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April 2021

# QIAseq<sup>®</sup> DIRECT SARS-CoV-2 Handbook

Targeted whole viral genome library preparation  
direct from purified RNA

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# Kit Contents

<b>QIAseq DIRECT SARS-CoV-2 Kit</b> <b>Catalog no.</b> <b>Number of reactions</b>	<b>A</b> <b>333891</b> <b>96</b>	<b>B</b> <b>333892</b> <b>96</b>	<b>C</b> <b>333893</b> <b>96</b>	<b>D</b> <b>333894</b> <b>96</b>
RP Primer	1 tube	1 tube	1 tube	1 tube
EZ Reverse Transcriptase	1 tube	1 tube	1 tube	1 tube
RNase Inhibitor	1 tube	1 tube	1 tube	1 tube
Multimodal RT Buffer, 5x	1 tube	1 tube	1 tube	1 tube
QIAseq 2X HiFi MM	4 tubes	4 tubes	4 tubes	4 tubes
DIRECT SARS-CoV-2 Pool 1	1 tube	1 tube	1 tube	1 tube
DIRECT SARS-CoV-2 Pool 2	1 tube	1 tube	1 tube	1 tube
QIAseq DIRECT UDI Set A, B, C, or D	1 set (A)	1 set (B)	1 set (C)	1 set (D)
Nuclease-Free Water	1 bottle	1 bottle	1 bottle	1 bottle
QIAseq Beads	1 bottle	1 bottle	1 bottle	1 bottle

<b>QIAseq DIRECT SARS-CoV-2 HT</b> <b>Catalog no.</b> <b>Number of reactions</b>	<b>A-D</b> <b>333898</b> <b>384</b>
RP Primer	4 tubes
EZ Reverse Transcriptase	4 tubes
RNase Inhibitor	4 tubes
Multimodal RT Buffer, 5x	4 tubes
QIAseq 2X HiFi MM	16 tubes
DIRECT SARS-CoV-2 Pool 1	4 tubes
DIRECT SARS-CoV-2 Pool 2	4 tubes
QIAseq DIRECT UDI Set A, B, C, and D	4 sets (A, B, C and D)
Nuclease-Free Water	4 bottles
QIAseq Beads	4 bottles

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## Important: Additional Required Products

- Potential options for concentration readings:
  - Qubit® Fluorometer (Thermo Fisher Scientific)  
Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific cat. no. Q32851 or Q32854)  
Qubit Assay Tubes (Thermo Fisher Scientific cat. no. Q32856)
  - NanoDrop™ Spectrophotometer (Thermo Fisher Scientific)
  - QIAxpert® System (QIAGEN®)

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## Shipping and Storage

The QIAseq DIRECT SARS-CoV-2 Kit is shipped in two boxes. Box 1 (enzymes, reagents, and UDI plate) are shipped on dry ice or blue ice and should be stored immediately upon receipt at  $-30$  to  $-15^{\circ}\text{C}$ . Box 2 (QIAseq Beads) is shipped on cold packs and should be stored immediately upon receipt at  $2$  to  $8^{\circ}\text{C}$ .

## Intended Use

The QIAseq DIRECT SARS-CoV-2 Kit is intended for molecular biology applications. This product is 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.

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## 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](http://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 DIRECT SARS-CoV-2 Kit is 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 comprised 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, streamlined library preparation solutions remain elusive for SARS-CoV-2 assessment. The QIAseq DIRECT SARS-CoV-2 Kit represents a rapid library prep, enabling high-throughput SARS-CoV-2 mutation surveillance on Illumina® instruments.

## Principle and procedure

QIAseq DIRECT SARS-CoV-2 utilizes a streamlined, four-hour workflow for enrichment and library prep of the SARS-CoV-2 virus genome (Figure 1).



**Figure 1. QIAseq DIRECT SARS-CoV-2 workflow.**

### cDNA synthesis and SARS-CoV-2 enrichment

The QIAseq DIRECT SARS-CoV-2 workflow begins with random-primed cDNA synthesis (no rRNA depletion or poly-A selection is required). This reaction is flexible with regard to input RNA; 5 µl viral RNA input is required as a starting point, regardless of viral titer.

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Following cDNA synthesis, multiplexed primer pools are used in a high-fidelity multiplex PCR reaction to prepare two pools of approximately 225–275 bp QIAseq DIRECT SARS-CoV-2 amplicons. The two enriched pools per sample are then pooled into a single tube and purified using a QIAseq Bead cleanup.

## Library amplification and sample indexing

Following quantification and normalization, SARS-CoV-2 enriched samples are amplified and sample-indexed using a high-fidelity amplification reaction. During this reaction, unique dual indexes (UDIs) are added to the samples. UDIs effectively mitigate 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 UDIs, please refer to Appendix A: QIAseq DIRECT Unique Dual Indexes.

## Next-generation sequencing

The QIAseq DIRECT SARS-CoV-2 libraries are compatible with Illumina NGS platforms including iSeq® 100, MiniSeq®, MiSeq®, NextSeq® 500/550, HiSeq® 2500, HiSeq 3000/4000, and NovaSeq™ 6000. Dual 10-bp indexes and 149-bp paired-end reads are required. Table 1 describes the number of clusters/flow cell per Illumina Instrument.



**Table 1. Illumina sequencers and clusters/flow cell**

<b>Instrument</b>	<b>Version</b>	<b>Clusters/flow cell (M)</b>
iSeq 100	i1 Reagents	4
MiniSeq	Mid Output	8
MiniSeq	High Output	25
MiSeq	v2 Reagents	15
MiSeq	v3 Reagents	25
NextSeq 500/550	Mid Output	130
NextSeq 500/550	High Output	400
HiSeq 2500	Rapid Run v2 with cBot	150 (per lane)
HiSeq 3000/4000		312.5 (per lane)
NovaSeq 6000	SP	800
NovaSeq 6000	S1	1600

## Data analysis

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

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# 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

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## Laboratory equipment

- Single-channel pipette
- Multichannel pipette
- Magnetic racks for magnetic beads separation: DynaMag™-96 Side Magnet (Thermo Fisher Scientific, cat. no. 12331D)
- Microcentrifuge
- Thermal cycler
- Vortexer
- Library QC: Bioanalyzer®, TapeStation, Fragment Analyzer (Agilent®), or similar.

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# 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 products, notes, recommendations, and stopping points.

# Protocol: cDNA Synthesis

## Important points before starting

- Use 5 µl viral RNA input, regardless of viral titer.
- Set up cDNA synthesis reaction on ice.
- Do not vortex any first-strand synthesis reagents or reaction mixtures.
- Use a thermal cycler with a heated lid.

## Procedure

1. Thaw template RNA on ice. Gently 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.

**Note:** RNase Inhibitor and EZ Reverse Transcriptase should be removed from the freezer just before use and placed on ice. After use, immediately return the enzymes to the freezer.

3. **Important:** Dilute the RP Primer 11-fold. As an example, 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.

On ice, prepare the cDNA synthesis reaction according to Table 2. Briefly centrifuge, mix by pipetting up and down 10 times, 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 cDNA synthesis reaction**

Component	Volume/reaction (µl)
Template RNA	5
RP Primer (11-fold diluted)	1
Multimodal RT Buffer, 5x	4
Nuclease-free water	8
RNase Inhibitor	1
EZ Reverse Transcriptase	1
<b>Total volume</b>	<b>20</b>

4. Incubate as described in Table 3.

**Table 3. cDNA synthesis incubation**

Step	Temperature (°C)	Incubation time
1	42	30 min
2	85	5 min
3	4	Hold

5. Proceed to “Protocol: SARS-CoV-2 Enrichment”. Alternatively, the samples can be stored at –30 to –15°C in a constant-temperature freezer.

# Protocol: SARS-CoV-2 Enrichment

## Important points before starting

- A total of 5  $\mu$ l (two, 2.5  $\mu$ l aliquots) of the cDNA from “Protocol: cDNA Synthesis” is the starting material for the target enrichment.
- Set up the reactions on ice.
- Do not vortex any reagents or reactions.
- Use a thermal cycler with a heated lid.
- Equilibrate the QIAseq Beads to room temperature (15–25°C) for 20–30 min before use.
- Vortex the QIAseq 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

1. Prepare the reagents required for target enrichment.
  - 1a. Thaw the DIRECT SARS-CoV-2 Pool 1 and DIRECT SARS-CoV-2 Pool 2 at room temperature.
  - 1b. Thaw the QIAseq 2X HiFi MM on ice.

**Note:** If a precipitate is present, bring to room temperature, and dissolve the precipitate by mixing with pipettor.
  - 1c. Mix by flicking the tubes, and then centrifuge briefly.
2. On ice, prepare two target enrichment reactions per sample according to Table 4. Briefly centrifuge, mix by pipetting up and down 10 times, 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 SARS-CoV-2 enrichment reactions**

Component	Pool 1: Volume/reaction (µl)	Pool 2: Volume/reaction (µl)
cDNA from "Protocol: cDNA Synthesis"	2.5	2.5
DIRECT SARS-CoV-2 Pool 1	2	—
DIRECT SARS-CoV-2 Pool 2	—	2
QIAseq 2X HiFi MM	12.5	12.5
Nuclease-free water	8	8
<b>Total volume</b>	<b>25</b>	<b>25</b>

**Table 5. Target enrichment cycling conditions**

Step	Time	Temperature (°C)	Number of cycles
Heat activation	2 min	98	1
Denaturation	20 s	98	31 *
Annealing/extension	3 min	63	
Hold	∞	4	Hold

\* 35 cycles is also an option. In testing with control RNA samples, where cDNA has been synthesized from 20 copies of SARS-CoV-2 (translates to 2.5 copies being added to each SARS-CoV-2 enrichment reaction), 31 cycles has nonetheless proven sufficient.

3. Incubate as described in Table 5.
4. After amplification, combine the entire contents of "Pool 1" and "Pool 2" PCR reactions for each biological sample into a single well of a plate, giving a volume of 50 µl.
5. Add 50 µl QIAseq Beads to each 50 µl combined sample. Briefly centrifuge, mix by pipetting up and down 12 times, and centrifuge briefly again.  
**Note:** Vortex the QIAseq Beads thoroughly to ensure they are well resuspended; the solution should be a homogenous brown color.
6. Incubate for 5 min at room temperature.



7. Centrifuge the plate until the beads are pelleted (2 min), and then place the plate 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.

**Tip:** It may be valuable to discard the supernatant twice. The contents settle after the first discard.

8. With the plate still on the magnetic stand, add 200  $\mu$ l of 80% ethanol. Wait 1 min and carefully remove and discard the wash. Repeat the wash, for a total of 2 ethanol washes. Remove excess ethanol as much as possible.

**Important:** To, completely remove all traces of the ethanol wash after the second wash, immediately briefly centrifuge and return the tubes to the magnetic stand. Remove the ethanol with a 200  $\mu$ l pipette first, and then use a 10  $\mu$ l pipette to remove any residual ethanol. This should be done quickly.

9. With the plate still on the magnetic stand, air dry at room temperature for 4–8 min (until the beads start to crack and pellet loses its shine).
10. Remove the plate from the magnetic stand and elute the DNA from the beads by adding 30  $\mu$ l nuclease-free water. Mix well by pipetting up and down at least 12 times and incubate for 2 min.
11. Centrifuge the plate until the beads are pelleted (2 min), and then return the plate to the magnetic rack until the solution has cleared.
12. Transfer 28  $\mu$ l to a clean plate. This is now “enriched SARS-CoV-2”.
13. Proceed to “Protocol: SARS-CoV-2 Enrichment Quantification and Normalization”. Alternatively, the samples can be stored at  $-30$  to  $-15^{\circ}\text{C}$  in a constant-temperature freezer.

# Protocol: SARS-CoV-2 Enrichment Quantification and Normalization

## Important points before starting

- A small portion (approx. 1–2  $\mu$ l) of “enriched SARS-CoV-2” from “Protocol: SARS-CoV-2 Enrichment” is the starting material for quantification and normalization.

## Procedure

1. Using a small portion (approx. 1–2  $\mu$ l) of the sample, quantify the enrichment reaction using a Qubit, NanoDrop, QIAxpert, or similar instrument. To do this, follow the respective user manual for your chosen instrument.

**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 requantify.

2. Once concentrations have been determined, dilute an aliquot of each “enriched SARS-CoV-2” to a common concentration using nuclease-free water, in a volume of 23  $\mu$ l or less. The diluted samples should be pipetted into a clean, empty PCR plate.

**Note:** The goal is to add 100 ng of “enriched SARS-CoV-2” during library amplification and indexing; nonetheless, if 100 ng is not achievable, less can be used.

3. Proceed to “Protocol: Library Amplification and Indexing”.

# Protocol: Library Amplification and Indexing

## Important points before starting

- The QIAseq DIRECT UDI Sets have pierceable foil seals, and the indexes must be pipetted from the plate into separate reaction plates. To prevent cross-contamination, each well is single use.
- Set up the reactions on ice.
- Do not vortex any reagents or reactions.
- Use a thermal cycler with a heated lid.
- Equilibrate the QIAseq Beads to room temperature (15–25°C) for 20–30 min before use.
- Vortex the QIAseq 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

1. Prepare the reagents required for library amplification and indexing.
  - 1a. Thaw the QIAseq DIRECT UDI Index Set A, B, C, or D at room temperature. Mix by gently vortexing the plate and then centrifuge briefly.
  - 1b. Thaw the QIAseq 2X HiFi MM on ice.

**Note:** If a precipitate is present, bring to room temperature, and dissolve the precipitate by mixing with pipettor.
  - 1c. Mix by flicking the tubes, and then centrifuge briefly.
2. Program a thermal cycler with a heated lid according to Table 6.

**Table 6. Library amplification and indexing cycling conditions**

Step	Temperature (°C)	Time	Number of cycles
Initial denaturation	98	2 min	1
3-step cycling			
Denaturation	98	20 s	
Annealing	60	30 s	6*
Extension	72	30 s	
Final extension	72	1 min	1
Hold	4	∞	Hold

\* Based on input of 100 ng of “enriched SARS-CoV-2”. If 50 ng is added, perform 7 cycles. If 10 ng is added, perform 9 cycles.

- For the QIAseq DIRECT UDI plates, pierce the foil seal associated with each well that will be used, and transfer 2 µl (each well contains a forward primer and a reverse primer, each with a unique index) to the diluted “enriched SARS-CoV-2” sample plate prepared in “Protocol: SARS-CoV-2 Enrichment Quantification and Normalization”.

**Important:** Only one UDI pair should be used per amplification reaction.

**Important:** The QIAseq DIRECT UDI index plates are stable for a maximum of 10 freeze–thaw cycles. If all 96-wells have not been used at one time, cover used wells with foil and return to the freezer. Do not reuse wells from the QIAseq DIRECT UDI index plates once the foil seals have been pierced. This would risk significant cross-contamination.

- On ice, prepare the library amplification and indexing reaction according to Table 7. Briefly centrifuge, mix by pipetting up and down 10 times, 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 7. Reaction mix for library amplification and indexing**

Component	Volume/reaction
Diluted "enriched SARS-CoV-2" sample	Variable (100 ng*)
Index from QIAseq DIRECT UDI index plate (A, B, C, or D) Plate	2 µl
QIAseq 2X HiFi MM	25 µl
Nuclease-free water	Variable
<b>Total reaction volume</b>	<b>50 µl</b>

\* Based on input of 100 ng of "enriched SARS-CoV-2". 50 ng or 10 ng can also be added. If lower amounts have been added, adjust the number of cycles accordingly in Table 6.

- Transfer the plate to the thermal cycler and start the program.
- Once PCR is complete, add 55 µl of resuspended QIAseq Beads to each 50 µl reaction. Briefly centrifuge, mix by pipetting up and down 12 times, centrifuge briefly again.

**Note:** Vortex the QIAseq Beads thoroughly to ensure they are well resuspended; the solution should be a homogenous brown color.

- Incubate the mixture for 5 min at room temperature.
- Centrifuge the plate until the beads are pelleted (2 min), and then place the plate onto a magnetic rack. After the solution has cleared (2 min or longer), carefully remove and discard the supernatant.

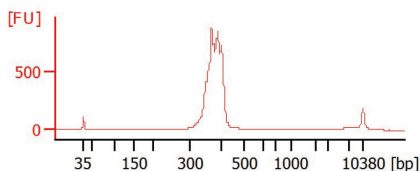
**Important:** Do not discard the beads as they contain the DNA of interest.

**Tip:** It may be valuable to discard the supernatant twice. The contents settle after the first discard.

- With the plate still on the magnetic stand, add 200 µl of 80% ethanol. Wait 1 min and carefully remove and discard the wash. Repeat the wash, for a total of 2 ethanol washes. Remove excess ethanol as much as possible.

**Important:** To completely remove all traces of the ethanol wash after the second wash, immediately briefly centrifuge and return the plate 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. This should be done quickly.

10. With the plate still on the magnetic stand, air dry at room temperature for 4–8 min (until the beads start to crack and pellet loses its shine).
11. Remove the plate from the magnetic stand and elute the DNA from the beads by adding 25 µl nuclease-free water. Mix well by pipetting up and down at least 12 times and incubate for 2 min.
12. Centrifuge the plate until the beads are pelleted (2 min), and then return the plate to the magnetic rack until the solution has cleared.
13. Transfer 23 µl to a clean plate. This is the “SARS-CoV-2 library”. If not proceeding immediately, the sample can be stored at –30 to –15°C.
14. Assess the quality of the library using a Bioanalyzer, TapeStation, or Fragment Analyzer. Check for the expected size distribution of library fragments. An example library is shown in Figure 2:



**Figure 2.** Example QIAseq DIRECT SARS-CoV-2 library.

15. Quantify and normalize the “SARS-CoV-2 library”, and proceed to “Protocol: Sequencing Setup on an Illumina Instrument”. Alternatively, the purified “SARS-CoV-2 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

“SARS-CoV-2 libraries” are compatible with Illumina NGS platforms, including iSeq100, MiniSeq, MiSeq, NextSeq 500/550, HiSeq 2500, HiSeq 3000/4000, and NovaSeq 6000.

## Important points before starting

- **Important:** To make sequencing preparation convenient, download Illumina compatible sample sheets for different sequencing instruments on [www.qiagen.com](http://www.qiagen.com), and refer to “Appendix A: QIAseq DIRECT Unique Dual Indexes”.
- **Important:** 149 bp paired-end sequencing with dual 10 bp indexes should be used.
- 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

- Dilute the individual “SARS-CoV-2 libraries” to a concentration of 4 nM, then combine libraries with different sample indexes in equimolar amounts. The recommended starting final loading concentration of the pooled “SARS-CoV-2 libraries” to load onto a MiSeq is 8 pM, or 1.5 pM on a MiniSeq, or 1.5 pM on a NextSeq instrument.
- Dilute the individual “SARS-CoV-2 libraries” to a concentration of 10 nM, then combine libraries with different sample indexes in equimolar amounts. The recommended final loading concentration of the pooled “SARS-CoV-2 libraries” to load onto a NovaSeq instrument is between 175 and 265 pM.

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## 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 NGS 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 in our Technical Support Center: [www.qiagen.com/FAQ/FAQList.aspx](http://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 or protocols in this handbook (for contact information, visit [support.qiagen.com](http://support.qiagen.com)).

## Comments and suggestions

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### 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.   |
| b) Residual ethanol after QIAseq Bead washes             | Ensure that (1) all ethanol has been removed and (2) QIAseq Beads have been thoroughly dried, according to the protocols listed in the handbook. |

### Dimers observed in final library traces

- |  |   |
|--|---|
| a) Residual ethanol after QIAseq Bead washes | Residual ethanol during QIAseq Bead washes can also result in library dimers. Ensure that (1) all ethanol has been removed and (2) QIAseq Beads have been thoroughly dried, according to the protocols listed in the handbook.  |
| b) Ultra-low viral titer                     | With ultra-low viral titers (20 copies and under), there is the possibility of dimers. As ultra-low viral titer samples conceptually do not require as many sequencing reads as higher viral titer samples, this should not present an issue during next-generation sequencing. |

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# Appendix A: QIAseq DIRECT Unique Dual Indexes

## Generation of sample sheets for Illumina instruments

Index sequences for QIAseq DIRECT Unique Dual Indexes 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 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 DIRECT Unique Dual Indexes 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.

## QIAseq DIRECT UDI Layouts

The layouts of the single-use QIAseq DIRECT UDI plates is shown in Figure 3. 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**.

	1	2	3	4	5	6	7	8	9	10	11	12
A	17RUI-001	17RUI-009	17RUI-017	17RUI-025	17RUI-033	17RUI-041	17RUI-049	17RUI-057	17RUI-065	17RUI-073	17RUI-081	17RUI-089
B	18RUI-001	18RUI-009	18RUI-017	18RUI-025	18RUI-033	18RUI-041	18RUI-049	18RUI-057	18RUI-065	18RUI-073	18RUI-081	18RUI-089
C	17RUI-002	17RUI-010	17RUI-018	17RUI-026	17RUI-034	17RUI-042	17RUI-050	17RUI-058	17RUI-066	17RUI-074	17RUI-082	17RUI-090
D	17RUI-003	17RUI-011	17RUI-019	17RUI-027	17RUI-035	17RUI-043	17RUI-051	17RUI-059	17RUI-067	17RUI-075	17RUI-083	17RUI-091
E	18RUI-003	18RUI-011	18RUI-019	18RUI-027	18RUI-035	18RUI-043	18RUI-051	18RUI-059	18RUI-067	18RUI-075	18RUI-083	18RUI-091
F	17RUI-004	17RUI-012	17RUI-020	17RUI-028	17RUI-036	17RUI-044	17RUI-052	17RUI-060	17RUI-068	17RUI-076	17RUI-084	17RUI-092
G	18RUI-004	18RUI-012	18RUI-020	18RUI-028	18RUI-036	18RUI-044	18RUI-052	18RUI-060	18RUI-068	18RUI-076	18RUI-084	18RUI-092
H	17RUI-005	17RUI-013	17RUI-021	17RUI-029	17RUI-037	17RUI-045	17RUI-053	17RUI-061	17RUI-069	17RUI-077	17RUI-085	17RUI-093
I	18RUI-005	18RUI-013	18RUI-021	18RUI-029	18RUI-037	18RUI-045	18RUI-053	18RUI-061	18RUI-069	18RUI-077	18RUI-085	18RUI-093
J	17RUI-006	17RUI-014	17RUI-022	17RUI-030	17RUI-038	17RUI-046	17RUI-054	17RUI-062	17RUI-070	17RUI-078	17RUI-086	17RUI-094
K	18RUI-006	18RUI-014	18RUI-022	18RUI-030	18RUI-038	18RUI-046	18RUI-054	18RUI-062	18RUI-070	18RUI-078	18RUI-086	18RUI-094
L	17RUI-007	17RUI-015	17RUI-023	17RUI-031	17RUI-039	17RUI-047	17RUI-055	17RUI-063	17RUI-071	17RUI-079	17RUI-087	17RUI-095
M	18RUI-007	18RUI-015	18RUI-023	18RUI-031	18RUI-039	18RUI-047	18RUI-055	18RUI-063	18RUI-071	18RUI-079	18RUI-087	18RUI-095
N	17RUI-008	17RUI-016	17RUI-024	17RUI-032	17RUI-040	17RUI-048	17RUI-056	17RUI-064	17RUI-072	17RUI-080	17RUI-088	17RUI-096
O	18RUI-008	18RUI-016	18RUI-024	18RUI-032	18RUI-040	18RUI-048	18RUI-056	18RUI-064	18RUI-072	18RUI-080	18RUI-088	18RUI-096

	1	2	3	4	5	6	7	8	9	10	11	12
A	ITRUDI-497	ITRUDI-105	ITRUDI-113	ITRUDI-121	ITRUDI-129	ITRUDI-137	ITRUDI-145	ITRUDI-153	ITRUDI-161	ITRUDI-169	ITRUDI-177	ITRUDI-185
	SRUDI-497	SRUDI-105	SRUDI-113	SRUDI-121	SRUDI-129	SRUDI-137	SRUDI-145	SRUDI-153	SRUDI-161	SRUDI-169	SRUDI-177	SRUDI-185
B	ITRUDI-498	ITRUDI-106	ITRUDI-114	ITRUDI-122	ITRUDI-130	ITRUDI-138	ITRUDI-146	ITRUDI-154	ITRUDI-162	ITRUDI-170	ITRUDI-178	ITRUDI-186
	SRUDI-498	SRUDI-106	SRUDI-114	SRUDI-122	SRUDI-130	SRUDI-138	SRUDI-146	SRUDI-154	SRUDI-162	SRUDI-170	SRUDI-178	ITRUDI-186
C	ITRUDI-499	ITRUDI-107	ITRUDI-115	ITRUDI-123	ITRUDI-131	ITRUDI-139	ITRUDI-147	ITRUDI-155	ITRUDI-163	ITRUDI-171	ITRUDI-179	ITRUDI-187
	SRUDI-499	SRUDI-107	ITRUDI-115	ITRUDI-123	ITRUDI-131	ITRUDI-139	ITRUDI-147	ITRUDI-155	ITRUDI-163	ITRUDI-171	ITRUDI-179	ITRUDI-187
D	ITRUDI-100	ITRUDI-108	ITRUDI-116	ITRUDI-124	ITRUDI-132	ITRUDI-140	ITRUDI-148	ITRUDI-156	ITRUDI-164	ITRUDI-172	ITRUDI-180	ITRUDI-188
	SRUDI-100	SRUDI-108	ITRUDI-116	ITRUDI-124	ITRUDI-132	ITRUDI-140	ITRUDI-148	ITRUDI-156	ITRUDI-164	ITRUDI-172	ITRUDI-180	ITRUDI-188
E	ITRUDI-101	ITRUDI-109	ITRUDI-117	ITRUDI-125	ITRUDI-133	ITRUDI-141	ITRUDI-149	ITRUDI-157	ITRUDI-165	ITRUDI-173	ITRUDI-181	ITRUDI-189
	SRUDI-101	SRUDI-109	ITRUDI-117	ITRUDI-125	ITRUDI-133	ITRUDI-141	ITRUDI-149	ITRUDI-157	ITRUDI-165	ITRUDI-173	ITRUDI-181	ITRUDI-189
F	ITRUDI-102	ITRUDI-110	ITRUDI-118	ITRUDI-126	ITRUDI-134	ITRUDI-142	ITRUDI-150	ITRUDI-158	ITRUDI-166	ITRUDI-174	ITRUDI-182	ITRUDI-190
	SRUDI-102	SRUDI-110	ITRUDI-118	ITRUDI-126	ITRUDI-134	ITRUDI-142	ITRUDI-150	ITRUDI-158	ITRUDI-166	ITRUDI-174	ITRUDI-182	ITRUDI-190
G	ITRUDI-103	ITRUDI-111	ITRUDI-119	ITRUDI-127	ITRUDI-135	ITRUDI-143	ITRUDI-151	ITRUDI-159	ITRUDI-167	ITRUDI-175	ITRUDI-183	ITRUDI-191
	SRUDI-103	ITRUDI-111	ITRUDI-119	ITRUDI-127	ITRUDI-135	ITRUDI-143	ITRUDI-151	ITRUDI-159	ITRUDI-167	ITRUDI-175	ITRUDI-183	ITRUDI-191
H	ITRUDI-104	ITRUDI-112	ITRUDI-120	ITRUDI-128	ITRUDI-136	ITRUDI-144	ITRUDI-152	ITRUDI-160	ITRUDI-168	ITRUDI-176	ITRUDI-184	ITRUDI-192
	SRUDI-104	ITRUDI-112	ITRUDI-120	ITRUDI-128	ITRUDI-136	ITRUDI-144	ITRUDI-152	ITRUDI-160	ITRUDI-168	ITRUDI-176	ITRUDI-184	ITRUDI-192

	1	2	3	4	5	6	7	8	9	10	11	12
A	IRU(1)-13	IRU(2)-31	IRU(3)-209	IRU(4)-217	IRU(5)-225	IRU(6)-233	IRU(7)-241	IRU(8)-249	IRU(9)-257	IRU(10)-265	IRU(11)-273	IRU(12)-281
B	ISRU(1)-13	ISRU(2)-31	ISRU(3)-209	ISRU(4)-217	ISRU(5)-225	ISRU(6)-233	ISRU(7)-241	ISRU(8)-249	ISRU(9)-257	ISRU(10)-265	ISRU(11)-273	ISRU(12)-281
	IRU(1)-194	IRU(2)-262	IRU(3)-210	IRU(4)-218	IRU(5)-226	IRU(6)-234	IRU(7)-242	IRU(8)-250	IRU(9)-258	IRU(10)-266	IRU(11)-274	IRU(12)-282
	ISRU(1)-194	ISRU(2)-262	ISRU(3)-210	ISRU(4)-218	ISRU(5)-226	ISRU(6)-234	ISRU(7)-242	ISRU(8)-250	ISRU(9)-258	ISRU(10)-266	ISRU(11)-274	ISRU(12)-282
C	IRU(1)-195	IRU(2)-203	IRU(11)-211	IRU(8)-219	IRU(12)-227	IRU(2)-235	IRU(10)-243	IRU(5)-251	IRU(9)-259	IRU(1)-267	IRU(7)-275	IRU(13)-283
	ISRU(1)-195	ISRU(2)-203	ISRU(11)-211	IRU(8)-219	IRU(12)-227	ISRU(2)-235	ISRU(10)-243	IRU(5)-251	ISRU(9)-259	ISRU(1)-267	IRU(7)-275	IRU(13)-283
D	IRU(1)-196	IRU(2)-204	IRU(12)-212	IRU(2)-220	IRU(12)-228	IRU(2)-236	IRU(12)-244	IRU(2)-252	IRU(2)-260	IRU(8)-268	IRU(2)-276	IRU(2)-284
	ISRU(1)-196	ISRU(2)-204	IRU(12)-212	IRU(2)-220	IRU(12)-228	ISRU(2)-236	IRU(12)-244	IRU(2)-252	IRU(2)-260	IRU(8)-268	IRU(2)-276	IRU(2)-284
E	IRU(1)-197	IRU(2)-205	IRU(13)-213	IRU(2)-221	IRU(13)-229	IRU(2)-237	IRU(13)-245	IRU(2)-253	IRU(2)-261	IRU(9)-269	IRU(2)-277	IRU(14)-285
	ISRU(1)-197	ISRU(2)-205	IRU(13)-213	IRU(2)-221	IRU(13)-229	ISRU(2)-237	IRU(13)-245	IRU(2)-253	IRU(2)-261	IRU(9)-269	IRU(2)-277	IRU(14)-285
F	IRU(1)-206	IRU(2)-206	IRU(14)-214	IRU(2)-222	IRU(14)-230	IRU(2)-238	IRU(2)-246	IRU(2)-254	IRU(2)-262	IRU(2)-270	IRU(2)-278	IRU(2)-286
	ISRU(1)-206	ISRU(2)-206	IRU(14)-214	IRU(2)-222	IRU(14)-230	ISRU(2)-238	IRU(2)-246	IRU(2)-254	IRU(2)-262	IRU(2)-270	IRU(2)-278	IRU(2)-286
G	IRU(1)-199	IRU(2)-207	IRU(2)-215	IRU(2)-223	IRU(2)-231	IRU(2)-239	IRU(2)-247	IRU(2)-255	IRU(2)-263	IRU(2)-271	IRU(2)-279	IRU(2)-287
	ISRU(1)-199	ISRU(2)-207	IRU(2)-215	IRU(2)-223	IRU(2)-231	ISRU(2)-239	IRU(2)-247	IRU(2)-255	IRU(2)-263	IRU(2)-271	IRU(2)-279	IRU(2)-287
H	IRU(1)-200	IRU(2)-208	IRU(2)-216	IRU(2)-224	IRU(2)-232	IRU(2)-240	IRU(2)-248	IRU(2)-256	IRU(2)-264	IRU(2)-272	IRU(2)-280	IRU(2)-288
	ISRU(1)-200	ISRU(2)-208	IRU(2)-216	IRU(2)-224	ISRU(2)-232	IRU(2)-240	IRU(2)-248	IRU(2)-256	IRU(2)-264	IRU(2)-272	IRU(2)-280	IRU(2)-288

	1	2	3	4	5	6	7	8	9	10	11	12
A	TRUDU-289	TRUDU-291	TRUDU-305	TRUDU-313	TRUDU-321	TRUDU-329	TRUDU-337	TRUDU-345	TRUDU-353	TRUDU-361	TRUDU-369	TRUDU-377
	ESRUZ-289	ESRUZ-291	ESRUZ-305	ESRUZ-313	ESRUZ-321	ESRUZ-329	ESRUZ-337	ESRUZ-345	ESRUZ-353	ESRUZ-361	ESRUZ-369	ESRUZ-377
B	TRUDU-290	TRUDU-292	TRUDU-306	TRUDU-314	TRUDU-322	TRUDU-330	TRUDU-338	TRUDU-346	TRUDU-354	TRUDU-362	TRUDU-370	TRUDU-378
	ESRUZ-290	ESRUZ-292	ESRUZ-306	ESRUZ-314	ESRUZ-322	ESRUZ-330	ESRUZ-338	ESRUZ-346	ESRUZ-354	ESRUZ-362	ESRUZ-370	ESRUZ-378
C	TRUDU-291	TRUDU-299	TRUDU-307	TRUDU-315	TRUDU-323	TRUDU-331	TRUDU-339	TRUDU-347	TRUDU-355	TRUDU-363	TRUDU-371	TRUDU-379
	ESRUZ-291	ESRUZ-299	ESRUZ-307	ESRUZ-315	ESRUZ-323	ESRUZ-331	ESRUZ-339	ESRUZ-347	ESRUZ-355	ESRUZ-363	ESRUZ-371	ESRUZ-379
D	TRUDU-292	TRUDU-300	TRUDU-308	TRUDU-316	TRUDU-324	TRUDU-332	TRUDU-340	TRUDU-348	TRUDU-356	TRUDU-364	TRUDU-372	TRUDU-380
	ESRUZ-292	ESRUZ-300	ESRUZ-308	ESRUZ-316	ESRUZ-324	ESRUZ-332	ESRUZ-340	ESRUZ-348	ESRUZ-356	ESRUZ-364	ESRUZ-372	ESRUZ-380
E	TRUDU-293	TRUDU-301	TRUDU-309	TRUDU-317	TRUDU-325	TRUDU-333	TRUDU-341	TRUDU-349	TRUDU-357	TRUDU-365	TRUDU-373	TRUDU-381
	ESRUZ-293	ESRUZ-301	ESRUZ-309	ESRUZ-317	ESRUZ-325	ESRUZ-333	ESRUZ-341	ESRUZ-349	ESRUZ-357	ESRUZ-365	ESRUZ-373	ESRUZ-381
F	TRUDU-294	TRUDU-302	TRUDU-310	TRUDU-318	TRUDU-326	TRUDU-334	TRUDU-342	TRUDU-350	TRUDU-358	TRUDU-366	TRUDU-374	TRUDU-382
	ESRUZ-294	ESRUZ-302	ESRUZ-310	ESRUZ-318	ESRUZ-326	ESRUZ-334	ESRUZ-342	ESRUZ-350	ESRUZ-358	ESRUZ-366	ESRUZ-374	ESRUZ-382
G	TRUDU-295	TRUDU-303	TRUDU-311	TRUDU-319	TRUDU-327	TRUDU-335	TRUDU-343	TRUDU-351	TRUDU-359	TRUDU-367	TRUDU-375	TRUDU-383
	ESRUZ-295	ESRUZ-303	ESRUZ-311	ESRUZ-319	ESRUZ-327	ESRUZ-335	ESRUZ-343	ESRUZ-351	ESRUZ-359	ESRUZ-367	ESRUZ-375	ESRUZ-383
H	TRUDU-296	TRUDU-304	TRUDU-312	TRUDU-320	TRUDU-328	TRUDU-336	TRUDU-344	TRUDU-352	TRUDU-360	TRUDU-368	TRUDU-376	TRUDU-384
	ESRUZ-296	ESRUZ-304	ESRUZ-312	ESRUZ-320	ESRUZ-328	ESRUZ-336	ESRUZ-344	ESRUZ-352	ESRUZ-360	ESRUZ-368	ESRUZ-376	ESRUZ-384

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# Ordering Information

Product	Contents	Cat. no.
QIAseq DIRECT SARS-CoV-2 Kit A	For 96 reactions: Contains all buffers and reagents for cDNA synthesis, high-fidelity SARS-CoV-2 amplicon enrichment, and high-fidelity library amplification; includes index set A with UDIs 1-96 (pierceable foil seal allowing usage of defined parts of plate)	333891
QIAseq DIRECT SARS-CoV-2 Kit B	For 96 reactions: Contains all buffers and reagents for cDNA synthesis, high-fidelity SARS-CoV-2 amplicon enrichment, and high-fidelity library amplification; includes index set B with UDIs 97-192 (pierceable foil seal allowing usage of defined parts of plate)	333892
QIAseq DIRECT SARS-CoV-2 Kit C	For 96 reactions: Contains all buffers and reagents for cDNA synthesis, high-fidelity SARS-CoV-2 amplicon enrichment, and high-fidelity library amplification; includes index set C with UDIs 193-288 (pierceable foil seal allowing usage of defined parts of plate)	333893

Product	Contents	Cat. no.
QIAseq DIRECT SARS-CoV-2 Kit D	For 96 reactions: Contains all buffers and reagents for cDNA synthesis, high-fidelity SARS-CoV-2 amplicon enrichment, and high-fidelity library amplification; includes index set D with UDIs 289-384 (pierceable foil seal allowing usage of defined parts of plate)	333894
QIAseq DIRECT SARS-CoV-2 HT (A–D)	For 384 reactions: Contains all buffers and reagents for cDNA synthesis, high-fidelity SARS-CoV-2 amplicon enrichment, and high-fidelity library amplification; includes index sets A, B, C and D with UDIs 1-384 (pierceable foil seal allowing usage of defined parts of plates)	333898

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# Document Revision History

Date	Changes
04/2021	Initial revision

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