



# xGen™ SARS-CoV-2 Amplicon Panel

## xGen SARS-CoV-2 S Gene Amplicon Panel

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# REVISION HISTORY

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Version	Release date	Description of changes
2	May 2022	Updated "off-bead" PCR bead ratio and analysis modes in Appendix B
1	December 2021	Initial release

## Table of contents

Revision history	2
Overview	5
Supported applications and sample types	5
Research applications	5
Research sample types	5
Workflow	6
Consumables and equipment	7
Consumables from IDT—Kit contents	7
Customizable IDT consumables	7
Consumables from IDT—Reagents	8
Consumables from other suppliers	9
Equipment	9
Guidelines	10
Reagent handling	10
cDNA synthesis and input considerations	10
Avoid cross-contamination	11
Size selection during cleanups	11
Notes on automation	11
Protocol	12
Perform multiplex PCR	12
Prepare panel-specific, multiplex PCR reaction mix	12
Prepare Indexing PCR	13
Perform post-multiplex PCR cleanup	13
Perform Indexing PCR	14
Perform post-indexing PCR cleanup	14
Perform library quantification	15
Introduction to Normalase treatment	16

Perform Normalase I step	17
Perform equal volume library pooling	18
Perform Normalase II step	18
Perform Normalase inactivation	19
Perform calibration of Normalase pools	19
<b>Appendixes</b>	<b>20</b>
Appendix A: Sequencing recommendations	20
Appendix B: Sequencing data analysis	20
Dockerized data analysis workflow	20
Appendix C: Low viral load input recommendations	21
Perform multiplex PCR	21
Perform indexing PCR	21
Appendix D: Indexing primer sequences	21
Appendix E: Plate usage guidelines	22
Appendix F: xGen Amplicon UDI primer plate specifications	22
Appendix G: xGen Amplicon UDI primer plate layouts	23

# OVERVIEW

The xGen™ SARS-CoV-2 Kits provide the reagents for creating next generation sequencing (NGS) workflows on Illumina® sequencing platforms for research purposes. These kits leverage patented, multiplex PCR technology, enabling library construction from first- or second-strand cDNA using a single pool of tiled, multiplexed primer pairs to target the 29.9 kilobase viral genome, or S gene. Primers were designed against the NCBI Reference Sequence NC\_045512.2 (severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome).

Features	Specifications
Panel information	<b>xGen SARS-CoV-2 Amplicon Panel</b> 99.7% (29,828 of 29,903 total bases) 345 amplicons, sized 116–255 bp (average 150 bp)
	<b>xGen SARS-CoV-2 S Gene Amplicon Panel</b> 100% (3,819 of 3,819 S gene DNA bases) 43 amplicons, sized 140–150 bp (average 146 bp)
Input material	1st or 2nd strand cDNA Minimum 10–100+ viral copies (RT-qPCR Ct value 30–40)
Time	2-hr workflow: cDNA-to-library
	3-hr workflow: cDNA-to-normalized library pool
Components	Reagent G1 (Target-specific multiplex primer pool)* PCR and library prep reagents xGen Normalase reagents (optional, included) Dual-indexed primers*
<b>Note: Kits do not include RT module or magnetic beads</b>	
Multiplexing capability	Up to 96 Combinatorial Dual Index (CDI) Up to 1536 Unique Dual Index (UDI)
Recommended read depth	250–500K reads/library for xGen SARS-CoV-2 Amplicon Panel 200K reads/library for xGen SARS-CoV-2 S Gene Amplicon Panel

\*Customizable Reagent G1 is available. Contact us for more information at [applicationsupport@idtdna.com](mailto:applicationsupport@idtdna.com).

## Supported applications and sample types

### Research applications

- Identification
- Variant calling
- Epidemiological studies
- Public health surveillance

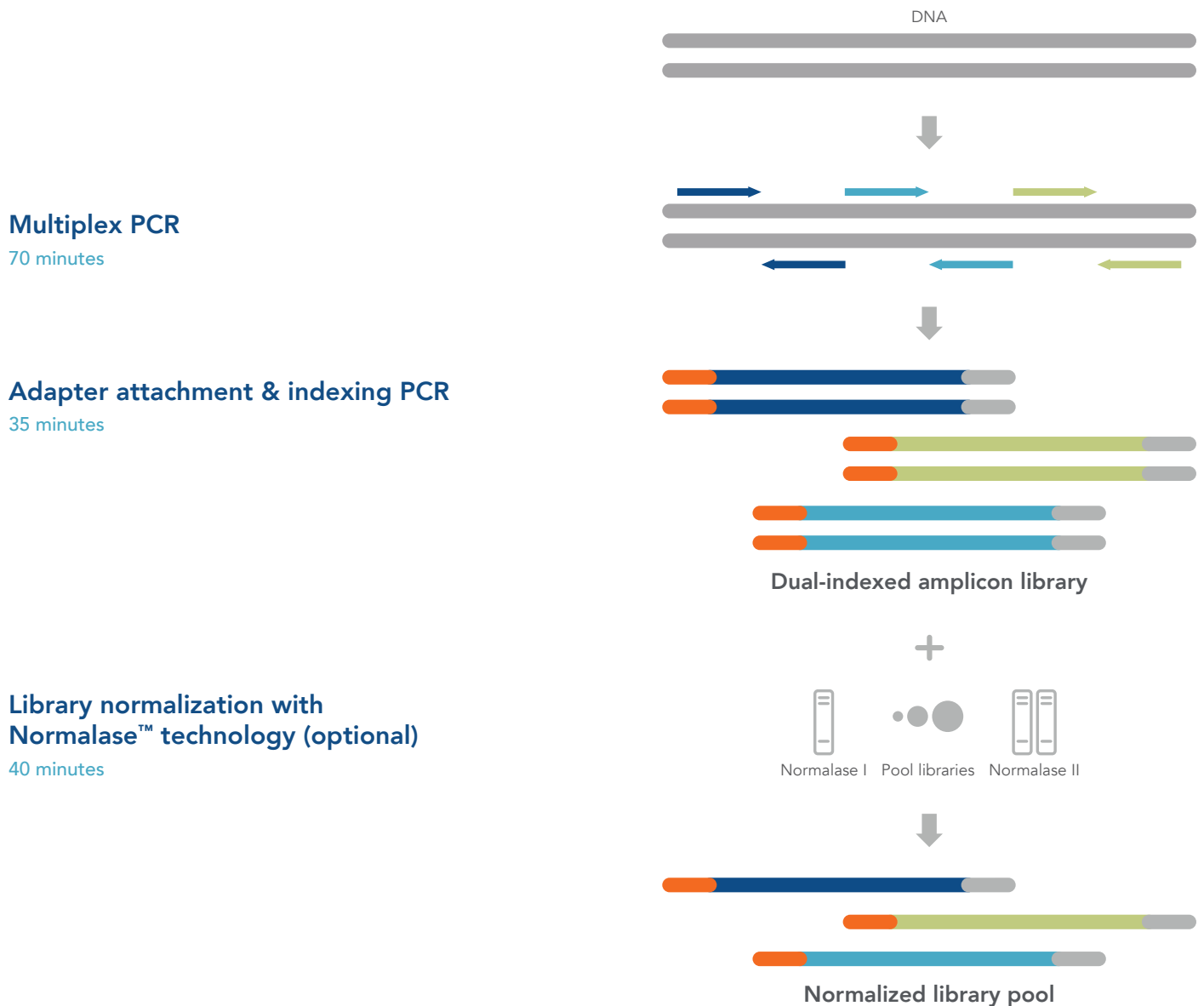
### Research sample types

- Nasopharyngeal/oropharyngeal swabs
- Sputum
- Bronchoalveolar lavage (BAL)
- Stool
- Wastewater

# WORKFLOW

The xGen Amplicon Panels use a rapid, 2-hr workflow to prepare ready-to-sequence libraries. They utilize multiple, overlapping amplicons in a single tube. The PCR1 + PCR2 workflow generates comprehensive libraries, from as low as 10–100 viral genome copies. The libraries may be quantified with conventional methods, such as Qubit™ (Thermo Fisher Scientific), or an Agilent® Bioanalyzer®. The libraries can be normalized by manual pooling, or enzymatically, using the included xGen Normalase reagents.

This protocol includes instructions for a 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 Panels workflow.** A dual-index library is prepared from cDNA in three main steps: 1) multiplex PCR, 2) adapter attachment with indexing PCR, and 3) an optional Normalase step to produce equimolar library pools.

# CONSUMABLES AND EQUIPMENT

These xGen SARS-CoV-2 Amplicon Panel kits contain sufficient reagents for the preparation of 96 libraries (including 10% excess volume).

## Consumables from IDT—Kit contents

Workflow stage	Component	96 rxns	Storage (°C)
Pre-PCR	● Reagent G2	317 µL	–20
	● Enzyme G3	1584 µL	–20
	● Pre-PCR TE	6 mL	Room temperature
Post-PCR	● Reagent I1	348 µL	–20
	● Enzyme I2	53 µL	–20
	● Enzyme I3	15 µL	–20
	● Enzyme I4	2640 µL	–20
	● Post-PCR TE	6 mL	Room temperature
	PEG NaCl	6 mL	Room temperature
Normalase Module	● Buffer S1	454 µL	–20
	● Reagent S2	21 µL	–20
	● Enzyme S3	53 µL	–20
	● Buffer N1	101 µL	–20
	● Enzyme N2	10 µL	–20
	● Reagent X1	21 µL	–20

## Customizable IDT consumables

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

## Consumables from IDT—Reagents

Component	Product name	Index number	Reaction size (rxn)	Catalog number
xGen Core Reagents	xGen Amplicon Core	N/A	96	10009827
Reagent G1 (Panel-specific primers)*	xGen SARS-CoV-2 Amplicon Panel	N/A	96	10009832
	xGen SARS-CoV-2 S Gene Amplicon Panel	N/A	96	10009831
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	4 x 96	10009846
	xGen Amplicon UDI Primer Set 2	SU385-SU768	4 x 96	10009851
	xGen Amplicon UDI Primer Set 3	SU769-SU1152	4 x 96	10009852
	xGen Amplicon UDI Primer Set 4	SU1153-SU1536	4 x 96	10009853

\*Select one option for G1 and one option for the single-use indexing primers (CDI or UDI).

Each UDI primer set contains four 96-well plates. Each plate contains 96 premixed primer pairs.



## Consumables from other suppliers

Item	Supplier	Catalog number
SPRIselect® or AMPure® XP beads, or equivalent	Beckman Coulter	B23317/B23318/B23319 or A63880/A63881/A63882
Reverse transcription module that supports 2-step RT-PCR, includes random primers and has processivity >1 kb, such as the Superscript® IV First-Strand Synthesis System, or equivalent	Thermo Fisher Scientific, or equivalent	18091050
Aerosol-resistant pipette tips, ranging from 1–1000 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 equivalent	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 1–1000 µL	Various suppliers	Varies

\*All xGen Amplicon Panel libraries are tested with a Bio-Rad® T100 thermal cycler. Other instruments may not produce identical results.

# GUIDELINES

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## Reagent handling

- Upon receipt, store the xGen Amplicon Panel products at –20°C, except for the PEG solution and TE, which are stored at room temperature.
- Separate the multiplex PCR Reagents (keep in the pre-PCR area) from the Indexing and Normalase Reagents (keep in post-PCR area). For more details on using designated lab areas, see the [Avoid cross-contamination](#) section.
- To maximize use of enzyme reagents, remove enzyme tubes from –20°C storage and place on ice for 10 minutes before pipetting. Attempting to pipette enzymes at –20°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 Mix 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 before incubations reduces yields and performance of this product.

## cDNA synthesis and input considerations

The xGen SARS-CoV-2 Amplicon Panels support first- or second-strand cDNA as input. Select a cDNA synthesis module that supports two-step RT-PCR, includes random primers, and has a processivity >1 kb.

The xGen Amplicon Panels have been demonstrated with synthetic SARS-CoV-2 genome (Twist Biosciences Cat. No. 102024). As few as 10–100 viral genome copies were mixed with 50 ng of Universal Human Reference RNA (Agilent 740000) and used as input with the Superscript™ IV First-Strand Synthesis System (Thermo Fisher Scientific Cat. No. 18091050). Follow the manufacturer's protocol when using the random primers and associated specifications, with a single modification of increasing the RT incubation step (50°C) from 10 minutes to a minimum of 30 minutes. Optional host gDNA/RNA removal steps and the optional RNase H step were not performed. An input of 10 µL of the resulting cDNA was used directly in the amplicon multiplex PCR step, as described in this protocol.

 **Note:** For samples with less than ~1000 viral copies, or with Ct >28, we suggest an alternative procedure in [Appendix C: Low viral load input recommendations](#).

## Avoid cross-contamination

**!** **Important:** To reduce the risk of cDNA 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 treatment) reagents. Move samples to the 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. Use caution when opening your sample tubes after the multiplex PCR step. Follow the instructions below 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 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 PCR reagents (keep in pre-PCR area) from the Indexing and Normalase reagents (keep in post-PCR area).

## Size selection during cleanups

We developed this protocol using SPRIselect® beads from Beckman Coulter (B23317/B23318/B23319); however, these can be substituted with Agencourt AMPure® XP beads (Beckman Coulter, Cat. Nos. A63880/A63881/A63882). Other bead-based cleanup products may not perform as described in this protocol.

Make sure the beads and samples are at room temperature before use. At no time should “with bead” samples be stored on ice, as this affects binding to the magnetic beads. Briefly vortex beads to homogenize before use. Also, make sure the beads and samples never completely dry during processing.

## Notes on automation

This protocol is readily automatable. A 10% overage volume of reagents is supplied to accommodate automation. Contact us at [applicationsupport@idtdna.com](mailto:applicationsupport@idtdna.com) if you require additional reagent overage volume or would like to learn about our custom packaging options.

While IDT does not supply automated liquid handling instruments or consumables, our automation team collaborates with automation solution providers and customers to develop automated scripts for use of our kits with liquid handling platforms routinely used in NGS library preparation. Contact us at [applicationsupport@idtdna.com](mailto:applicationsupport@idtdna.com) to discuss automating the xGen Amplicon Kits with your automated liquid handling system.

# PROTOCOL

## Perform multiplex PCR

1. Pre-program the thermal cycler for Multiplex PCR and allow the block to reach 98°C before loading samples (confirm lid heating is turned ON and is set to 105°C).

Preprogram thermal cycler		
Multiplex PCR	98°C, 30 sec	
	98°C, 10 sec	
	61°C, 5 min	4 cycles
	65°C, 1 min	
	98°C, 10 sec	18 cycles*
	64°C, 1 min	
	65°C, 1 min	
	4°C, Hold	

\* The PCR cycle number can be increased for samples that may give low yields. See [Appendix C: Low viral load input recommendations](#) for adjusted thermocycling programs.

2. Gently rock (Enzyme G3) at room temperature for 5 minutes or until all solutes appear to be in solution. Place back on ice for the remainder of this protocol.
3. Load 10 µL of cDNA 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 reaction mix

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

Reagents	Volume per sample (µL)
• Reagent G1*	2
• Reagent G2	3
• Enzyme G3	15
<b>Total volume</b>	<b>20</b>

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

4. Mix the Master Mix by briefly vortexing and then add 20 µL of the Multiplex PCR Reaction Mix to each 10 µL input cDNA sample on ice. Briefly vortex, quick spin, then place in the thermal cycler and run the program.
 


**Important:** Move samples to post-PCR area before opening tubes.
5. Near the completion of the thermal cycler run, prepare the Indexing Reaction Mix in the post-PCR area.

## Prepare Indexing PCR

1. Assemble this reaction mix on ice and keep it cold until adding it to samples in the Indexing PCR step. All components of the PCR reaction mix, except the indexing primers, should be made when running multiple samples in parallel.
2. Before mixing reagents, calculate the total volume of the Master Mix based on the number of reactions required, with appropriate overage for pipetting. We suggest preparing at least 10 reactions at any one time to maintain a volume of Enzyme I3 that can be accurately pipetted. Keep prepared Master Mix on ice.

Reagents	Volume per sample (μL)
• Reagent I1	3.3
• Enzyme I2	0.5
• Enzyme I3	0.1
• Enzyme I4	25
<b>Total volume</b>	<b>28.9</b>



**Important:** Keep prepared Master Mix ON ICE during size selection and post-multiplex PCR cleanup, refer to step 1.

## Perform post-multiplex PCR cleanup

1. Make sure that the beads and samples are at room temperature. Briefly vortex beads to homogenize before use.
2. Add 30 μL (ratio: 1.0X) of magnetic beads to each 30 μL sample. Mix by vortexing. Pulse-spin the samples in a microcentrifuge to collect contents. Make sure that bead-sample suspension droplets are left on the sides of the tube. If droplets are visible, pulse-spin the samples in a microcentrifuge to collect contents.
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 the sample is on the magnet, remove and discard the supernatant without disturbing the pellet (approximately 5 μL may be left behind) using a clean pipette tip. Leave tubes on the magnet.
6. Add 180 μL of freshly prepared 80% ethanol solution to the pellet while it is still on the magnet without disturbing the pellet. Incubate for 30 seconds, then carefully remove the ethanol solution using a clean pipette tip.
7. Repeat step 6, 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 pipette tip.
9. Resuspend each bead pellet in 17.4 μL Post-PCR TE Buffer. If an off-bead PCR is preferred, place tubes back on the magnet and transfer the 17.4 μL of eluate to a fresh tube.



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

## Perform Indexing PCR

1. Program the thermal cycler with the Indexing PCR program and allow the block to reach 37°C before loading samples (confirm lid heating is turned ON and is set to reach 105°C).

Thermal cycler program		
Indexing PCR	37°C, 20 min	9 cycles*
	98°C, 30 sec	
	98°C, 10 sec	
	60°C, 30 sec	
	66°C, 1 min	
	4°C, Hold	

\* The PCR cycle number can be increased for samples that may give low yields. See [Appendix C: Low viral load input recommendations](#) for adjusted thermal cycler programs suggested for low inputs.

2. If using CDIs, add 2 µL xGen Amplicon CD Index D50X and 1.7 µL xGen Amplicon CD Index S7XX to each sample (See [Appendix E: Plate usage guidelines](#), See [Appendix C: Low viral load input recommendations](#)).

**OR**

If using UDIs, add 3.7 µL of xGen Amplicon UD indexing primer pair to each sample (See [Appendix E: Plate usage guidelines](#)).

3. Add 28.9 µL of the Indexing PCR Reaction Mix, stored on ice, to each sample. Mix thoroughly by pipetting (total volume 50 µL).
4. Place tubes in the thermal cycler and run the program.

## Perform post-indexing PCR cleanup


1. Make sure that the PEG NaCl solution is at room temperature. Briefly vortex the PEG NaCl solution to homogenize before use.
2. Add 32.5 µL (ratio: 0.65X) of PEG NaCl solution to each 50 µL sample. Mix by vortexing. Make sure no bead-sample suspension droplets are left on the sides of the tube. If droplets are visible, pulse-spin the samples in a microcentrifuge to collect contents. If performing an "off bead" PCR, use a 32.5 µL (ratio: 0.65X) 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 the sample is on the magnet, remove and discard the supernatant without disturbing the pellet (approximately 5 µL may be left behind) using a clean pipette tip. Leave tubes on the magnet.
6. Add 180 µL of freshly prepared 80% ethanol solution to the pellet while it is still on the magnet and do not disturb the pellet.
7. Repeat step 6 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. Immediately add 20 µL of post-PCR TE buffer and resuspend the pellet, mix well by pipetting up and down until homogenous. Incubate at room temperature for 2 minutes, off the magnet.
10. After incubation, place sample back on the magnet and transfer the clean, 20 µL 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, wait for a pellet to form, and transfer the eluate again.



**Safe Stop:** Store freshly prepared libraries at -20°C.

## Perform library quantification

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

 **Note:** For normalization using the Normalase method, 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, as described below.

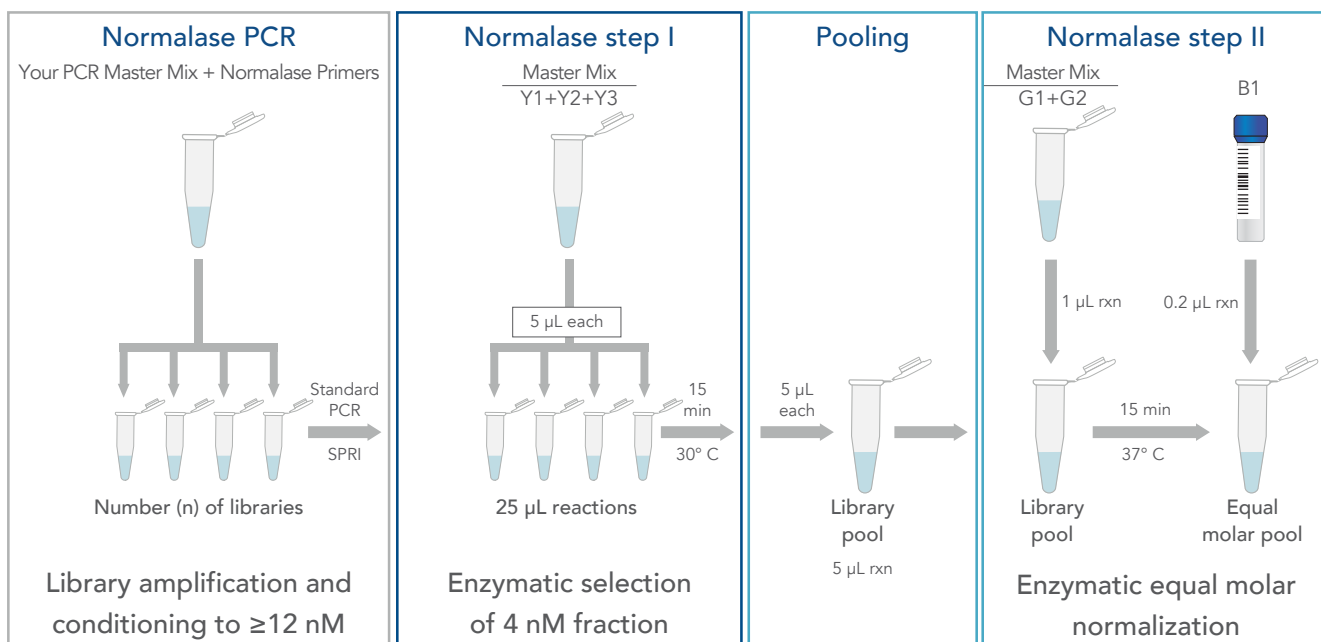
# INTRODUCTION TO NORMALASE TREATMENT

This guide provides instructions for optional, enzymatic normalization of multiplexed xGen Amplicon NGS libraries for equimolar pools and balanced sample representation in sequencing. The protocol is designed for xGen Amplicon libraries that produce consistent amplified library yields  $\geq 12$  nM after indexing PCR. Normalase treatment generates an equimolar library pool. 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.

A simple [calculator](#) for converting between ng/ $\mu$ L and nM is available. For the SARS-CoV-2 panels, use a finished library size of 285 bp (for use in the base pair length column).

The Normalase 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. Normalase workflow schematic.** Normalase I Master Mix is added to samples and incubated at 30°C for 15 minutes. Next, library pooling is performed. Normalase II Master Mix is added to the library pool and incubated at 37°C for 15 minutes. Reagent X1 inactivates the reaction, and a final equimolar pool is produced.



## Notes:

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



## Perform Normalase I step

To make sure that the 12 nM threshold has been met for each library after indexing PCR:

- Spot check library yields using any fluorometric method (i.e., Qubit™ fluorometer) or electrophoretic method (i.e., Bioanalyzer® machine).
- Perform a Normalase workflow modification, that will require a 6 nM threshold to obtain a 2 nM Normalase pool.
  1. Prepare the Normalase I Master Mix as described in the table below. The mix can be prepared at room temperature and stored on ice until use, if prepared in advance. Make sure that it is thoroughly mixed by moderate vortexing, followed by a pulse-spin to collect contents before use. Preset a thermal cycler using the program listed below.

### Thermal cycler program

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

2. For libraries with a yield >6 nM, or to obtain a final pool of 2 nM (instead of 4 nM), use half of the specified volume of Reagent S2 and add an equal volume of TE, thus reducing concentration by two-fold. Then, follow the protocol as written.

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 made for a minimum of 10 reactions to ensure pipetting accuracy.

3. Using a calibrated P10 pipette, add 5 μL of Normalase I Master Mix to each 20 μL library eluate at room temperature and thoroughly mix by moderate vortexing for 5 seconds.
4. Spin the sample tube in a microcentrifuge to collect contents. Place tube in the thermal cycler and run the program.

## Perform equal volume library pooling

Sufficient Normalase II reagents are supplied so that this step can be repeated to enable various re-pooling combinations. Only 5  $\mu$ L of post-Normalase I library (out of a 25  $\mu$ L volume) is used for pooling.

-  **Important:** The stability of normalized pools (after Normalase II) is limited. Our testing defined a maximum storage time of four weeks since the resulting normalized pools contain single-stranded DNA. If re-sequencing is required after four weeks, re-pool the Normalase I libraries and repeat Normalase II and inactivation for best results.
-  **Note:** If pooling fewer than five libraries, see the [Normalase Module protocol](#) for low-plex pooling suggested changes.
-  **Note:** If pooling 5  $\mu$ L per sample does not generate a normalized pool of sufficient volume for the instrument loading, see the [Normalase Module protocol](#) for suggested changes to compensate for pooling high sample volumes.
-  **Important:** Consider the desired number of reads for each sample, and pool only samples together that have the same required depth. For example, samples that require 50,000 reads can be pooled together, whereas samples requiring 1 million reads should be combined in a separate pool. Adjust the 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  $\mu$ L of each individual library into one 0.2 mL PCR tube if pooling 30 libraries or less (this achieves a final volume of up to 186  $\mu$ L). If pooling more than 30 libraries, use a 1.5 mL microcentrifuge tube, as the volume will exceed the PCR tube maximum volume.

To ensure even pooling, use a calibrated P10 pipette for best results.

- Thoroughly mix the samples by pipetting up and down, spin the library pools in a microcentrifuge, and proceed to the Normalase II reaction.


## Perform Normalase II step

- Preset a thermal cycler program as described below. Alternatively, if using a 1.5 mL microcentrifuge tube, set a heat block to 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

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

Reagents	Per library ( $\mu$ L)	16 libraries ( $\mu$ L)	96 libraries ( $\mu$ L)
• Buffer N1	0.96	15.36	92.16
• Enzyme N2	0.04	0.64	3.84
<b>Total volume</b>	<b>1</b>	<b>16</b>	<b>96</b>

-  **Note:** Prepare Normalase II Master Mix for a minimum of 16 samples, even if you are processing fewer than 16 samples, to avoid pipetting extremely low volumes. For best results, use a calibrated P2 pipette for adding Enzyme N2. Although sufficient reagents are supplied for up to five repeated Normalase II reactions per sample, repeatedly processing a lower number of samples will result in significant loss of Normalase II reagent volume.

- Add 1  $\mu$ L of Normalase II Master Mix for each library within the prepared pool.
- Mix samples by vortexing for 5 seconds, then spin down the library pools in a microcentrifuge to collect contents.
- Place 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 described below. Alternatively, if using a 1.5 mL microcentrifuge tube, set a heat block to 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 for each library within the prepared pool, as indicated in this table.

Reagent	Per library (µL)	16-plex pool (µL)	96-plex pool (µL)
• Reagent X1	0.2	3.2	19.2

- Place the library pools in the thermal cycler and advance the program to the 2-minute incubation step, or place the 1.5 mL microcentrifuge tubes into the heat block. If using a 1.5 mL microcentrifuge tube, set a heat block to 95°C to incubate library pools.

 **Important:** Do not incubate the samples longer than 2 minutes.

- The final, multiplexed library pools are now equimolar. Proceed to qPCR quantification of the Normalase pools and sequencing. It is not necessary to perform an additional purification step.

## Perform calibration of Normalase pools

Final library pools are ssDNA and cannot be quantified by dsDNA-based fluorometric methods or fragment analysis. Therefore, using qPCR quantification (such as the KAPA® Library Quantification Kit from Roche, or an equivalent product) on the final Normalase pool(s) is an alternative method of quantification for these samples.

The Normalase 4 nM formulation may not conform exactly with your qPCR assay quantification, due to variability in different qPCR assays and laboratory practices. Load the final pool based on results from a qPCR assay that reproducibly predicts the required number of reads on your sequencing instrument. In other Illumina® platforms, library types, and insert sizes, testing of different loading concentration may be required to achieve the number of reads supported by the 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 to concentrate pools and then proceed to qPCR quantification and loading.

Once the sequencer has been calibrated according to the Normalase output, and you have ensured that your samples meet the minimum threshold for Normalase, an option is to use qPCR on the final pool to “feel” test if the Normalase workflow produced the expected results.

# APPENDIXES

## Appendix A: Sequencing recommendations

xGen Amplicon libraries may be sequenced using paired-end sequencing on Illumina® instruments. Due to the lengths of the generated library fragments, we suggest using 2x150 paired-end reads. The depth of coverage required will depend on your research application. Be sure to use either 8 (CDI) or 10 (UDI) sequencing cycles for each index read.

For the SARS-CoV-2 Panel, >50,000 reads per library are recommended for determination of the presence/absence (+/-) confirmation, whereas 250–500K reads per library are recommended for variant calling. The following table shows examples of the number of libraries that can be multiplexed to achieve this depth per sequencing run.

Panel	Application	Libraries per sequencing run					
		MiSeq®			MiniSeq®		
		v2 Nano	v2 Micro	v2	v3	Mid-output	High-output
SARS-CoV-2 panels	+/- confirmation	40	160	600	1000	320	1000
	Variant calling	8	32	120	200	64	120
S gene	Variant calling	40	160	600	1000	320	600

Due to the complexity of the libraries, a PhiX spike-in is not required on MiSeq® or MiniSeq® (Illumina) instruments. The NextSeq550® (Illumina®) may be sensitive to low complexity libraries, so PhiX or another suitable, high-complexity library spike-in may be required. Contact Illumina® technical support for further information regarding instrument compatibility with low-complexity sequences.

## Appendix B: Sequencing data analysis

### Dockerized data analysis workflow

Those using an xGen Amplicon SARS-CoV-2 Panel who are comfortable with command line tools can use the IDT ready-to-use variant calling workflow, with all tools and reference files pre-installed and configured in a Docker image. The tool is available to run on a local Linux machine.

#### Features

- [DockerHub](#) public repository hosting one container image with tools preloaded.
- Analysis workflow includes a run script, which handles all Docker commands, requiring only the input FASTQ files and the panel master file as arguments.
- Analysis pipeline features two modes: offline mode (default) and online mode.

#### Requirements

- Linux server with Docker installed.
- At least 8 GB of RAM (≥ 32 GB recommended).

#### Analysis overview

1. Adapter trimming and filtering out of reads < 30 bases long (Trimmomatic)
2. Alignment (BWA MEM)
3. Primer trimming (Primerclip)
4. On-target and coverage metrics calculation and reporting
5. Variant calling (GATK Haplotype Caller)

Follow this [Download Link](#) for the dockerized analysis workflow. For more detailed information about primer trimming, review the [Primerclip Technical Note](#) or by contacting [applicationsupport@idtdna.com](mailto:applicationsupport@idtdna.com).

## Appendix C: Low viral load input recommendations

If samples contain a low number of viral copies, use the following adjustments to alter the number of cycles for both the Multiplex PCR and Indexing PCR thermal cycling programs.

### Perform multiplex PCR

Program the thermal cycler with the Multiplex PCR program, described below, and allow the block to reach 98°C before loading samples (confirm lid heating is turned ON and is set to reach 105°C).

Pre-program thermal cycler		
Multiplex PCR	98°C, 30 sec	
	98°C, 10 sec	
	61°C, 5 min	4 cycles
	65°C, 1 min	
	98°C, 10 sec	24 cycles
	64°C, 1 min	
	65°C, 1 min	
	4°C, Hold	

### Perform indexing PCR

Program the thermal cycler with the Indexing PCR program, described below, and allow the block to reach 37°C before loading samples (confirm lid heating is turned ON and is set to reach 105°C).

Thermal cycler program		
Indexing PCR	37°C, 20 min	
	98°C, 30 sec	
	98°C, 10 sec	
	60°C, 30 sec	5 cycles
	66°C, 1 min	
	4°C, Hold	

## Appendix D: Indexing primer sequences

These are the full-length, indexing primer sequences and the underlined text indicates the location of the index sequences (8 bp for CDI, 10 bp for UDI). These sequences represent the adapter sequences following completion of Indexing PCR.

- Index 1 (i7) Adapter:

5' – GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXXXX(XX)ATCTCGTATGCCGTCTTCTGCTTG – 3'

- Index 2 (i5) Adapter:

5' – AATGATACGGCGACCACCGAGATCTACACYYYYYYYY(YY)ACACTCTTCCCTACACGACGCTCTTCCGATCT – 3'

Use the [Index Master List](#) for preparing your Illumina® sequencing sample sheet on your instrument of choice. Contact [applicationsupport@idtdna.com](mailto:applicationsupport@idtdna.com) if you need assistance confirming compatibility of your own primers with the xGen Amplicon workflow or contact your local sales representative or distributor to inquire about the purchase of Normalase Indexing Primers that use customized index sequences.

## Appendix E: Plate usage guidelines

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

Carefully pierce the foil seal for the intended wells before adding primer mix to the reactions. Piercing the foil ahead of time avoids accidental clogging of pipette tips and avoids the introduction of foil into the reaction. In addition, pre-piercing the foil reduces the resistance to multichannel pipettes, which can result in undesired movement of the plate and 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 with a plate puncher.

During **Indexing PCR**, use 3.7 µL of a unique xGen Amplicon indexing primer pair (SU001-SU1536 UDIs) to amplify and index each library. The UDI primer pair must be added individually to each sample.

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

## Appendix F: xGen Amplicon UDI primer plate specifications

This product is dispensed in a 96-well plate. Physical specifications are below.

Plate dimensions	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°
Well offset	
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 G: xGen Amplicon UDI primer plate layouts

xGen Amplicon UDI Primer Plate Cat. Nos., 10009846, 10009851, 10009852, and 10009853 are sold as a bundle of 4 x 96-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 SARS-CoV-2 Amplicon Panel

### xGen SARS-CoV-2 S Gene Amplicon Panel

Technical support: [applicationsupport@idtdna.com](mailto:applicationsupport@idtdna.com)

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