# xGen Amplicon Panels for viral genome sequencing

**Protocol for the following xGen products:**

- xGen Amplicon Core Kit
- xGen SARS-CoV-2 Amplicon Panel
- xGen SARS-CoV-2 S Gene Amplicon Panel
- xGen Monkeypox Virus Amplicon Panel*
- xGen Amplicon Indexing Primers (CDI or UDI)
- xGen Respiratory Virus Amplicon Panel
- xGen HIV Amplicon Panel

# REVISION HISTORY

| Version | Release date | Description of changes |
|---|---|---|
| 4 | September 2024 | Updated to include Respiratory and HIV panel information and instructions |
| 3 | August 2022 | Updated to include monkeypox panel information and instructions |
| 2 | May 2022 | Updated "off-bead" PCR bead ratio and analysis modes in Appendix B |
| 1 | December 2021 | Initial release |

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# OVERVIEW

The xGen™ Amplicon Panels for viral genome sequencing provide reagents to perform next generation sequencing (NGS) workflows on Illumina™ sequencing platforms for research purposes. These kits leverage patented, multiplex PCR technology, enabling library construction from first/second-strand cDNA or genomic DNA using a single pool of tiled, multiplexed primer pairs to target whole viral genomes, or genomic regions of interest. Primers for SARS-CoV-2 were designed against the NCBI Reference Sequence NC_045512.2 (severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome). Primers for monkeypox were designed against the Reference Sequence ON568298 (West African Clade sample collected 5/19/22 in Germany, Antwerpen et al. Virological. 2022).

| Features | Specifications |
|---|---|
| Panel information | **xGen SARS-CoV-2 Amplicon Panel**<br>99.7% (29,828 of 29,903 total bases)<br>345 amplicons, sized 116–255 bp (average 150 bp) |
| | **xGen SARS-CoV-2 S Gene Amplicon Panel**<br>100% (3,819 of 3,819 S gene DNA bases)<br>43 amplicons, sized 140–150 bp (average 146 bp) |
| | **xGen Monkeypox Virus Amplicon Panel**<br>Full coverage from positions 6760-190905 (ITRs excluded)<br>1892 amplicons 93–246 bp (average 150 bp) |
| | **xGen Respiratory Virus Amplicon Panel**<br>1199 amplicons, sized 92–255 bp |
| | **xGen HIV Amplicon Panel**<br>107 amplicons, average amplicon size 102 bp |
| Input material for RNA viruses | 1st or 2nd strand cDNA from total nucleic acid extracted from biological samples<br>Minimum 10–100+ viral SARS CoV-2 genome copies (RT-qPCR Ct value 30–40)<br>Minimum 10–100 viral copies for Respiratory panel<br>Minimum 1,000 double stranded viral genome copies for HIV panel |
| Input material for DNA viruses | Viral genomic DNA<br>Minimum 300+ viral monkeypox genome copies |
| Time | 2.5-hr workflow: sample-to-library |
| | 3.5-hr workflow: sample-to-normalized library pool |
| Components | Reagent G1 (Target-specific multiplex primer pool)*<br>PCR and library prep reagent<br>xGen Normalase™ reagents (optional, included)<br>Dual-indexed primers*<br>**Note: Kits do not include RT module or magnetic beads** |
| Multiplexing capability | Up to 96 Combinatorial Dual Index (CDI)<br>Up to 1536 Unique Dual Index (UDI) |
| Recommended read depth | 250–500K reads/library for xGen SARS-CoV-2 Amplicon Panel<br>200K reads/library for xGen SARS-CoV-2 S Gene Amplicon Panel<br>500K reads/library for xGen Monkeypox Virus Amplicon Panel<br>200K reads/library for xGen Respiratory Virus Amplicon Panel<br>500,000 total reads per library for xGen HIV Amplicon Panel |

* Customizable Reagent G1 is available. For more information, go to http://www.idtdna.com/ContactUs.

# Supported applications and sample types

## Research applications

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

## Potential sample types total nucleic acid extracted from:

- Nasopharyngeal/oropharyngeal swabs
- Sputum
- Bronchoalveolar lavage (BAL)
- Stool
- Lesion material (crusts or swabs)
- Wastewater

## Positive Control samples:

- Stabilized lysates such as those available from BEI Resources
- Synthetic viral genome fragments and plasmids from various sources

# 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 for SARS-CoV-2, or 300 viral genome copies for monkeypox.

Due to the single tube workflow, the overlapping amplicon designs produce super-amplicons which maintain full target coverage when a novel mutation occurs over a priming sequence. Therefore xGen Amplicon is ideal for targeted sequencing of rapidly evolving viral genomes, see Superamplicon tech note for more information

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 multiple sample library pool.

### Multiplex PCR
70 minutes

### Adapter attachment & indexing PCR
35 minutes

### Library normalization with Normalase™ technology (optional)
40 minutes

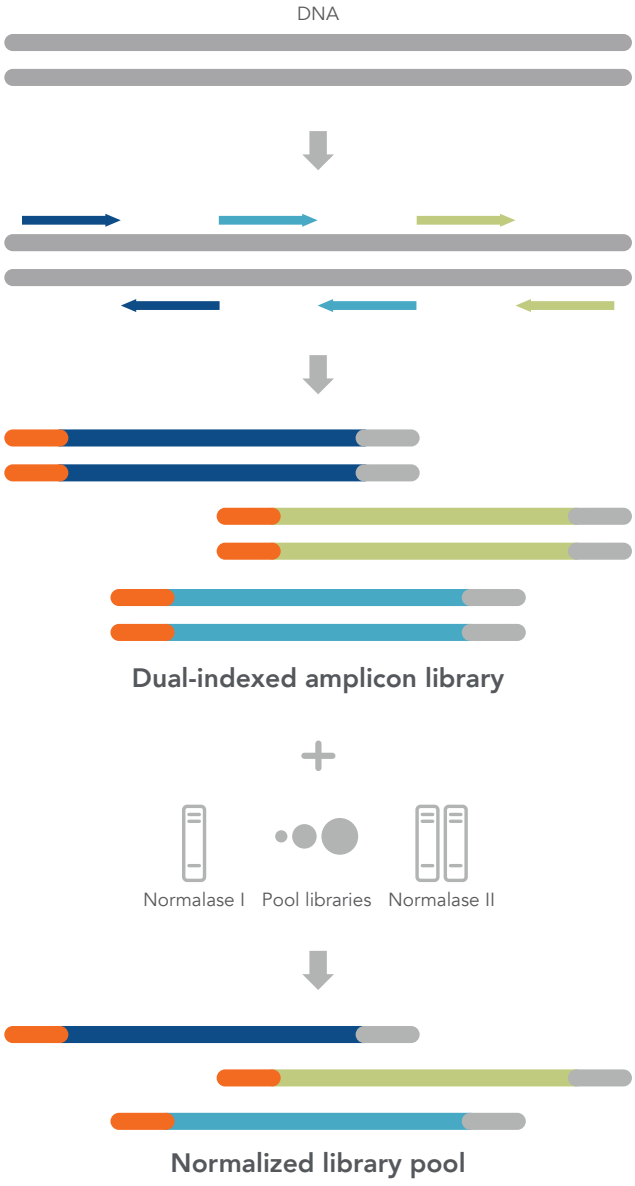


**Figure 1. xGen Amplicon Panels workflow.** A dual-index library is prepared from viral samples in three main steps: 1) multiplex PCR, 2) adapter attachment with indexing PCR, and 3) an optional Normalase step to produce equimolar library pools.

# CONSUMABLES AND EQUIPMENT

These xGen Amplicon Panels for viral genome sequencing 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 Monkeypox Virus Amplicon Panel | N/A | 96 | 10015906 |
| | xGen Respiratory Virus Panel | N/A | 96 | 10017901 |
| | xGen HIV Amplicon Panel | N/A | 96 | 10020328 |
| 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—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 |

* RT module only required for RNA viral genomes, including SARS-CoV-2

## 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

## 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.

**Important:** 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.

## Input considerations and optional cDNA synthesis

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. This panel has 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).

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.

**Important:** 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.

**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.

The xGen Monkeypox Virus Panel supports viral genomic DNA as input. A cDNA synthesis upstream module is not required. This panel has been demonstrated with BEI Monkeypox DNA NR-4928 (DQ011157). As few as 300 viral genome copies were mixed with 10 ng of Coriell NA12878 human genomic DNA and used as input for multiplexed PCR. Contact us for recommendations when using a lower viral copy number.

For more information about the Respiratory or HIV panels refer to the Respiratory Virus Amplicon Panel and the HIV Amplicon Panel protocol addendums.

If using an RT module other than SSIV, please consider using SSIV as a positive control to confirm compatibility of the alternate reagents.

When using the Respiratory or other panel that targets more than one viral genome, be aware that the library output will represent the relative abundance of viral copy number within a sample of mixed viral composition. For example, if the sample has a high copy number of one genome and low copy of another, deeper sequencing will be required to obtain reads for the less abundant genome. Alternatively, custom panels unique to one or more viral genomes can be used to avoid the requirement for higher read depth, contact tech support for more information on experimental design.

## Avoid cross-contamination

**Important:** To reduce the risk of sample 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 guidelines 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.

**Important:**

- 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.
- Make sure the beads and samples never completely dry during processing to avoid sample loss during cleanup steps.

## Notes on automation

This protocol is readily automatable. A 10% overage volume of reagents is supplied to accommodate automation. Contact us 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 to discuss automating the xGen Amplicon Kits with your automated liquid handling system.

# PROTOCOL

**Important:** For best results, it is essential to keep all multiplex PCR reagents and reaction set up on ice until placing into a pre-heated thermal cycler. Failure to keep reagents on ice will lead to low yields and poor performance.

## 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 | 4 cycles |
| | 61°C, 5 min | |
| | 65°C, 1 min | |
| | 98°C, 10 sec | SARS CoV-2:18 cycles* |
| | 64°C, 1 min | Monkeypox: 20 cycles |
| | 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 SARS-CoV-2 and 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.

   **Important:** Keep all tubes on ice during assembly of the Master Mix and the reaction, until placed in thermal cycler. Neglecting to store on ice reduces yields and performance of this product.

## 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 |
| Total volume | 20 |

* 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 |
| Total volume | 28.9 |

**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. Do not allow beads to over-dry and crack or sample recovery will be reduced.

**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 could affect 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 | |
| | 98°C, 30 sec | |
| | 98°C, 10 sec | SARS CoV-2: 9 cycles* |
| | 60°C, 30 sec | Monkeypox: 7 cycles |
| | 66°C, 1 min | Respiratory: 5 cycles<br>HIV: 9 cycles |
| | 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 ≥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; alternatively, if there is concern that not all samples will reach 12 nM, adjusting Normalase chemistry to require a minimum of only 6 nM can be performed.

For the SARS-CoV-2 and monkeypox panels, use a finished library size of 285 bp (for use in the base pair length column).

**Note:** Use this simple calculator for converting between ng/µL and nM.

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 ≥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

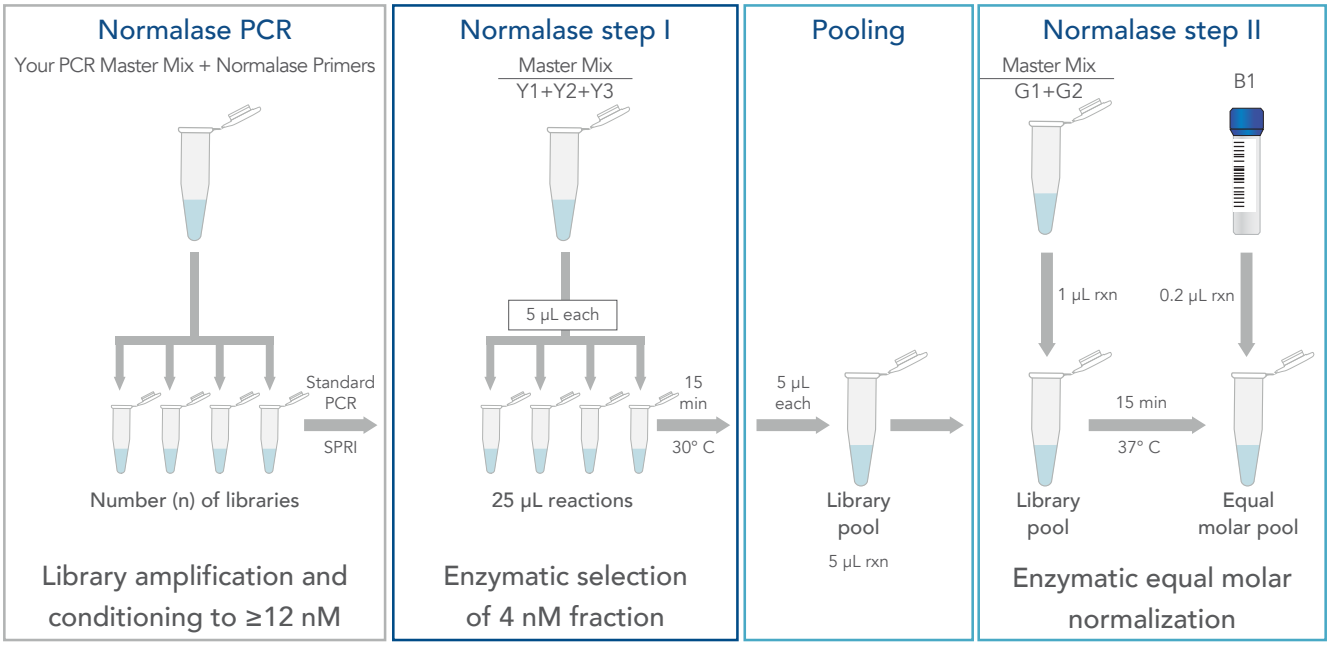


**Figure 2. Normalase workflow schematic.** Normalase I Master Mix is added to samples and incubated at 30°C for 15 minutes. Next, library pooling is performed. Normalase II Master Mix is added to the library pool and incubated at 37°C for 15 minutes. Reagent X1 inactivates the reaction, and a final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

**Notes:**

- The Normalase product specification is defined by cluster density of the Normalase pool when loaded on a MiSeq v2 flow cell (Illumina) at 12 pM to achieve a cluster density of 1000–1200 K/mm$^2$ and a CV ≤15% within a pool.
- Across Illumina 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 |
| Total volume | 5 | 80 | 480 |

**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 µL of post-Normalase I library (out of a 25 µL 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 µL 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.

1. Following the Normalase I incubation, generate a library pool (or pools) by placing 5 µL 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 µL). 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.
2. 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

1. 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 |

2. 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 (µL) | 16 libraries (µL) | 96 libraries (µL) |
|---|---|---|---|
| • Buffer N1 | 0.96 | 15.36 | 92.16 |
| • Enzyme N2 | 0.04 | 0.64 | 3.84 |
| Total volume | 1 | 16 | 96 |

**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.

3. Add 1 µL of Normalase II Master Mix for each library within the prepared pool.
4. Mix samples by vortexing for 5 seconds, then spin down the library pools in a microcentrifuge to collect contents.
5. 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

1. 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<br>2 min at 95°C with lid kept at 95°C<br>Hold at 4°C | 2 min at 95°C |

2. 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 |

3. 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.

4. 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 2 x 150 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.

When sequencing viral genomes that have significant nucleotide variation from the reference genome used for panel design, super-amplicon formation is critical to obtaining full genome coverage. In some cases, performing 2 x 250 paired-end reads will provide more complete genome coverage.For more information, see xGen™ Amplicon panels leverage super amplicon technology and product page for HIV panel data.

For the SARS-CoV-2 Panel 250–500K reads per library are recommended for strain identification/variant calling. For the Monkeypox Panel, 500k reads per library are recommended for strain identification/variant calling. The following table shows examples of the number of libraries that can be multiplexed to achieve this depth per sequencing run.

| | | Libraries per sequencing run | | | | | |
|---|---|---|---|---|---|---|---|
| | | MiSeq™* | | | | MiniSeq™ | |
| Panel | Application | v2 Nano | v2 Micro | v2 | v3 | Mid-output | High-output |
| SARS-CoV-2 panels | Variant calling | 8 | 32 | 120 | 200 | 64 | 120 |
| S gene | Variant calling | 40 | 160 | 600 | 1000 | 320 | 600 |
| Monkeypox | Variant calling§ | 4 | 16 | 60 | 100 | 129 | 100 |

* 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.

§ Read count expected to produce > 95% of target space at 10X read depth or higher.

For questions for Respiratory or HIV panels please contact technical support.

# 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. Contact us if you need further assistance for the analysis.

**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 Contact us.

**Important:** Ensure that your aligner settings do not filter out the longer super-amplicon reads, as they can be important for achieving full genome coverage over the panel target sequences.

# 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 | 4 cycles |
| | 61°C, 5 min | |
| | 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 | 8 cycles |
| | 60°C, 30 sec | |
| | 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′ – GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXX(XX)ATCTCGTATGCCGTCTTCTGCTTG – 3′

- Index 2 (i5) Adapter:

5′ – AATGATACGGCGACCACCGAGATCTACACYYYYYYYY(YY)ACACTCTTTCCCTACACGACGCTCTTCCGATCT – 3′

Use the Index Master List for preparing your Illumina sequencing sample sheet on your instrument of choice. Contact us 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 |

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