February 2022

QIAseq® SARS-CoV-2 Primer Panel Handbook

Targeted whole viral genome library preparation

Sample to Insight

QIAGEN

Contents

Kit Contents

QIAseq SARS-CoV-2 Primer Panel	**(24)**	**(96)**
Catalog no.	**333895**	**333896**
Number of reactions	**24**	**96**
RP Primer	1 x 12 µl	1 x 96 µl
EZ Reverse Transcriptase	1 x 36 µl	1 x 150 µl
RNase Inhibitor	1 x 96 µl	1 x 144 µl
Multimodal RT Buffer, 5x	2 x 60 µl	1 x 480 µl
QIAseq 2X HiFi MM	1 x 720 µl	2 x 1440 µl
QIAseq SARS-CoV-2 Pool 1*	1 x 90 µl	1 x 360 µl
QIAseq SARS-CoV-2 Pool 2*	1 x 90 µl	1 x 360 µl
Nuclease-free Water	1 tube	10 ml

* Based on primer sequences and a study (1) from the ARTIC network. In addition, coverage of region 19205-19616 has been augmented. Materials 333895 and 333896 contain the ARTIC V3 Panel Design.

QIAseq SARS-CoV-2 Region Booster	**(96)**
Catalog no.	**333897**
Number of reactions	**96**
ARTIC Region Booster Pool 1*	1 x 360 µl
ARTIC Region Booster Pool 2*	1 x 360 µl

* Booster primers intended for use with the ARTIC V3 Workflow (333896).

QIAseq SARS-CoV-2 ARTIC Panel	**(96)**
Catalog no.	**333883**
Number of reactions	**96**
RP Primer	1 x 96 µl
EZ Reverse Transcriptase	1 x 150 µl
RNase Inhibitor	1 x 144 µl
Multimodal RT Buffer, 5x	1 x 480 µl
QIAseq 2X HiFi MM	2 x 1440 µl
ARTIC Primer Pool 1*	1 x 360 µl
ARTIC Primer Pool 2*	1 x 360 µl
Nuclease-free Water	10 ml

* Configurable material that contains customer selected version of ARTIC Primer panel designed. This product contains ARTIC V4.1 or above.

Important: Additional Required Products

- QIAseq FX DNA Library CDI Kit (QIAGEN, cat. no. 180483 or 180484) or

  QIAseq FX DNA Library UDI Kit (QIAGEN, cat. no. 180477, 180479, 180480, 180481, or 180482)
- Agencourt® AMPure® XP Beads (Beckman Coulter®, cat. no. A63880 or A63881) for bead-based library purification
- Buffer EB (QIAGEN, cat. no. 19086)
- **Optional product**: QIAseq Library Quant Assay Kit (QIAGEN, cat. no. 333314) or QIAseq Library Quant Array Kit (QIAGEN, cat. no. 333304)

  Qubit® dsDNA HS Assay Kit (Thermo Fisher Scientific, cat. no. Q32851 or Q32854)

Shipping and Storage

The QIAseq SARS-CoV-2 Primer Panel, ARTIC Region Booster Panel, or ARTIC Panel should be stored immediately upon receipt at –30 to –15°C.

Intended Use

The QIAseq SARS-CoV-2 Primer Panel, ARTIC Region Booster Panels, and ARTIC Panels are intended for molecular biology applications. The QIAseq SARS-CoV-2 Primer Panel, ARTIC Region Booster Panels, and ARTIC Panels are not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the QIAseq SARS-CoV-2 Primer Panel, ARTIC Region Booster Panels, and ARTIC Panel are tested against predetermined specifications to ensure consistent product quality.

Introduction

This kit is specially designed to aid in the research of the SARS-CoV-2 virus, which is the causative agent of coronavirus disease 2019 (COVID-19). Viruses consist of nucleic acid (viral genome) and a limited number of proteins that aid with entry into the host cells, replication of the genome, and production of virions. While viral genomes can be composed of RNA or DNA, SARS-CoV-2 is encoded by RNA. The size of the entire SARS-CoV-2 virus genome is under 30 kb and can be mixed with host RNA when isolating from a human sample, making it challenging to reconstruct the whole genome of the virus.

While next-generation sequencing (NGS) has become a vital tool, library preparation remains a key bottleneck in the NGS workflow. The QIAseq SARS-CoV-2 Primer panels and boosters are multiplexed PCR primer sets for whole-genome amplification of SARS-CoV-2, which produce approximately 400 bp amplicons in two PCR enrichment pools that tile the SARS-CoV-2 genome. Products 333895 and 333896 are based on ARTIC V3 primer sequences and a study (1) from the ARTIC network. In addition, coverage of region 19205-19616 has been augmented. The ARTIC V3 S Gene Booster (cat. no. 333897 SBP-101Z) is based in part on primer sequences from the ARTIC network V4 build, and it is designed to augment coverage of the S gene imparted by products 333895 and 333896. The ARTIC V3 S Gene Booster avoids high frequency variants in Alpha, Beta, Gamma, Delta, as well as B.1.429, B.1.525, and B.1.617.1. ARTIC V4.1 Panel (or above) (cat. no. 333883) is based on ARTIC network primer sequences. Using the QIAseq FX DNA Library Kit, the amplicons from the QIAseq SARS-CoV-2 Primer Panel are brought within the length requirements to perform sequencing on Illumina® instruments.

The QIAseq FX technology incorporates enzymatic DNA fragmentation into a streamlined, optimized protocol that does not require sample cleanup between fragmentation and adapter ligation, which saves time and prevents errors. Optimized enzyme and buffer compositions ensure efficient library construction with a wide range of input amounts and high sequencing library yield. The streamlined QIAseq FX protocol can be performed optionally at room

temperature, enabling straightforward automation of the library preparation on various liquid-handling platforms.

Principle and procedure

The QIAseq SARS-CoV-2 Primer Panel utilizes a 2-stage PCR workflow for targeted enrichment of the entire SARS-CoV-2 virus genome (Figure 1).

Figure 1A. Schematic of QIAseq SARS-CoV-2 Primer Panel Workflow

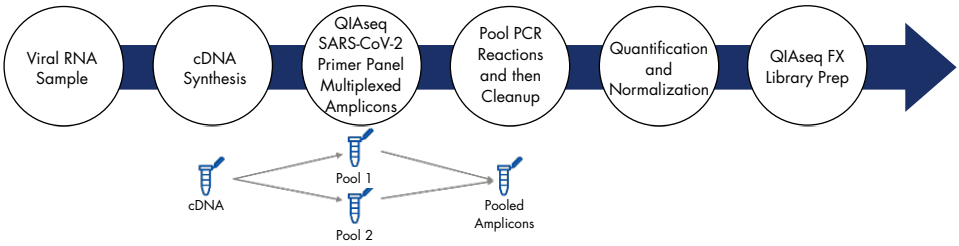

Figure 1B. Schematic of QIAseq FX DNA Library Kit Workflow

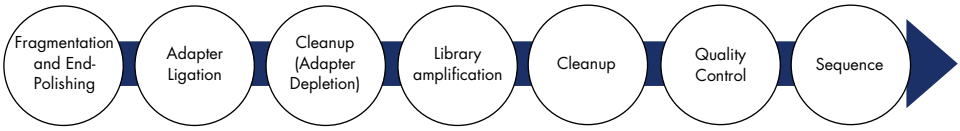

Figure 1. Scheme of optimized QIAseq SARS-CoV-2 Primer Panel inclusive of QIAseq FX DNA library construction. (A) The QIAseq SARS-CoV-2 Primer Panel workflow illustrates the conversion of viral RNA to cDNA. The cDNA is then used as the input into the multiplex PCR reaction. The amplified amplicons are then pooled and purified. (B) Using the QIAseq FX DNA Library Kit, purified amplicons from the multiplexed QIAseq SARS-CoV-2 Primer Panel are converted to sequencing libraries by employing a one-tube library construction step.

cDNA Synthesis and Targeted Enrichment:

The QIAseq SARS-CoV-2 Primer Panel workflow begins with reverse-transcribing total viral RNA into cDNA using random priming (no rRNA depletion or poly-A selection is required). 5 µl viral RNA input is the recommended starting amount; more RNA volume can be added.

Following cDNA synthesis, a high-fidelity multiplex PCR reaction is performed to prepare two pools of 400 bp QIAseq SARS-CoV-2 Primer Panel amplicons. In addition, coverage of region 19205-19616 has been augmented. Products 333895 and 333896 are based on ARTIC V3 primer sequences and a study (1) from the ARTIC network. In addition, coverage of region 19205-19616 has been augmented. The ARTIC V3 S Gene Booster (cat. no. 333897 SBP-101Z) is based in part on primer sequences from the ARTIC network V4 build, and it is designed to augment coverage of the S gene imparted by products 333895 and 333896. The ARTIC V3 S Gene Booster avoids high frequency variants in Alpha, Beta, Gamma, Delta, as well as B.1.429, B.1.525, and B.1.617.1. ARTIC V4.1 Panel or above (cat. no. 333883) is based on ARTIC network primer sequences. The two enriched pools per sample are then pooled into a single tube and purified. The procedure then moves to the QIAseq FX DNA library construction.

QIAseq FX DNA Library Construction:

Purified, target-enriched samples from the QIAseq SARS-CoV-2 Primer Panel are first enzymatically sheared into smaller fragments. The desired median fragment size is 250 bp. The fragmented DNA is then directly end-repaired, and an 'A' is added to the 3' ends during the FX reaction, making the DNA fragments ready for adapter ligation. Following this step, Illumina platform-specific adapters are ligated to both ends of the DNA fragments. These adapters contain sequences essential for binding dual-barcoded libraries to a flow cell for sequencing. To ensure maximum yields, a high-fidelity amplification step is then performed using the reagents included in the QIAseq FX DNA Library Kit. The proprietary HiFi PCR Master Mix can evenly amplify DNA regions with vastly different GC contents, minimizing amplification bias caused by PCR.

Next-generation Sequencing

QIAseq SARS-CoV-2 Primer Panel is compatible with Illumina NGS platforms including iSeq® 100, MiniSeq®, MiSeq®, NextSeq® 500/550, NextSeq 1000/2000, HiSeq® 2500, HiSeq 3000/4000, and NovaSeq® 6000. When using Illumina NGS systems, 151-bp paired-end

reads are required for the QIAseq FX DNA CDI libraries and dual 8-bp indices, while 149-bp paired-end reads are required for the QIAseq FX DNA UDI libraries and dual 10-bp indices. **Important**: As a starting point, we recommend allocating 1M reads per sample. Through testing, it is possible to reduce the read allocation per sample. Table 1 describes the number of samples that can be multiplexed (assumes 10% duplicates).

Table 1. Sample multiplexing recommendations

Instrument	Version	Clusters/flow cell (M)	Samples/flow cell
iSeq 100	i1 Reagents	4	4
MiniSeq	Mid Output	8	7
MiniSeq	High Output	25	22
MiSeq	v2 Reagents	15	13
MiSeq	v3 Reagents	25	22
NextSeq 500/550	Mid Output	130	117
NextSeq 500/550	High Output	400	360
NextSeq 1000/2000	P1	100	90
NextSeq 1000/2000	P2	400	360
NextSeq 1000/2000	P3	1200	1080
HiSeq 2500	Rapid Run v2 with cBot	150 (per lane)	135
HiSeq 3000/4000		312.5 (per lane)	281
NovaSeq 6000	SP	800	720
NovaSeq 6000	S1	1600	1440

Indexing recommendations

Sample multiplexing is one of the most important NGS tools for increasing throughput and reducing costs. It works by combining multiple samples to be processed together in a single sequencing run; as a consequence, sequencing reads need to be demultiplexed by reassigning each single read to its original source library. This is facilitated by the integration of index sequences into the individual adapter molecules. Use different sample indexes for all samples.

The QIAseq FX DNA Library CDI/UDI Kits include a fully compatible indexing solution containing a QIAseq Y-Adapter plate with either combinatorial dual-index adapters (CDI) or unique dual-index adapters (UDI). We recommend using the QIAseq Dual-Index Y-Adapter Plates delivered with the kit. Each QIAseq FX DNA Library CDI/UDI Kit includes one of the following:

- QIAseq Combinatorial Dual-Index (CDI) Y-Adapter Plate (24)
- QIAseq Combinatorial Dual-Index (CDI) Y-Adapter Plate (96)
- QIAseq Unique Dual-Index (UDI) Y-Adapter Plate (24)
- QIAseq Unique Dual-Index (UDI) Y-Adapter Plate A, B, C, or D (96)

This is achieved using either the QIAseq CDI Y-Adapters or QIAseq UDI Y-Adapters to generate QIAseq FX, QIAseq SARS-CoV-2 enriched amplicon libraries. CDI adapters use twelve i7 and eight i5 barcode motives that can be combined to form up to 96 combinatory dual indices. In contrast, QIAseq UDI Adapters use a fixed combination of two unique barcode motives per adapter molecule. Therefore, each single-index motive is only used once on any UDI adapter plate. The QIAseq FX DNA CDI library adapters are described in References

Itokawa K, Sekizuka T., Hashino M., Tanaka R., and Kuroda M. (2020). A proposal of an alternative primer for the ARTIC Network's multiplex PCR to improve coverage of SARS-CoV-2 genome sequencing. https://doi.org/10.1101/2020.03.10.985150.

Appendix A: QIAseq Dual-Index Y-Adapters. To multiplex more than 96 libraries in a single sequencing run, combine kits with different UDI Y-adapter plates. For example, combining the QIAseq FX DNA Library UDI-A (or B or C or D) Kit (96) Kit will allow the generation of 384 libraries with different sample indexes for 384-plex sequencing. Use of UDI adapters effectively mitigates the risk of read misassignment due to index hopping. This is enabled by filtering misassigned reads during the demultiplexing of individual samples, thus generating highly accurate output data. For more information on QIAseq Y-Adapter Plates, please refer to References

Itokawa K, Sekizuka T., Hashino M., Tanaka R., and Kuroda M. (2020). A proposal of an alternative primer for the ARTIC Network's multiplex PCR to improve coverage of SARS-CoV-2 genome sequencing. https://doi.org/10.1101/2020.03.10.985150.

Appendix A: QIAseq Dual-Index Y-Adapters.

Data Analysis

Downstream NGS data can be analyzed with the QIAGEN CLC Genomics Workbench.

Equipment and Reagents to be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Consumables and reagents

- Nuclease-free pipette tips and tubes
- PCR tubes (0.2 ml individual tubes or 8-well tube strips) (VWR®, cat. no. 20170-012 or 93001-118) or plates
- 1.5 ml LoBind® tubes (Eppendorf®, cat. no. 022431021)
- 100% ethanol (ACS grade)
- Nuclease-free Water
- Ice
- Agilent® High Sensitivity DNA Kit (Agilent, cat. no. 5067-4626) or High Sensitivity D1000 ScreenTape (Agilent, cat. no. 5067-5584) or High Sensitivity D5000 ScreenTape (Agilent, cat. no. 5067-5592)

Laboratory equipment

- Single-channel pipette
- Multichannel pipette
- Magnetic racks for magnetic beads separation, e.g., MagneSphere® Technology Magnetic Separation Stand (Promega®, cat. no. Z5342) or DynaMag™-96 Side Magnet (Thermo Fisher Scientific, cat. no. 12331D)
- Microcentrifuge
- Thermal cycler
- Vortexer
- Qubit 4 Fluorometer or Qubit Flex Fluorometer (Thermo Fisher Scientific)
- Capillary electrophoresis device, (e.g., QIAGEN QIAxcel®, Agilent 2100 Bioanalyzer®, or similar to evaluate the DNA fragmentation profile [optional])

Important Notes

General precautions

- Observe Good Laboratory Practices to minimize cross-contamination of nucleic acid products.
- Always use PCR tubes, microcentrifuge tubes, and pipette tips that are certified sterile and DNase- and RNase-free.
- Before starting, wipe down work area and pipettes with an RNase and DNA cleaning product such as RNase Away® (Thermo Fisher Scientific) or LookOut® DNA Erase (Sigma-Aldrich).
- For consistent genome amplification and library construction and amplification, ensure that the thermal cycler used in this protocol is in good working order and has been calibrated according to the manufacturer's specifications.
- Please read the entire protocol before beginning. Take note of required product, notes, recommendations, and stopping points.
- **Recommended library quantification methods**: QIAGEN's QIAseq Library Quant Assay Kit (QIAGEN, cat. no. 333314) or QIAseq Library Quant Array Kit (QIAGEN, cat. no. 333304), which both contain laboratory-verified forward and reverse primers together with a DNA standard, is highly recommended for accurate quantification of the prepared QIAseq SARS-CoV-2 in conjunction with QIAseq FX DNA libraries.

Protocol: cDNA Synthesis

Important points before starting

- **Use 5 µl viral RNA input as a starting point.**
- Set up cDNA synthesis reaction on ice.
- Use a thermal cycler with a heated lid.
- **Note**: Depending on the application, the RNA/primer mixture (Table 2) and RNA/primer incubation (Table 3) may be able to be skipped. Instead, the RNA and the 11-fold diluted RP Primer could be assembled as part of Table 4. For this, please perform an equivalency experiment where both conditions are tested.

Procedure: cDNA Synthesis

1. Thaw template RNA on ice. Gently yet thoroughly mix, then briefly centrifuge to collect residual liquid from the sides of the tubes and return to ice.
2. Prepare the reagents required for cDNA synthesis.
   2a. Thaw RP Primer (random hexamer), Multimodal RT Buffer, and Nuclease-free Water at room temperature (15–25°C).
   2b. Mix thoroughly and then briefly centrifuge to collect residual liquid from the sides of the tubes. Prolonged storage of Multimodal RT Buffer at –30 to –15°C can cause white precipitate to form. This is normal. Just ensure to mix until precipitate is fully dissolved.
   2c. Thaw RNase Inhibitor and EZ Reverse Transcriptase on nice. Mix by flicking the tubes, briefly centrifuge, and return to ice. After use, immediately return the enzymes to the freezer.
3. **Important**: Dilute the RP Primer 11-fold. Add 2 µl of RP Primer to 20 µl of Nuclease-free Water to create 22 µl of "**RP-Primer (11-fold diluted)**". Briefly centrifuge and vortex to mix.
4. On ice, prepare the cDNA synthesis reaction according to Table 2. Briefly centrifuge, "Gently yet thoroughly" vortex the reaction to mix, and centrifuge briefly again.

   Note: If setting up more than one reaction, prepare a volume of master mix 10% greater than what is required for the total number of reactions.

Table 2. Setup of RNA/primer mixture

Component	Volume/reaction
Template RNA	5 µl
RP Primer **(11-fold diluted)**	1 µl
Nuclease-free Water	6 µl
Total volume	**12 µl**

5. Incubate as described in Table 3.

Table 3. RNA/primer incubation

Step	Temperature	Incubation time
1	65°C	5 min
2	Ice	1 min

6. On ice, prepare the cDNA synthesis reaction according to Table 4. Briefly centrifuge, "Gently yet thoroughly" vortex the reaction to mix, and centrifuge briefly again.
   Note: If setting up more than one reaction, prepare a volume of master mix 10% greater than what is required for the total number of reactions.

Table 4. Setup of cDNA synthesis reaction

Component	Volume/reaction
Template RNA/RP Primer Mixture (already in tube)	12 µl
Multimodal RT Buffer, 5x	4 µl
Nuclease-free Water	2 µl
RNase Inhibitor	1 µl
EZ Reverse Transcriptase	1 µl
Total volume	**20 µl**

7. Incubate as described in Table 5.

Table 5. cDNA synthesis incubation

Step	Temperature	Incubation time
1	42°C	50 min
2	70°C	15 min
3	4°C	Hold

8. Proceed to "Protocol: Target Enrichment". Alternatively, the samples can be stored at –30 to –15°C in a constant-temperature freezer.

Protocol: Target Enrichment

Important points before starting

- 5 µl (two, 2.5 µl aliquots) of the cDNA from "Protocol: cDNA Synthesis" is the starting material for the Target Enrichment.
- Set up the reactions on ice.
- Use a thermal cycler with a heated lid.
- Equilibrate Agencourt AMPure XP beads to room temperature (15–25°C) for 20–30 min before use.
- Vortex AMPure XP beads thoroughly to ensure they are well resuspended; the solution should be a homogenous brown color. If a delay in the protocol occurs, simply vortex the beads.
- **Important**: Prepare fresh 80% ethanol daily.

Procedure: Target Enrichment

1. Prepare the reagents required for target enrichment.
   1a. Thaw QIAseq SARS-CoV-2 Pool 1 and QIAseq SARS-CoV-2 Pool 2 at room temperature.
   1b. Thaw QIAseq 2X HiFi MM on ice.
   Note: If a precipitate is present, bring to room temperature for 5 min, and dissolve the precipitate by mixing with pipettor.
   1c. Mix thoroughly by flicking the tubes, and then centrifuge briefly.
2. On ice, prepare the two target enrichment reactions according to Table 6. Briefly centrifuge, "Gently yet thoroughly" vortex the reaction to mix, and centrifuge briefly again.

   Note: If setting up more than one reaction, prepare a volume of master mix 10% greater than what is required for the total number of reactions.

Table 6. Setup of target enrichment reactions

Component	Enrichment 1: Volume/reaction	Enrichment 2: Volume/reaction
cDNA from "Protocol: cDNA Synthesis"	2.5 µl	2.5 µl
QIAseq SARS-CoV-2 Pool 1*	3 µl	– µl
QIAseq SARS-CoV-2 Pool 2*	– µl	3 µl
ARTIC Region Booster Pool 1†	3 µl	– µl
ARTIC Region Booster Pool 2†	– µl	3 µl
QIAseq 2X HiFi MM	12.5 µl	12.5 µl
Nuclease-free Water	Bring total volume to 25 µl	Bring total volume to 25 µl
Total volume	**25 µl**	**25 µl**

* QIAseq SARS-CoV-2 Pool 1 and Pool 2 can be replaced by ARTIC V4.1 or above. ARTIC Region Boosters should only be combined with the appropriate QIAseq SARS-CoV-2 Pool ARTIC Version in the Enrichment reactions.

† ARTIC Region Boosters should only be combined with compatible Artic QIAseq SARS-CoV-2 Pools in the Enrichment reactions. For example, the ARTIC V3 S Gene Booster should only be combined with the ARTIC V3 Panel (333896; 333895). It is not compatible with Artic V4.1 (333883 SRP-101Z). Additionally, both ARTIC V3 S Gene Booster Pools 1 and 2 should be used, one per its respective reaction. They should not be used alone.

3. Incubate as described in Table 7.

Table 7. Target enrichment cycling conditions

Step	Time	Temperature	Number of cycles
Heat activation	2 min	98°C	1
Denaturation	20 s	98°C	35
Annealing/Extension	5 min	65°C	
Hold	∞	4°C	Hold

4. After amplification, combine the entire contents of "Pool 1" and "Pool 2" PCR reactions for each biological sample into a single tube, giving a volume of 50 µl. Briefly centrifuge, "Gently yet thoroughly" vortex the reaction to mix, and centrifuge briefly again.

5. Add 50 µl AMPure XP beads to each of the 50 µl combined sample. "Gently yet thoroughly" vortex the reaction to mix.

   Note: Vortex AMPure XP beads thoroughly to ensure they are well resuspended; the solution should be a homogenous brown color.

6. Pulse centrifuge and incubate for 5 min at room temperature.
7. Place the tube onto a magnetic rack. After the solution has cleared (2 min or longer), carefully remove and discard the supernatant.

   Important: Do not discard the beads, as they contain the DNA of interest.

8. With the tubes still on the magnetic stand, add 200 µl of 80% ethanol. Carefully remove and discard the wash. Repeat the wash, for a total of 2 ethanol washes. Remove excess ethanol as much as possible.

   Important: Completely remove all traces of the ethanol wash after the second wash. Briefly centrifuge and return the tubes to the magnetic stand. Remove the ethanol with a 200 µl pipette first, and then use a 10 µl pipette to remove any residual ethanol.

9. With the tube (caps opened) still on the magnetic stand, air-dry at room temperature for 5–10 min or until the pellet loses its shine.

   Note: If the pellet dries completely, it will crack and become difficult to resuspend.

10. Remove the tube from the magnetic stand and elute the DNA from the beads by adding 30 µl Buffer EB. "Gently yet thoroughly" vortex (triturate if necessary) to mix, briefly centrifuge, and incubate for 2 min.
11. Return the tube to the magnetic rack until the solution has cleared.
12. Transfer 28 µl to a clean tube. This is the target enriched sample.
13. Proceed to "Protocol: Target Enrichment Quantification and Normalization". Alternatively, the samples can be stored at –30 to –15°C in a constant-temperature freezer.

Protocol: Target Enrichment Quantification and Normalization

Important points before starting

- A small portion (approximately 1 to 2 µl) of the target enriched sample from "Protocol: Target Enrichment" is the starting material for quantification and normalization.
- Normalize the samples in Buffer EB.

Procedure: Target Enrichment Quantification and Normalization

1. Using a small portion (approximately 1 to 2 µl) of the sample, quantify the amount of enrichment using a dsDNA HS assay Kit with either a Qubit 4 Fluorometer, Qubit Flex Fluorometer, or similar instrument. To do this, follow the respective user manual for your chosen fluorometer.
   Note: Ensure that the concentration reading is on the linear scale. If the concentration is not on the linear scale, dilute a small portion of the target enriched sample, and re-quantify.
2. Once concentration has been determined, dilute each target enriched sample to a common concentration (as an example, 10 ng/µl, 25 ng/µl, etc.) using Buffer EB.
3. Proceed to "Protocol: Fragmentation, End-Repair, and A-addition". Alternatively, the samples can be stored at –30 to –15°C in a constant-temperature freezer.

Protocol: Fragmentation, End-Repair, and A-addition

The reagents required for this protocol are found in the QIAseq FX DNA Library kits. This protocol describes the FX reaction for single-tube fragmentation, end-repair, and A-addition.

Important points before starting

- A normalized, target enriched sample from "Protocol: Target Enrichment Quantification and Normalization" is the starting material for the QIAseq FX DNA Library Kit preparation.
- Before setting up the reaction, it is critical to accurately determine the amount of the input DNA.
- **Important**: Make fresh 80% ethanol.
- Thaw reagents on ice. Once reagents are thawed, mix buffers thoroughly by vortexing quickly to avoid any localized concentrations. Briefly spin down vortexed reagents before use.
- Program thermal cyclers. For increased speed and convenience, all incubation steps of the protocol can be preprogrammed and saved in advance.

Procedure: Fragmentation, End-Repair, and A-addition

1. Program a thermal cycler according to Table 8 using the pre-determined FX fragmentation time for step 2. Be sure to use the instrument's heated lid, and if possible, set the temperature of the heated lid to 70°C.

Table 8. DNA fragmentation, end-repair, and A-addition program

Step	Incubation temperature	Incubation time
1	4°C	1 min
2	32°C	14 min*
3	65°C	30 min
4	4°C	Hold

* **Important**: The target-enriched sample is approximately 400 bp, and the desired fragment size is approximately 250 bp.

2. Start the program. When the thermal cycler block reaches 4°C, pause the program.
3. Prepare the FX reaction mix in a PCR plate or tube on ice according to Table 9 for >10 ng input DNA or Table 10 for 1–10 ng input DNA. Mix well by gently pipetting (do not vortex to mix).

Table 9. FX reaction mix setup (per sample) for >10 ng input DNA

Component	Volume/reaction
FX Buffer, 10x	5 µl
Purified DNA	Variable
Nuclease-free Water	Variable
Total without FX Enzyme Mix	**40 µl**

Table 10. FX reaction mix setup (per sample) for 1–10 ng input DNA

Component	Volume/reaction
FX Buffer, 10x	5 µl
Purified DNA	Variable
FX Enhancer	2.5
Nuclease-free Water	Variable
Total without FX Enzyme Mix	**40 µl**

4. Add 10 µl FX Enzyme Mix to each reaction and mix well by pipetting up-and-down 20 times.

   Important: It is critical to thoroughly mix the reaction.

   Important: It is critical to keep reactions on ice for the entire duration of the reaction setup.

5. Briefly spin down the PCR plate/tubes and immediately transfer to a pre-chilled thermal cycler (4°C). Resume the cycling program.
6. When the thermal cycler program is complete and the sample block has cooled to 4°C, remove samples and place them on ice.
7. Immediately proceed to "Protocol: Adapter Ligation".

Protocol: Adapter Ligation

The reagents required for this protocol are found in the QIAseq FX DNA Library kits. This protocol describes adapter ligation.

Things to do before starting

- The adapter layout of the 96-plex and 24-plex single use adapter plates are described in References
- Itokawa K, Sekizuka T., Hashino M., Tanaka R., and Kuroda M. (2020). A proposal of an alternative primer for the ARTIC Network's multiplex PCR to improve coverage of SARS-CoV-2 genome sequencing. https://doi.org/10.1101/2020.03.10.985150.
- Appendix A: QIAseq Dual-Index Y-Adapters.
- Equilibrate AMPure XP beads to room temperature (15–25°C) for 20–30 min before use.
- Vortex AMPure XP beads thoroughly to ensure they are well resuspended; the solution should be a homogenous brown color. If a delay in conducting the protocol occurs, simply re-vortex the beads.
- Vortex and spin down the thawed adapter plate before use.
- Thaw Ligation buffer, 5x and DNA ligase on ice. Thoroughly mix Ligation buffer 5x by vortexing and DNA ligase by flicking the tube. Briefly centrifuge.

Procedure: Adapter Ligation

1. Remove the protective adapter plate lid, pierce the foil seal for each adapter well to be used, and transfer 5 µl from one DNA adapter well to each 50 µl sample from the previous protocol. Track the barcodes from each adapter well used for each sample.

   Note: If the DNA input is <10 ng, dilute the adapters according to Table 11.

Table 11. Adapter dilution factors

Sample DNA amount	Adapter dilution

10–99 pg	1:1000
100–999 pg	1:100
1–9 ng	1:10

2. Replace the adapter plate lid and freeze unused adapters. The adapter plate is stable for a minimum of 10 freeze–thaw cycles.

   Important: Only 1 single adapter should be used per ligation reaction. Do not reuse adapter wells once the foil seal has been pierced.

3. Prepare the ligation master mix (per DNA sample, Table 12) in a separate PCR plate or tube on ice, and mix well by pipetting.

Table 12. Ligation master mix setup (per sample)

Component	Volume/reaction
Ligation buffer, 5x	20 µl
DNA ligase	10 µl
Nuclease-free Water	15 µl
Total	**45 µl**

4. Add 45 µl of the ligation master mix to each sample (for a total of 100 µl), and mix well by pipetting, and briefly centrifuge. Incubate the ligation reaction at 20°C for 15 min, and then heat inactivate the ligase at 65°C for 20 min.

   Important: Do not use a thermal cycler with a heated lid for the "20°C for 15 min" step.

5. Proceed immediately to adapter ligation cleanup using 0.8x (80 µl) AMPure XP beads.

   Note: In contrast to the protocol described in an ARTIC network study (1), the adapter ligations are kept separate here to facilitate equal sample loading and representation during NGS.

6. Add 80 µl of resuspended AMPure XP beads to each ligated sample, mix well by pipetting, and briefly centrifuge.
7. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand until the solution has cleared (2 min or longer), then carefully discard the supernatant.

8. Wash the beads by adding 200 µl of 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash, for a total of 2 ethanol washes. Remove as much excess ethanol as possible.

   Important: Completely remove all traces of the ethanol wash after the second wash. Briefly centrifuge and return the tubes to the magnetic stand. Remove ethanol with a 200 µl pipette first, then use a 10 µl pipette to remove any residual ethanol.

9. Incubate the beads on the magnetic stand for 5–10 min or until the beads are dry. Overdrying of the beads may result in lower DNA recovery. Remove beads from the magnetic stand.

10. Elute by resuspending in 52.5 µl of Buffer EB. "Gently yet thoroughly" vortex (triturate if necessary) to mix, briefly centrifuge, and incubate for 2 min. Pellet the beads on the magnetic stand, and carefully transfer 50 µl of supernatant into a new plate or tube.

11. Perform a second purification using 50 µl AMPure XP beads.

12. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand until the solution has cleared (2 min or longer), then carefully discard the supernatant.

13. Wash the beads by adding 200 µl of 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash, for a total of 2 ethanol washes. Remove as much excess ethanol as possible.

    Important: Completely remove all traces of the ethanol wash after the second wash. Briefly centrifuge and return the tubes to the magnetic stand. Remove the ethanol with a 200 µl pipette first, then use a 10 µl pipette to remove any residual ethanol.

14. Incubate the beads on the magnetic stand for 5–10 min or until the beads are dry. Overdrying of beads may result in lower DNA recovery. Remove beads from the magnetic stand.

15. Elute DNA by adding 26 µl Buffer EB. "Gently yet thoroughly" vortex (triturate if necessary) to mix, briefly centrifuge, and incubate for 2 min. Pellet the beads on the magnetic stand, and carefully collect 23.5 µl of purified DNA sample in a new tube.

16. Proceed to "Protocol: Library Amplification". Alternatively, the purified library can be safely stored at –30 to –15°C in a constant-temperature freezer.

Protocol: Library Amplification

The reagents required for this protocol are found in the QIAseq FX DNA Library Kit. This protocol is for high-fidelity amplification of the DNA library using the amplification reagents provided in the QIAseq FX DNA Library Kit.

Things to do before starting

- Thaw HiFi PCR Master Mix, 2x, and Primer Mix on ice. Once reagents are thawed, mix them thoroughly by vortexing quickly to avoid any localized concentrations.
- Always start with the cycling conditions specified in this protocol. The cycling has been optimized for use with QIAseq HiFi PCR Master Mix for even and high-fidelity amplification of sequencing libraries.

Procedure: Amplification of Library DNA

1. Program a thermal cycler with a heated lid according to Table 13.

Table 13. Library amplification cycling conditions

Step	Temperature	Time	Number of cycles
Initial denaturation	98°C	2 min	1
3-step cycling			
Denaturation	98°C	20 s	
Annealing	60°C	30 s	8 (100 ng input DNA)* 12 (10 ng input DNA)* 14 (1 ng input DNA)* 16 (100 pg input DNA)* 18 (20 pg input DNA)*
Extension	72°C	30 s	
Final extension	72°C	1 min	1
Hold	4°C	∞	Hold

* **Note**: Amplification cycles are recommended based on the amount of input DNA into the FX kit and quality.

2. Prepare a reaction mix on ice according to Table 14. Mix the components in a PCR tube or a 96-well PCR plate.

Table 14. Reaction mix for library amplification

Component	Volume/reaction
HiFi PCR Master Mix, 2x	25 µl
Primer Mix (10 µM each)	1.5 µl
Nuclease-free Water	4 µl
Library DNA	19.5 µl
Total reaction volume	**50 µl**

3. Transfer the PCR tube or plate to the thermal cycler and start the program.
4. Once PCR is complete, add 50 µl of resuspended AMPure XP beads to each reaction (50 µl) mix well by pipetting, and briefly centrifuge..
5. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand until the solution has cleared (2 min or longer), and then carefully discard the supernatant.
6. Wash the beads by adding 200 µl of 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash for a total of 2 ethanol washes. Remove as much excess ethanol as possible.

   Important: Completely remove all traces of the ethanol wash after this second wash. Briefly centrifuge and return the tubes to the magnetic stand. Remove the ethanol with a 200 µl pipette first, and then use a 10 µl pipette to remove any residual ethanol.
7. Incubate on the magnetic stand for 5–10 min or until the beads are dry. Over-drying of beads may result in lower DNA recovery. Remove from the magnetic stand.
8. Elute by resuspending in 25 µl of Buffer EB. "Gently yet thoroughly" vortex (triturate if necessary) to mix, briefly centrifuge, and incubate for 2 min. Pellet the beads on the magnetic stand, and carefully transfer 23 µl of the supernatant into a new tube. If not proceeding immediately, the sample can be stored at –30 to –15°C.

9. Assess the quality of the library using a capillary electrophoresis device such as QIAGEN QIAxcel or Agilent Bioanalyzer. Check for the expected size distribution of library fragments (approximately 400 bp hump, potentially with a spike at approximately 500 bp; see Figure 2) and for the absence of an adapters or adapter-dimers peak around 120 bp.

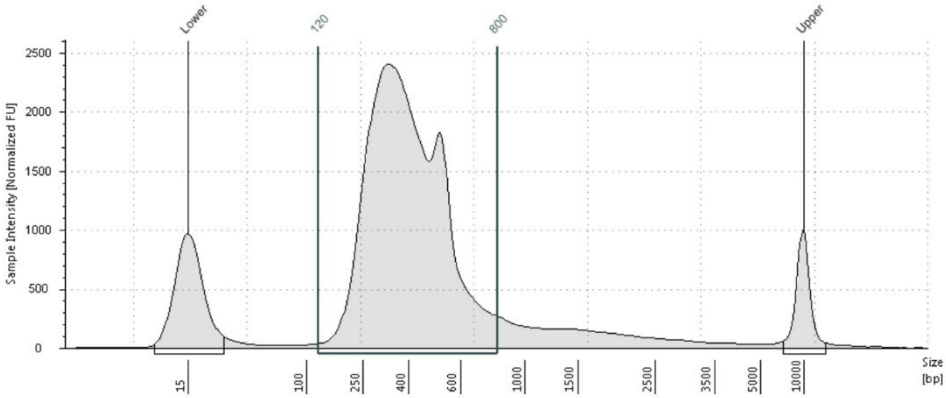

Figure 2. Example of QIAseq SARS-CoV-2 Library trace using an Agilent TapeStation with a High Sensitivity D5000 ScreenTape.

10. Quantify the library using a qPCR-based method, such as the QIAseq Library Quant Assay Kit (cat. no. 333314; not provided), or a comparable method.
11. Proceed to "Protocol: Library Amplification". Alternatively, the purified library can be safely stored at –30 to –15°C in a constant-temperature freezer until ready to use for sequencing.

Protocol: Sequencing Setup on an Illumina Instrument

QIAseq FX DNA libraries are compatible with Illumina NGS platforms, including iSeq100, MiniSeq, MiSeq, NextSeq 500/550, NextSeq 1000/2000, HiSeq 2500, HiSeq 3000/4000, and NovaSeq 6000.

Important: As a starting point, we recommend allocating 1M reads per sample.

Important points before starting

- **Important**: Recommendations for library dilution concentrations and library loading concentrations are based on QIAseq Library Quant System.
- **Important**: To make sequencing preparation more convenient, download Illumina-compatible sample sheets for different sequencing instruments on **www.qiagen.com**, and refer to References
- Itokawa K, Sekizuka T., Hashino M., Tanaka R., and Kuroda M. (2020). A proposal of an alternative primer for the ARTIC Network's multiplex PCR to improve coverage of SARS-CoV-2 genome sequencing. https://doi.org/10.1101/2020.03.10.985150.
- Appendix A: QIAseq Dual-Index Y-Adapters.
- **Important**: 151 bp paired-end sequencing should be used for QIAseq FX DNA CDI libraries and dual 8 bp indices on Illumina platforms.
- **Important**: 149 bp paired-end sequencing should be used for QIAseq FX DNA UDI libraries and dual 10 bp indices on Illumina platforms.
- For complete instructions on how to denature sequencing libraries and set up a sequencing run, please refer to the system-specific Illumina documents.

Sample dilution, pooling, sequencing, and data analysis

- QIAseq FX libraries yields are typically sufficient for an NGS sequencing run. Dilute the individual QIAseq FX DNA libraries to a concentration of 4 nM, then combine libraries with different sample indexes in equimolar amounts. The recommended starting concentration of the pooled QIAseq FX DNA libraries to load onto a MiSeq is 9 pM, or 1.6 pM on a NextSeq instrument.

Protocol: Data Analysis

- Downstream NGS data can be analyzed with QIAGEN CLC Genomics Workbench. Desktop or server versions are available.
- QIAGEN CLC Genomics Workbench is a comprehensive analysis package for the analysis and visualization of data from all major NGS platforms. The workbench supports and seamlessly integrates into a typical NGS workflow, and is available for Windows, Mac OS X, and Linux platforms. Incorporating cutting-edge technology and algorithms, QIAGEN CLC Genomics Workbench supports key next-generation sequencing features within genomics, transcriptomics, and epigenomics research fields. Additionally, it includes all the classical analysis tools of QIAGEN CLC Main Workbench.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

		Comments and suggestions
Low library yields		
a)	Suboptimal reaction conditions due to low RNA quality	Make sure to use high-quality RNA to ensure optimal activity of the library enzymes.
Unexpected signal peaks in capillary electrophoresis device traces		
a)	Presence of shorter peaks between 60 and 120 bp	These peaks represent library adapters and adapter-dimers that occur when there is no, or insufficient, adapter depletion after library preparation. As adapter-dimers can form clusters on the flow cell and will be sequenced, this will reduce the capacity of the flow cell for the library fragments, even though a low ratio of adapter-dimers versus library will not be a problem. Agencourt AMPure XP Beads or GeneRead® Size Selection Kit (cat. no. 180514) efficiently remove adapter-dimers, as well as free adapter molecules.
b)	Incorrect library fragment size after adapter ligation	During library preparation, adapters of approximately 60 bp are ligated to both ends of the DNA library fragments. This should be reflected on a capillary electrophoresis device by a shift in size of all library fragments of 120 bp. If using library adapters from other suppliers, refer to the size information given in the respective documentation. The absence of a clear size shift may indicate no, or only low, adapter ligation efficiency. Ensure that you use the parameters and incubation times described in the handbook for end-repair, A-addition, and ligation, as well as the correct amount of starting DNA.

References

1. Itokawa K, Sekizuka T., Hashino M., Tanaka R., and Kuroda M. (2020). A proposal of an alternative primer for the ARTIC Network's multiplex PCR to improve coverage of SARS-CoV-2 genome sequencing. https://doi.org/10.1101/2020.03.10.985150.

Appendix A: QIAseq Dual-Index Y-Adapters

Generation of sample sheets for Illumina instruments

Index sequences for QIAseq Unique and Combinatorial Dual-Index Y-Adapters are available for download at **www.qiagen.com**. Sequencing on the NextSeq, HiSeq X™, or HiSeq 3000/4000 system follows a different dual-indexing workflow than other Illumina systems. If you are manually creating sample sheets for these instruments, enter the reverse complement of the i5 index adapter sequence. If you are using Illumina Experiment Manager, BaseSpace®, or Local Run Manager for run planning, the software will automatically reverse complement index sequences when necessary.

Ready-to-use sample sheets containing all QIAseq CDI and UDI Y-Adapter barcode sequences are available for MiSeq, NextSeq, MiniSeq, HiSeq, and NovaSeq instruments. These can be conveniently downloaded from **www.qiagen.com.** These can be imported and edited using the Illumina Experiment Manager Software, Illumina Local Run Manager, or any text editor. Make sure to download the appropriate sample sheet for NextSeq, HiSeq X, or HiSeq 3000/4000 systems depending on whether you are using Local Run Manager or manually configuring the sequencing run

Unique Dual-Index Y-Adapters

The layout of the 24-plex and 96-plex (A/B/C/D) single-use UDI adapter plate is shown in Figure 3 and Figure 4. The index motives used in the QIAseq Unique Dual-Index Kits are listed at **www.qiagen.com**. To make sequencing preparation more convenient, you can download Illumina-compatible sample sheets for different sequencing instruments on **www.qiagen.com**.

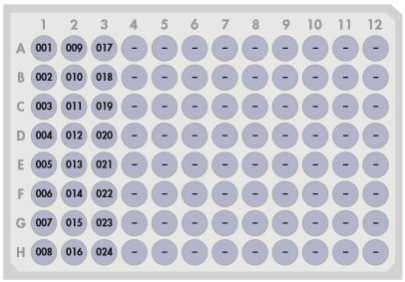

Figure 3. QIAseq UDI Y-Adapter Plate (24) layout (UDI 1–24).

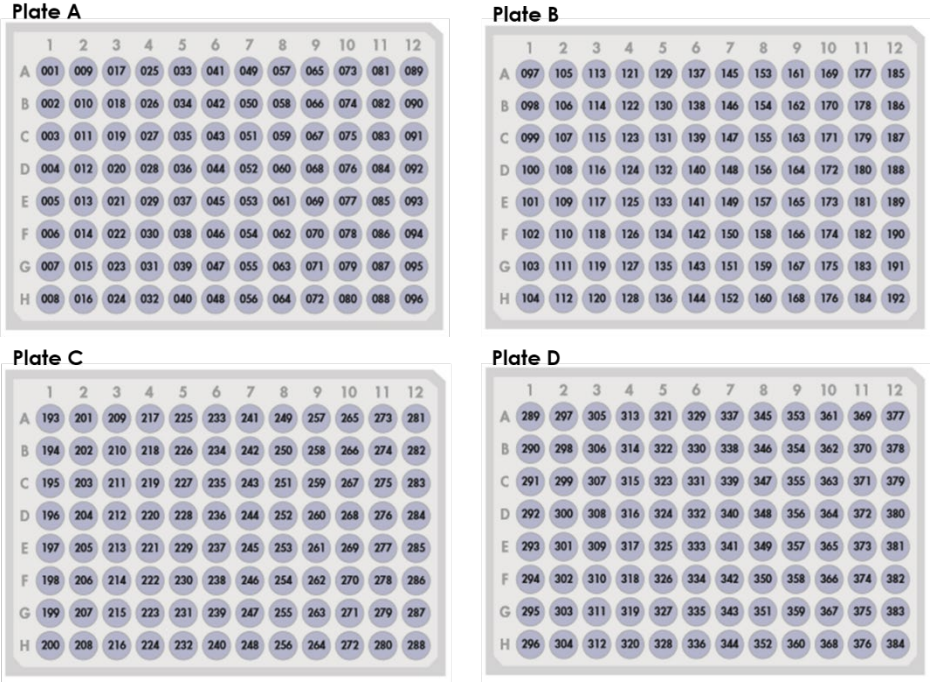

Figure 4. QIAseq UDI Y-Adapter Plates: Plate A (96) layout (UDI 1–96), Plate B (96) layout (UDI 97–192), Plate C (96) layout (UDI 193–288), and Plate D (96) layout (UDI 289–384).

Combinatorial Dual-Index Y-Adapters

The layout of the 96-plex and 24-plex single-use CDI adapter plate is shown in Figure 5 and Figure 6. The barcode sequences used in the QIAseq Combinatorial Dual-Index Kits are listed in Table 15. Indices 501-508 and 701-712 correspond to the respective Illumina adapter barcodes. To make sequencing preparation more convenient, you can download Illumina-compatible sample sheets for different sequencing instruments at **www.qiagen.com**.

Table 15. CDI Adapter barcodes used in the QIAseq CDI Y-Adapter FX DNA Library Kits (24 and 96-plex Adapter Plates)

Codes for entry on sample sheet				
D50X barcode name	**i5 bases for entry on sample sheet (MiSeq, HiSeq 2000/ 2500)**	**i5 bases for entry on sample sheet (MiniSeq, NextSeq, HiSeq 3000/ 4000)***	**D50X barcode name**	**i7 bases for entry on sample sheet**
D501	TATAGCCT	AGGCTATA	D701	ATTACTCG
D502	ATAGAGGC	GCCTCTAT	D702	TCCGGAGA
D503	CCTATCCT	AGGATAGG	D703	CGCTCATT
D504	GGCTCTGA	TCAGAGCC	D704	GAGATTCC
D505	AGGCGAAG	CTTCGCCT	D705	ATTCAGAA
D506	TAATCTTA	TAAGATTA	D706	GAATTCGT
D507	CAGGACGT	ACGTCCTG	D707	CTGAAGCT
D508	GTACTGAC	GTCAGTAC	D708	TAATGCGC
			D709	CGGCTATG
			D710	TCCGCGAA
			D711	TCTCGCGC
			D712	AGCGATAG

* **Note**: Sequencing on the MiniSeq, NextSeq, and HiSeq 3000/4000 systems follow a different dual-indexing workflow than other Illumina systems, which requires the reverse complement of the i5 index adapter sequence.

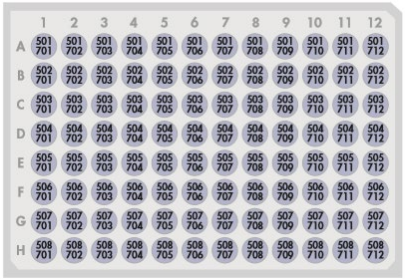

Figure 5. QIAseq CDI Y-Adapter Plate (96) layout (CDI 1–96).

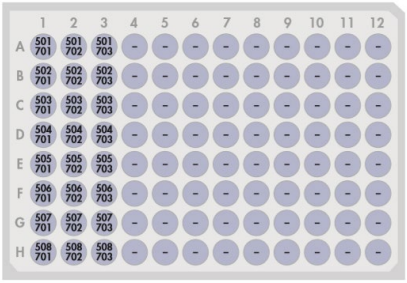

Figure 6. QIAseq CDI Y-Adapter Plate (24) layout (CDI 1–24).

Ordering Information

Product	Contents	Cat. no.
QIAseq SARS-CoV-2 Primer Panel (96)	For 96 reactions: Buffers and reagents for cDNA, and SARS-CoV-2 specific amplicon library amplification; for use with QIAGEN's QIAseq FX DNA Library Kits	333896
QIAseq SARS-CoV-2 Primer Panel (24)	For 24 reactions: Buffers and reagents for cDNA, and SARS-CoV-2 specific amplicon library amplification; for use with QIAGEN's QIAseq FX DNA Library Kits	333895
QIASeq SARS-CoV-2 Region Booster Panel	For 96 reactions: S Gene Booster Primer Pools 1 and 2 for the QIAseq SARS-CoV-2 Primer Panel	333897
QIAseq SARS-CoV-2 ARTIC Panel	For 96 reactions: ARTIC Panel for PCR amplicon tiling of the SARS-CoV-2 genome	333883
Related products		
QIAseq FX DNA Library UDI Kits for Illumina		
QIAseq FX DNA Library UDI-A Kit (96)	For 96 reactions: Buffers and reagents for DNA fragmentation, end-repair, A-addition, ligation, and library amplification; for use with Illumina instruments; includes a plate containing 96 adapters with different barcodes (pierceable foil seal allowing usage of defined parts of plate)	180479
QIAseq FX DNA Library UDI-B Kit (96)	For 96 reactions: Buffers and reagents for DNA fragmentation, end-repair, A-addition, ligation, and library amplification; for use with Illumina instruments; includes a plate containing 96 adapters with different barcodes (pierceable foil seal allowing usage of defined parts of plate)	180480

Product	Contents	Cat. no.
QIAseq FX DNA Library UDI-C Kit (96)	For 96 reactions: Buffers and reagents for DNA fragmentation, end-repair, A-addition, ligation, and library amplification; for use with Illumina instruments; includes a plate containing 96 adapters with different barcodes (pierceable foil seal allowing usage of defined parts of plate)	180481
QIAseq FX DNA Library UDI-D Kit (96)	For 96 reactions: Buffers and reagents for DNA fragmentation, end-repair, A-addition, ligation, and library amplification; for use with Illumina instruments; includes a plate containing 96 adapters with different barcodes (pierceable foil seal allowing usage of defined parts of plate)	180482
QIAseq FX DNA Library UDI Kit (24)	For 24 reactions: Buffers and reagents for DNA fragmentation, end-repair, A-addition, ligation, and library amplification; for use with Illumina instruments; includes a plate containing 24 adapters with different barcodes (pierceable foil seal allowing usage of defined parts of plate)	180477
QIAseq FX DNA Library CDI Kits for Illumina		
QIAseq FX DNA Library CDI Kit (96)	For 96 reactions: Buffers and reagents for DNA fragmentation, end-repair, A-addition, ligation, and library amplification; for use with Illumina instruments; includes a plate containing 96 adapters with different barcodes (pierceable foil seal allowing usage of defined parts of plate)	180484
QIAseq FX DNA Library CDI Kit (24)	For 24 reactions: Buffers and reagents for DNA fragmentation, end-repair, A-addition, ligation, and library amplification; for use with Illumina instruments; includes a plate containing 24 adapters with different barcodes (pierceable foil seal allowing usage of defined parts of plate)	180483
QIAseq Y-Adapter Kits for Illumina		
QIAseq UDI Y-Adapter Kit A (96)	Unique Dual-Index Adapters for Illumina (1–96)	180312

Product	Contents	Cat. no.
QIAseq UDI Y-Adapter Kit B (96)	Unique Dual-Index Adapters for Illumina (97–192)	180314
QIAseq UDI Y-Adapter Kit C (96)	Unique Dual-Index Adapters for Illumina (193–288)	180316
QIAseq UDI Y-Adapter Kit D (96)	Unique Dual-Index Adapters for Illumina (289–384)	180318
QIAseq UDI Y-Adapter Kit (24)	Unique Dual-Index Adapters for Illumina (1–24)	180310
QIAseq CDI Y-Adapter Kit (96)	Combinatorial Dual-Index Adapters for Illumina	180303
QIAseq CDI Y-Adapter Kit (24)	Combinatorial Dual-Index Adapters for Illumina	180301
QIAseq Library Quantification Kits for use with Illumina instruments		
QIAseq Library Quant Assay Kit	Laboratory-verified forward and reverse primers for 500 x 25 µl reactions (500 µl); DNA Standard (100 µl); Dilution Buffer (30 ml); SYBR® Green Mastermix (1.35 ml x 5)	333314
QIAseq Library Quant Array Kit	Plate containing dried assay reagents for quantification of libraries prepared for Illumina; SYBR Green Mastermix (1.35 ml x 2)	333304

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Notes

Document Revision History

Date	Changes
06/2020	Initial release.
03/2021	Added a statement for additional enrichment primers in "Kit Contents" and "Introduction". Changed the volume of “QIAseq 2X HiFi MM” in the “Kit Contents” section. Changed the optional library amplification protocol (Appendix B) into a main protocol ("Protocol: Library Amplification"). Deleted Appendix C (for fragmentation optimization) and all its citations. Removed a comment/suggestion for "low library yields" in the "Comments and suggestions" section. Emphasized the need to thoroughly mix a reaction in "Procedure: Fragmentation, End-Repair, and A-addition". Added a figure to show an example of QIAseq SARS-CoV-2 Library trace using an Agilent TapeStation (Figure 2).
02/2022	Implemented changes for the addition of the QIAseq SARS-CoV-2 Region Booster (cat. no. 333897) and the QIAseq SARS-CoV-2 ARTIC Panel (cat. no. 333883). Updated the Introduction section and protocols. Added NextSeq 1000/2000 in Table 1. Added footnotes in Table 6, to provide further instructions/information on the QIAseq SARS-CoV-2 Pool and ARTIC Region Booster Pool. Updated the Ordering Information section. Editorial and layout changes.