieve Normalase calibration. Across other Illumina platforms, library types, and insert sizes, optimization of loading concentration may be required to achieve the optimal number of reads supported by the sequencing flow cell of choice. If you have chosen the 6 nM to 2 nM option but require a higher pool concentration for your sequencer, perform a 2.0X SPRI cleanup to concentrate pools and then proceed to qPCR quantification and loading.

Once you have calibrated your sequencer loading procedure to the Normalase output and have established that your samples meet the minimum threshold for Normalase, qPCR of the final pool is optional but still recommended. For example, a Normalase workflow error may have occurred that would lead to unexpected results.

## APPENDIX A: SEQUENCING RECOMMENDATIONS

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xGen Amplicon Panel libraries may be sequenced using paired-end sequencing on Illumina instruments. We strongly recommend using 2 x 150 paired-end reads. The depth of coverage required will depend on the application (refer to the [Overview](#) section). Be sure to use either 8 (CD) or 10 (UD) sequencing cycles for each index read.

See [Appendix E](#) for panel-specific information on panel content and size, as well as multiplexed sequencing recommendations.

Due to the complexity of the libraries, no PhiX spike-in is required on the MiSeq™ or MiniSeq™ (Illumina) instruments. The NextSeq™ 550 (Illumina) may be sensitive to low complexity and PhiX or another suitable high-complexity library spike-in may be required. Contact Illumina technical support for further information regarding sequencing instrument compatibility with low-complexity sequences.

# APPENDIX B: SEQUENCING DATA ANALYSIS

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xGen Amplicon data analysis can be performed with your favorite NGS analysis toolset on Linux, cloud, or desktop applications. Similar to any amplicon data analysis, the most critical step is primer trimming or clipping to remove or soft-clip the synthetic primer sequences used in the PCR amplification step. IDT recommends post-alignment primer clipping using Fgbio [TrimPrimers](#) or [Primerclip™](#) tool.

## Analysis overview

Below are the commonly used suggested analysis steps:

1. Illumina Adapter trimming and filtering out reads <30 bases long ([Trimmomatic](#))
2. Alignment ([BWA-MEM](#), (see minimap2 for long-read alignment))
3. Primer trimming ([Primerclip](#) or [TrimPrimers](#))
4. On-target and coverage uniformity calculation using [Picard tools](#)
5. Variant calling of your choice ([GATK Haplotype Caller](#) for germline calls, lofreq, mutect or Vardict for somatic or low frequency variant calls)



**Tip:** For more detailed information about primer trimming, review the [Primerclip Application Note](#).

## APPENDIX C: INDEXED ADAPTER SEQUENCES

The full-length adapter sequences are below, where the underlined text indicates the location of the index sequences which are 8 bp for CDI and 10 bp for UDI. These sequences represent the adapter sequences following completion of the indexing PCR step.

### Index 1 (i7) adapters:

5' – GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXXXX(XX)ATCTCGTATGCCGTCTTCTGCTTG – 3'

### Index 2 (i5) adapters:

5' – AATGATACGGCGACCACCGAGATCTACACYYYYYYYY(YY)AACTCTTTCCCTACACGACGCTCTTCCGATCT – 3'

Refer to the accompanying [Index Sequences Master List](#) for index sequences for preparing your Illumina sequencing sample sheet on the instrument of your choice.

## APPENDIX D: PRIMER SEQUENCES

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For reference, the primer sequences are below. These primers include full-length Illumina adapter and index sequences.

**i7 primer:** Replace 8 CDI or 10 UDI **X's** with the **REVERSE COMPLEMENT** of the specified i7 index sequence in the Index Master List:

5' – CAAGCAGAAGACGGCATACGAGAT**XXXXXXXXXX(XX)**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT – 3'

**i5 primer:** Replace 8 CDI or 10 UDI **Y's** with the specified Forward Strand Workflow i5 index sequence in the Index Master List:

5' – AATGATACGGCGACCACCGAGATCTACAC**YYYYYYYY(YY)**ACACTCTTCCCTACACGACGCTCTTCCGATCT – 3'

Go to [www.idtdna.com/ContactUs](http://www.idtdna.com/ContactUs) if you would like assistance confirming compatibility of your own primers with the xGen Amplicon Panel workflow, or your local sales representative or distributor to inquire about the purchase of custom Normalase Indexing Primers that use your own index sequences.

# APPENDIX E: MULTIPLEX SEQUENCING RECOMMENDATIONS

Panels	Number of amplicons	Average amplicon size (bp)	Total target size (kb)	Number of multiplexing on MiSeq v2 standard	
				Germline 500X average read depth	Somatic 5000X average read depth
Oncology panels					
xGen 56G Oncology Amplicon Panel v2	263	138	1.5	220	22
xGen 57G Pan-Cancer Amplicon Panel	285	139	25.9	200	20
xGen BRCA1 BRCA2 Amplicon Panel	246	148	22.9	240	24
xGen BRCA1 BRCA2 PALB2 Amplicon Panel	302	149	28.9	190	19
xGen EGFR Pathway Amplicon Panel	17	136	1.5	3520*	352
xGen TP53 Amplicon Panel	21	140	1.8	2850*	285
xGen Lung Amplicon Panel	208	144	18.0	280	28
xGen Colorectal Amplicon Panel	186	143	17.0	320	32
xGen Lynch Syndrome Amplicon Panel	180	140	15.0	330	33
xGen Myeloid Amplicon Panel	478	142	42.0	120	12
Inherited Disease Panels					
xGen CFTR Amplicon Panel	87	143	10.0	384	38
xGen ACE2 Gene Amplicon Panel	41	150	4.0	1463	146
Sample tracking panel					
xGen Sample_ID Amplicon Panel	104	145	N/A—SNPs only	576	N/A

\* Requires custom indexes



## APPENDIX F: PLATE USAGE GUIDELINES

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Before piercing the foil and pipetting out the necessary indexes, be sure to thaw the plate at room temperature, vortex briefly, then centrifuge for one minute to spin down the primer reagents to the bottom of the plate wells.

Carefully pre-pierce the foil seal for the intended well(s) prior to pipetting the primer mix out of the plate to add to your reaction(s). Pre-piercing the foil avoids accidental clogging of pipette tips used for liquid pipetting, as well as the introduction of foil into the reaction. In addition, pre-piercing the foil reduces the resistance of multi-channel pipettors, which can result in undesired movement of the plate that may cause cross-contamination of reagents. The foil may be pre-pierced with pipette tips (e.g., 8-channel or 12-channel), 8-tube strips, an unskirted 96 well plate, or a plate puncher.

During the Indexing PCR step, use 3.7  $\mu$ L of a unique indexing primer pair (SU001-SU1536 UDIs) to amplify and index each library, where the UDI primer pair must be added individually to each sample.

Libraries made with uniquely indexed adapters may be pooled prior to cluster generation, subjected to Normalase chemistry, and co-sequenced on the same Illumina flow cell.

## APPENDIX G: xGEN AMPLICON UDI PRIMER PLATE SPECIFICATIONS AND DIMENSIONS

This product is dispensed in a 96-well plate with these specifications:

Plate dimension	Low-profile 96-well skirted plates
Length at base plane	127.76 mm
Width at base plane	85.48 mm
Height overall	16.06 mm
Well depth	14.81 mm
Well diameter at opening	5.46 mm
Well diameter at bottom of conical section	2.64 mm
Well volume	200 µL
Well spacing	9.00 mm
Well angle	17.5°
<b>Well offset</b>	
Left edge to well A1	14.38 mm
Top edge to well A1	11.24 mm
Left edge to H12	113.38 mm
Top edge to H12	74.24 mm

# APPENDIX H: xGEN AMPLICON UDI PRIMER PLATE LAYOUTS

xGen Amplicon UDI Primer Plate Cat. Nos., 10009846, 10009851, 10009852, and 10009853 are sold as a bundle of 4x96-well plates. xGen Amplicon UDI Primer Plate Cat. Nos., 10009847, 10009848, 10009849, and 10009850 are sold separately as individual plates.

xGen Amplicon UDI Primer Plate Catalog No. **10009846** includes the following four plates:

## xGen Amplicon UDI Primer Plate Catalog No. 10009847

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU001	SU009	SU017	SU025	SU033	SU041	SU049	SU057	SU065	SU073	SU081	SU089
B	SU002	SU010	SU018	SU026	SU034	SU042	SU050	SU058	SU066	SU074	SU082	SU090
C	SU003	SU011	SU019	SU027	SU035	SU043	SU051	SU059	SU067	SU075	SU083	SU091
D	SU004	SU012	SU020	SU028	SU036	SU044	SU052	SU060	SU068	SU076	SU084	SU092
E	SU005	SU013	SU021	SU029	SU037	SU045	SU053	SU061	SU069	SU077	SU085	SU093
F	SU006	SU014	SU022	SU030	SU038	SU046	SU054	SU062	SU070	SU078	SU086	SU094
G	SU007	SU015	SU023	SU031	SU039	SU047	SU055	SU063	SU071	SU079	SU087	SU095
H	SU008	SU016	SU024	SU032	SU040	SU048	SU056	SU064	SU072	SU080	SU088	SU096

## xGen Amplicon UDI Primer Plate Catalog No. 10009848

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU097	SU105	SU113	SU121	SU129	SU137	SU145	SU153	SU161	SU169	SU177	SU185
B	SU098	SU106	SU114	SU122	SU130	SU138	SU146	SU154	SU162	SU170	SU178	SU186
C	SU099	SU107	SU115	SU123	SU131	SU139	SU147	SU155	SU163	SU171	SU179	SU187
D	SU100	SU108	SU116	SU124	SU132	SU140	SU148	SU156	SU164	SU172	SU180	SU188
E	SU101	SU109	SU117	SU125	SU133	SU141	SU149	SU157	SU165	SU173	SU181	SU189
F	SU102	SU110	SU118	SU126	SU134	SU142	SU150	SU158	SU166	SU174	SU182	SU190
G	SU103	SU111	SU119	SU127	SU135	SU143	SU151	SU159	SU167	SU175	SU183	SU191
H	SU104	SU112	SU120	SU128	SU136	SU144	SU152	SU160	SU168	SU176	SU184	SU192

## xGen Amplicon UDI Primer Plate Catalog No. 10009849

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU193	SU201	SU209	SU217	SU225	SU233	SU241	SU249	SU257	SU265	SU273	SU281
B	SU194	SU202	SU210	SU218	SU226	SU234	SU242	SU250	SU258	SU266	SU274	SU282
C	SU195	SU203	SU211	SU219	SU227	SU235	SU243	SU251	SU259	SU267	SU275	SU283
D	SU196	SU204	SU212	SU220	SU228	SU236	SU244	SU252	SU260	SU268	SU276	SU284
E	SU197	SU205	SU213	SU221	SU229	SU237	SU245	SU253	SU261	SU269	SU277	SU285
F	SU198	SU206	SU214	SU222	SU230	SU238	SU246	SU254	SU262	SU270	SU278	SU286
G	SU199	SU207	SU215	SU223	SU231	SU239	SU247	SU255	SU263	SU271	SU279	SU287
H	SU200	SU208	SU216	SU224	SU232	SU240	SU248	SU256	SU264	SU272	SU280	SU288

## xGen Amplicon UDI Primer Plate Catalog No. 10009850

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU289	SU297	SU305	SU313	SU321	SU329	SU337	SU345	SU353	SU361	SU369	SU377
B	SU290	SU298	SU306	SU314	SU322	SU330	SU338	SU346	SU354	SU362	SU370	SU378
C	SU291	SU299	SU307	SU315	SU323	SU331	SU339	SU347	SU355	SU363	SU371	SU379
D	SU292	SU300	SU308	SU316	SU324	SU332	SU340	SU348	SU356	SU364	SU372	SU380
E	SU293	SU301	SU309	SU317	SU325	SU333	SU341	SU349	SU357	SU365	SU373	SU381
F	SU294	SU302	SU310	SU318	SU326	SU334	SU342	SU350	SU358	SU366	SU374	SU382
G	SU295	SU303	SU311	SU319	SU327	SU335	SU343	SU351	SU359	SU367	SU375	SU383
H	SU296	SU304	SU312	SU320	SU328	SU336	SU344	SU352	SU360	SU368	SU376	SU384

xGen Amplicon UDI Primer Plate Catalog No. **10009851** includes the following four plates:

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU385	SU393	SU401	SU409	SU417	SU425	SU433	SU441	SU449	SU457	SU465	SU473
B	SU386	SU394	SU402	SU410	SU418	SU426	SU434	SU442	SU450	SU458	SU466	SU474
C	SU387	SU395	SU403	SU411	SU419	SU427	SU435	SU443	SU451	SU459	SU467	SU475
D	SU388	SU396	SU404	SU412	SU420	SU428	SU436	SU444	SU452	SU460	SU468	SU476
E	SU389	SU397	SU405	SU413	SU421	SU429	SU437	SU445	SU453	SU461	SU469	SU477
F	SU390	SU398	SU406	SU414	SU422	SU430	SU438	SU446	SU454	SU462	SU470	SU478
G	SU391	SU399	SU407	SU415	SU423	SU431	SU439	SU447	SU455	SU463	SU471	SU479
H	SU392	SU400	SU408	SU416	SU424	SU432	SU440	SU448	SU456	SU464	SU472	SU480

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU481	SU489	SU497	SU505	SU513	SU521	SU529	SU537	SU545	SU553	SU561	SU569
B	SU482	SU490	SU498	SU506	SU514	SU522	SU530	SU538	SU546	SU554	SU562	SU570
C	SU483	SU491	SU499	SU507	SU515	SU523	SU531	SU539	SU547	SU555	SU563	SU571
D	SU484	SU492	SU500	SU508	SU516	SU524	SU532	SU540	SU548	SU556	SU564	SU572
E	SU485	SU493	SU501	SU509	SU517	SU525	SU533	SU541	SU549	SU557	SU565	SU573
F	SU486	SU494	SU502	SU510	SU518	SU526	SU534	SU542	SU550	SU558	SU566	SU574
G	SU487	SU495	SU503	SU511	SU519	SU527	SU535	SU543	SU551	SU559	SU567	SU575
H	SU488	SU496	SU504	SU512	SU520	SU528	SU536	SU544	SU552	SU560	SU568	SU576

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU577	SU585	SU593	SU601	SU609	SU617	SU625	SU633	SU641	SU649	SU657	SU665
B	SU578	SU586	SU594	SU602	SU610	SU618	SU626	SU634	SU642	SU650	SU658	SU666
C	SU579	SU587	SU595	SU603	SU611	SU619	SU627	SU635	SU643	SU651	SU659	SU667
D	SU580	SU588	SU596	SU604	SU612	SU620	SU628	SU636	SU644	SU652	SU660	SU668
E	SU581	SU589	SU597	SU605	SU613	SU621	SU629	SU637	SU645	SU653	SU661	SU669
F	SU582	SU590	SU598	SU606	SU614	SU622	SU630	SU638	SU646	SU654	SU662	SU670
G	SU583	SU591	SU599	SU607	SU615	SU623	SU631	SU639	SU647	SU655	SU663	SU671
H	SU584	SU592	SU600	SU608	SU616	SU624	SU632	SU640	SU648	SU656	SU664	SU672

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU673	SU681	SU689	SU697	SU705	SU713	SU721	SU729	SU737	SU745	SU753	SU761
B	SU674	SU682	SU690	SU698	SU706	SU714	SU722	SU730	SU738	SU746	SU754	SU762
C	SU675	SU683	SU691	SU699	SU707	SU715	SU723	SU731	SU739	SU747	SU755	SU763
D	SU676	SU684	SU692	SU700	SU708	SU716	SU724	SU732	SU740	SU748	SU756	SU764
E	SU677	SU685	SU693	SU701	SU709	SU717	SU725	SU733	SU741	SU749	SU757	SU765
F	SU678	SU686	SU694	SU702	SU710	SU718	SU726	SU734	SU742	SU750	SU758	SU766
G	SU679	SU687	SU695	SU703	SU711	SU719	SU727	SU735	SU743	SU751	SU759	SU767
H	SU680	SU688	SU696	SU704	SU712	SU720	SU728	SU736	SU744	SU752	SU760	SU768

xGen Amplicon UDI Primer Plate Catalog No. **10009852** includes the following four plates:

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU769	SU777	SU785	SU793	SU801	SU809	SU817	SU825	SU833	SU841	SU849	SU857
B	SU770	SU778	SU786	SU794	SU802	SU810	SU818	SU826	SU834	SU842	SU850	SU858
C	SU771	SU779	SU787	SU795	SU803	SU811	SU819	SU827	SU835	SU843	SU851	SU859
D	SU772	SU780	SU788	SU796	SU804	SU812	SU820	SU828	SU836	SU844	SU852	SU860
E	SU773	SU781	SU789	SU797	SU805	SU813	SU821	SU829	SU837	SU845	SU853	SU861
F	SU774	SU782	SU790	SU798	SU806	SU814	SU822	SU830	SU838	SU846	SU854	SU862
G	SU775	SU783	SU791	SU799	SU807	SU815	SU823	SU831	SU839	SU847	SU855	SU863
H	SU776	SU784	SU792	SU800	SU808	SU816	SU824	SU832	SU840	SU848	SU856	SU864

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU865	SU873	SU881	SU889	SU897	SU905	SU913	SU921	SU929	SU937	SU945	SU953
B	SU866	SU874	SU882	SU890	SU898	SU906	SU914	SU922	SU930	SU938	SU946	SU954
C	SU867	SU875	SU883	SU891	SU899	SU907	SU915	SU923	SU931	SU939	SU947	SU955
D	SU868	SU876	SU884	SU892	SU900	SU908	SU916	SU924	SU932	SU940	SU948	SU956
E	SU869	SU877	SU885	SU893	SU901	SU909	SU917	SU925	SU933	SU941	SU949	SU957
F	SU870	SU878	SU886	SU894	SU902	SU910	SU918	SU926	SU934	SU942	SU950	SU958
G	SU871	SU879	SU887	SU895	SU903	SU911	SU919	SU927	SU935	SU943	SU951	SU959
H	SU872	SU880	SU888	SU896	SU904	SU912	SU920	SU928	SU936	SU944	SU952	SU960

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU961	SU969	SU977	SU985	SU993	SU1001	SU1009	SU1017	SU1025	SU1033	SU1041	SU1049
B	SU962	SU970	SU978	SU986	SU994	SU1002	SU1010	SU1018	SU1026	SU1034	SU1042	SU1050
C	SU963	SU971	SU979	SU987	SU995	SU1003	SU1011	SU1019	SU1027	SU1035	SU1043	SU1051
D	SU964	SU972	SU980	SU988	SU996	SU1004	SU1012	SU1020	SU1028	SU1036	SU1044	SU1052
E	SU965	SU973	SU981	SU989	SU997	SU1005	SU1013	SU1021	SU1029	SU1037	SU1045	SU1053
F	SU966	SU974	SU982	SU990	SU998	SU1006	SU1014	SU1022	SU1030	SU1038	SU1046	SU1054
G	SU967	SU975	SU983	SU991	SU999	SU1007	SU1015	SU1023	SU1031	SU1039	SU1047	SU1055
H	SU968	SU976	SU984	SU992	SU1000	SU1008	SU1016	SU1024	SU1032	SU1040	SU1048	SU1056

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU1057	SU1065	SU1073	SU1081	SU1089	SU1097	SU1105	SU1113	SU1121	SU1129	SU1137	SU1145
B	SU1058	SU1066	SU1074	SU1082	SU1090	SU1098	SU1106	SU1114	SU1122	SU1130	SU1138	SU1146
C	SU1059	SU1067	SU1075	SU1083	SU1091	SU1099	SU1107	SU1115	SU1123	SU1131	SU1139	SU1147
D	SU1060	SU1068	SU1076	SU1084	SU1092	SU1100	SU1108	SU1116	SU1124	SU1132	SU1140	SU1148
E	SU1061	SU1069	SU1077	SU1085	SU1093	SU1101	SU1109	SU1117	SU1125	SU1133	SU1141	SU1149
F	SU1062	SU1070	SU1078	SU1086	SU1094	SU1102	SU1110	SU1118	SU1126	SU1134	SU1142	SU1150
G	SU1063	SU1071	SU1079	SU1087	SU1095	SU1103	SU1111	SU1119	SU1127	SU1135	SU1143	SU1151
H	SU1064	SU1072	SU1080	SU1088	SU1096	SU1104	SU1112	SU1120	SU1128	SU1136	SU1144	SU1152

xGen Amplicon UDI Primer Plate Catalog No. **10009853** includes the following four plates:

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU1153	SU1161	SU1169	SU1177	SU1185	SU1193	SU1201	SU1209	SU1217	SU1225	SU1233	SU1241
B	SU1154	SU1162	SU1170	SU1178	SU1186	SU1194	SU1202	SU1210	SU1218	SU1226	SU1234	SU1242
C	SU1155	SU1163	SU1171	SU1179	SU1187	SU1195	SU1203	SU1211	SU1219	SU1227	SU1235	SU1243
D	SU1156	SU1164	SU1172	SU1180	SU1188	SU1196	SU1204	SU1212	SU1220	SU1228	SU1236	SU1244
E	SU1157	SU1165	SU1173	SU1181	SU1189	SU1197	SU1205	SU1213	SU1221	SU1229	SU1237	SU1245
F	SU1158	SU1166	SU1174	SU1182	SU1190	SU1198	SU1206	SU1214	SU1222	SU1230	SU1238	SU1246
G	SU1159	SU1167	SU1175	SU1183	SU1191	SU1199	SU1207	SU1215	SU1223	SU1231	SU1239	SU1247
H	SU1160	SU1168	SU1176	SU1184	SU1192	SU1200	SU1208	SU1216	SU1224	SU1232	SU1240	SU1248

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU1249	SU1257	SU1265	SU1273	SU1281	SU1289	SU1297	SU1305	SU1313	SU1321	SU1329	SU1337
B	SU1250	SU1258	SU1266	SU1274	SU1282	SU1290	SU1298	SU1306	SU1314	SU1322	SU1330	SU1338
C	SU1251	SU1259	SU1267	SU1275	SU1283	SU1291	SU1299	SU1307	SU1315	SU1323	SU1331	SU1339
D	SU1252	SU1260	SU1268	SU1276	SU1284	SU1292	SU1300	SU1308	SU1316	SU1324	SU1332	SU1340
E	SU1253	SU1261	SU1269	SU1277	SU1285	SU1293	SU1301	SU1309	SU1317	SU1325	SU1333	SU1341
F	SU1254	SU1262	SU1270	SU1278	SU1286	SU1294	SU1302	SU1310	SU1318	SU1326	SU1334	SU1342
G	SU1255	SU1263	SU1271	SU1279	SU1287	SU1295	SU1303	SU1311	SU1319	SU1327	SU1335	SU1343
H	SU1256	SU1264	SU1272	SU1280	SU1288	SU1296	SU1304	SU1312	SU1320	SU1328	SU1336	SU1344

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU1345	SU1353	SU1361	SU1369	SU1377	SU1385	SU1393	SU1401	SU1409	SU1417	SU1425	SU1433
B	SU1346	SU1354	SU1362	SU1370	SU1378	SU1386	SU1394	SU1402	SU1410	SU1418	SU1426	SU1434
C	SU1347	SU1355	SU1363	SU1371	SU1379	SU1387	SU1395	SU1403	SU1411	SU1419	SU1427	SU1435
D	SU1348	SU1356	SU1364	SU1372	SU1380	SU1388	SU1396	SU1404	SU1412	SU1420	SU1428	SU1436
E	SU1349	SU1357	SU1365	SU1373	SU1381	SU1389	SU1397	SU1405	SU1413	SU1421	SU1429	SU1437
F	SU1350	SU1358	SU1366	SU1374	SU1382	SU1390	SU1398	SU1406	SU1414	SU1422	SU1430	SU1438
G	SU1351	SU1359	SU1367	SU1375	SU1383	SU1391	SU1399	SU1407	SU1415	SU1423	SU1431	SU1439
H	SU1352	SU1360	SU1368	SU1376	SU1384	SU1392	SU1400	SU1408	SU1416	SU1424	SU1432	SU1440

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU1441	SU1449	SU1457	SU1465	SU1473	SU1481	SU1489	SU1497	SU1505	SU1513	SU1521	SU1529
B	SU1442	SU1450	SU1458	SU1466	SU1474	SU1482	SU1490	SU1498	SU1506	SU1514	SU1522	SU1530
C	SU1443	SU1451	SU1459	SU1467	SU1475	SU1483	SU1491	SU1499	SU1507	SU1515	SU1523	SU1531
D	SU1444	SU1452	SU1460	SU1468	SU1476	SU1484	SU1492	SU1500	SU1508	SU1516	SU1524	SU1532
E	SU1445	SU1453	SU1461	SU1469	SU1477	SU1485	SU1493	SU1501	SU1509	SU1517	SU1525	SU1533
F	SU1446	SU1454	SU1462	SU1470	SU1478	SU1486	SU1494	SU1502	SU1510	SU1518	SU1526	SU1534
G	SU1447	SU1455	SU1463	SU1471	SU1479	SU1487	SU1495	SU1503	SU1511	SU1519	SU1527	SU1535
H	SU1448	SU1456	SU1464	SU1472	SU1480	SU1488	SU1496	SU1504	SU1512	SU1520	SU1528	SU1536

## xGen™ Amplicon Panels for oncology research, inherited disease research, and sample tracking

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