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Illumina COVIDSeq RUO Kits

Reference Guide

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For Research Use Only. Not for use in diagnostic procedures.

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Overview

Introduction

This guide explains how to detect the SARS-CoV-2 virus using either of two different research use only kits: the Illumina COVIDSeq Test (RUO) or the Illumina COVIDSeq Assay (96 Samples). The Illumina COVIDSeq Test supports sample processing for high throughput (HT) sequencing, while the Illumina COVIDSeq Assay is oriented toward sample processing for low throughput (LT) sequencing.

The Illumina COVIDSeq Test offers preparation of up to 3072 samples using the NovaSeq 6000 Sequencing System or up to 384 samples using the NextSeq 500/550 Sequencing Systems, NextSeq 550Dx Instrument in RUO mode, or NextSeq 1000/2000 Sequencing System. The Illumina COVIDSeq Assay offers preparation of up to 96 samples using the iSeq 100 Sequencing System, MiSeq Sequencing System or MiniSeq Sequencing System.

Input Recommendations

The Illumina COVIDSeq Test (RUO) and Illumina COVIDSeq Assay (96 Samples) support samples derived from nasopharyngeal (NP), oropharyngeal (OP), nasal swabs, and wastewater. Transport samples according to the governing regulations for the transport of etiologic agents applicable to your region.

Store samples according to the instructions from the manufacturer of tubes used for sample transport. Exceeding the storage times can negatively impact test results. Recommended extraction kits include the QIAamp Viral RNA Mini Kit and the Quick-DNA/RNA Viral Magbead Kit.

The following sample factors might affect SARS-CoV-2 detection:

- Sample collection methods, patient factors, and/or the stage of the infection.
- Viral RNA degradation during shipping and storage. RNA degradation can produce false-negative results.

Handle all specimens as infectious reagents.

Library Prep

Introduction

This chapter describes library preparation using either the Illumina COVIDSeq Test (RUO) or the Illumina COVIDSeq Assay (96 Samples).

- Confirm kit contents and make sure that you have the required equipment and consumables. Refer to *Consumables and Equipment* on page 20.

  The two kits contain minor differences in the names on reagent labeling. Reagents in the Illumina COVIDSeq Test (RUO) kit have HT on their labels to indicate use for high throughput sequencing. Reagents in the Illumina COVIDSeq Assay (96 Samples) do not. In addition, the reagent labeled ITB in Illumina COVIDSeq Test (RUO) is labeled IPB in Illumina COVIDSeq Assay (96 Samples).

- Follow the protocols in the order shown, using the specified volumes and incubation parameters.
- Make sure that reagents are not expired. Using expired reagents might negatively affect performance.
- When using the Illumina COVIDSeq Assay (96 Samples) or the Illumina COVIDSeq Test (RUO) for surveillance, a no template control (NTC) and a positive control are recommended for quality control but are not required. See *COVIDSeq Positive Control Preparation* on page 29 for preparation instructions. The positive control is not included in either Illumina COVIDSeq Test or Illumina COVIDSeq Assay. It is available separately from Illumina, part number 20041775.
- If performing library prep multiple times with the Illumina COVIDSeq Test (RUO), refer to the *Aliquot Procedure for Illumina COVIDSeq Test (RUO version) Kit Reagents* Illumina Technical Note.
- Do not allow more than eight freeze-thaw cycles for reagents kept at -20°C.

- Sequence libraries as soon as possible after pooling. Pooled libraries are stable for up to 30 days at -25°C to -15°C.

Tips and Techniques

Unless a safe stopping point is specified in the protocol, proceed immediately to the next step.

Avoiding Contamination

- Use proper laboratory practices to prevent nuclease and PCR product contamination. Nuclease and PCR product contamination can cause inaccurate and unreliable results.
- Perform library preparation in a RNase/DNase-free environment. Thoroughly decontaminate work areas with a RNAse/DNase-inhibiting solution, such as RNAseZap and DNAZap.
- Use fresh tips and fresh consumable labware between samples and dispensing reagents.
- Use aerosol-resistant tips to reduce the risk of carry-over and sample-to-sample cross-contamination.
- Due to the potential for contamination, take extreme care to make sure that well contents remain fully in the well. Do not splash contents.
- Do not use aerosol bleach sprays when performing library preparation. Trace bleach contamination can lead to assay failure.
- Use a unidirectional workflow when moving from pre-amplification to post-amplification environments.
- One or more no template controls (NTCs) are recommended per plate to monitor contamination.

Sealing and Unsealing the Plate

- Always seal the 96-well plate before the following steps in the protocol:
  - Shaking steps
  - Vortexing steps
  - Centrifuge steps
  - Thermal cycling steps
- To seal the plate, apply the adhesive cover to the plate and then seal with a wedge or rubber roller.
- Make sure the edges and wells are completely sealed to reduce the risk of cross-contamination and evaporation.
- Microseal 'B' adhesive seals are effective at -40°C to 110°C, and suitable for skirted or semiskirted PCR plates. Use Microseal 'B' for shaking, centrifuging, and long-term storage.
- Before unsealing:
  - Briefly centrifuge the 96-well plate at 1000 × g for 1 minute. For bead steps, centrifuge at 500 x g for 1 minute.
  - Place the plate on a flat surface before slowly removing the seal.

Plate Transfers

- When transferring volumes between plates, transfer the specified volume from each well of a plate to the corresponding well of the other plate.
- If beads are aspirated into the pipette tips, dispense back to the plate on the magnetic stand and wait until the liquid is clear (~2 minutes).

Centrifugation

- Centrifuge as needed at any step in the procedure to consolidate liquid or beads in the bottom of the well, and to prevent sample loss.

Handling Beads

- Pipette bead suspension slowly to prevent splashing and bubbles.
- When mixing, mix thoroughly.
- To avoid sample loss, confirm that no beads remain in pipette tips after resuspension and mixing steps.
- When washing beads:
  - Use the appropriate magnet for the plate.
  - Dispense liquid so that beads on the side of the wells are wetted.
  - Keep the plate on the magnet until the instructions specify to remove it.
  - Do not agitate the plate while on the magnetic stand. Do not disturb the bead pellet.

Extract RNA

This step extracts RNA from decontaminated viral transport medium tubes. You can extract RNA using the Quick-DNA/RNA Viral MagBead, Zymo Research, part # R2141 or the QIAamp Viral RNA Mini Kit, Qiagen, part # 52906. Follow the procedure corresponding to your extraction method.

If you plan to use the COVIDSeq Positive Control, make sure to follow the appropriate preparation procedure in *COVIDSeq Positive Control Preparation* on page 29.

Consumables

- **[QIAamp Viral RNA Mini Kit]** 1.7 ml LoBind tubes
- **[Quick-DNA/RNA Viral MagBead]** 2000 µl 96 deep well plate

Quick-DNA/RNA Viral MagBead Procedure

1. For each clinically-derived sample, add 400 µl sample to a new deep-well plate. For wastewater samples, add the maximum volume allowed in the extraction kit manual.
   If you plan to use controls, include one tube of diluted CPC (positive control) and ELB (no template control) per sample batch.

2. To extract RNA, use the Quick-DNA/RNA Viral MagBead. For information, see *Quick-DNA/RNA Viral MagBead Instruction Manual* from Zymo Research.
   Use the following protocol options:
   - Before adding MagBinding Beads, pipette up and down ten times to mix.
   - After adding 20 µl MagBinding Beads, pipette up and down ten times to mix, and then shake at 1500 rpm for 10 minutes.

QIAamp Viral RNA Mini Kit Procedure

1. For each clinically-derived sample, add 140 µl sample to new 1.7 ml microcentrifuge tube. For wastewater samples, add the maximum volume allowed in the extraction kit manual.
   If you plan to use controls, include one tube of diluted CPC (positive control) and ELB (no template control) per sample batch.
2. To extract RNA, use the QIAamp Viral RNA Mini Kit. For information, see *QIAmp Viral RNA Mini Handbook (document # HB-0354-007)* available on the QIAGEN website.
   Use the following protocol options:
   - Purify viral RNA using the spin protocol.
   - Incubate elution for at least 1 minute.
   - Elute in 30 µl Buffer AVE instead of 60 µl.

Anneal RNA

During this process the extracted RNA is annealed using random hexamers to prepare for cDNA synthesis.

If you plan to use the COVIDSeq Positive Control and have not yet prepared the control, make sure to follow the appropriate procedure in *COVIDSeq Positive Control Preparation* on page 29.

Consumables

- EPH3 (Elution Prime Fragment 3HC Mix)
- 96-well PCR Plate
- Microseal 'B' adhesive seals

About Reagents

- Vortex before each use

Preparation

1. Prepare the following consumables:

Reagent	Storage	Instructions
EPH3	-25°C to -15°C	Thaw at room temperature, and then invert to mix.

2. Save the following COVIDSeq Anneal program on the thermal cycler:
   - Choose the preheat lid option
   - Set the reaction volume to 17 µl
   - 65°C for 3 minutes
   - Hold at 4°C

Procedure

1. Label new PCR plate CDNA1.
2. Add 8.5 µl EPH3 to each well.
3. Add 8.5 µl eluted sample to each well.
4. Seal and shake at 1600 rpm for 1 minute.
5. Centrifuge at 1000 × g for 1 minute.
6. Place on the preprogrammed thermal cycler and run the COVIDSeq Anneal program.

Synthesize First Strand cDNA

This step reverse transcribes the RNA fragments primed with random hexamers into first strand cDNA using reverse transcriptase.

Consumables

- FSM (First Strand Mix)
- RVT (Reverse Transcriptase)
- 1.7 ml tubes (1 per 96-well sample plate)
- Microseal 'B' adhesive seal

Preparation

1. Prepare the following consumables:

Reagent	Storage	Instructions
FSM	-25°C to -15°C	Thaw and bring to room temperature. Invert to mix, and then keep on ice.
RVT	-25°C to -15°C	Invert to mix before use. Keep on ice.

2. Save the following COVIDSeq FSS program on the thermal cycler:

- Choose the preheat lid option
- Set the reaction volume to 25 µl
- 25°C for 5 minutes
- 50°C for 10 minutes
- 80°C for 5 minutes
- Hold at 4°C

Procedure

1. In a 1.7 ml tube, combine the following volumes to prepare First Strand cDNA Master Mix. Multiply each volume by the number of samples.
   - FSM (9 µl)
   - RVT (1 µl)

   Reagent overage is included to account for small pipetting errors.
2. Add 8 µl master mix to each well of the CDNA1 plate.
3. Seal and shake at 1600 rpm for 1 minute.
4. Centrifuge at 1000 × g for 1 minute.
5. Place on the preprogrammed thermal cycler and run the COVIDSeq FSS program.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 7 days.

Amplify cDNA

This step uses two separate PCR reactions to amplify cDNA.

Consumables

- IPM (Illumina PCR Mix)
- C5P1 (COVIDSeq v5.4.2 Primer Pool 1)
- C5P2 (COVIDSeq v5.4.2 Primer Pool 2)
- Nuclease-free water
- 15 ml tube (2 for four 96-well sample plates)
- 96-well PCR plates (2)
- Microseal 'B' adhesive seal

Preparation

1. Prepare the following consumables:

Reagent	Storage	Instructions
C5P1	-25°C to -15°C	Thaw at room temperature. Keep on ice until use.
C5P2	-25°C to -15°C	Thaw at room temperature. Keep on ice until use.
IPM	-25°C to -15°C	Thaw at room temperature, and then invert to mix. Keep on ice until use.

2. Save the following COVIDSeq PCR program on the thermal cycler:
   - Choose the preheat lid option
   - Set the reaction volume to 25 µl
   - 98°C for 3 minutes
   - 35 cycles of:
     - 98°C for 15 seconds
     - 63°C for 5 minutes
   - Hold at 4°C

Procedure

1. Label two new PCR plates COV1 and COV2.
   The plates represent two separate PCR reactions on each sample and control in the CDNA1 plate.
2. In a 15 ml tube, combine the following volumes to prepare COVIDSeq PCR 1 Master Mix and COVIDSeq PCR 2 Master Mix. Multiply each volume by the number of samples.
   Reagent overage is included to account for small pipetting errors.

Reagent	COVIDSeq PCR 1 Master Mix (µl)	COVIDSeq PCR 2 Master Mix (µl)
IPM	15	15
C5P1	4.3	N/A
C5P2	N/A	4.3
Nuclease-free water	4.7	4.7

3. Add 20 µl COVIDSeq PCR 1 Master Mix to each well of the COV1 plate corresponding to each well of the CDNA1 plate.
4. Add 5 µl first strand cDNA synthesis from each well of the CDNA1 plate to the corresponding well of the COV1 plate.
5. Add 20 µl COVIDSeq PCR 2 Master Mix to each well of the COV2 plate corresponding to each well of the CDNA1 plate.
6. Add 5 µl first strand cDNA synthesis from each well of the CDNA1 plate to the corresponding well of the COV2 plate.
7. Seal and shake at 1600 rpm for 1 minute.

8. Centrifuge at 1000 x g for 1 minute.
9. Place in the preprogrammed thermal cycler and run the COVIDSeq PCR program.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 3 days.

Tagment PCR Amplicons

This step uses EBLTS to tagment PCR amplicons, which is a process that fragments and tags the PCR amplicons with adapter sequences.

Consumables

- EBLTS (Enrichment BLT)
- TB1 (Tagmentation Buffer 1)
- Nuclease-free water
- 1.7 ml tube
- 15 ml tube (1 per four 96-well sample plates)
- 96-well PCR plate
- Microseal 'B' adhesive seal

About Reagents

- Store EBLTS upright at temperatures above 2°C. Make sure beads are always submerged in the buffer.
- If beads are adhered to the side or top of the 96-well plate, centrifuge at 500 × g for 1 minute, and then pipette to resuspend.

Preparation

1. Prepare the following consumables:

Reagent	Storage	Instructions
EBLTS	2°C to 8°C	Bring to room temperature. Vortex thoroughly before use.
TB1	-25°C to -15°C	Bring to room temperature. Vortex thoroughly before use.

2. If COV1 and COV2 plates were stored frozen, prepare as follows.
   a. Thaw at room temperature.
   b. Check seals, and then shake at 1600 rpm for 1 minute.
   c. Centrifuge at 1000 x g for 1 minute.
3. Save the following COVIDSeq TAG program on the thermal cycler:

- Choose the preheat lid option
- Set the reaction volume to 50 µl
- 55°C for 5 minutes
- Hold at 10°C

Procedure

1. Label a new PCR plate TAG1.
2. Combine COV1 and COV2 as follows.
   a. Transfer 10 µl from each well of the COV1 plate to the corresponding well of the TAG1 plate.
   b. Transfer 10 µl from each well of the COV2 plate to each well of the TAG1 plate containing COV1.
3. In a 15 ml tube, combine the following volumes to prepare Tagmentation Master Mix. Multiply each volume by the number of samples.
   – TB1 (12 µl)
   – EBLTS (4 µl)
   – Nuclease-free water (20 µl)
4. Add 30 µl master mix to each well in TAG1 plate.
5. Seal and shake at 1600 rpm for 1 minute.
6. Place on the preprogrammed thermal cycler and run the COVIDSeq TAG program.

Post Tagmentation Clean Up

This step washes the adapter-tagged amplicons before PCR amplification.

Consumables

- ST2 (Stop Tagment Buffer 2)
- TWB (Tagmentation Wash Buffer)
- Microseal 'B' adhesive seal

About Reagents

- Dispense ST2 and TWB slowly to minimize foaming.
- Dispense TWB directly onto beads.

Preparation

1. Prepare the following consumables:

Reagent	Storage	Instructions
ST2	Room temperature	Vortex before use.
TWB	2°C to 8°C	Vortex before use.

Procedure

1. Centrifuge the TAG1 plate at 500 x g for 1 minute.
2. Add 10 µl ST2 to each well of the TAG1 plate.
3. Seal and shake at 1600 rpm for 1 minute.
4. Incubate at room temperature for 5 minutes.
5. Centrifuge at 500 × g for 1 minute.
6. Place on the magnetic stand and wait until the liquid is clear (~3 minutes).
7. Inspect for bubbles on the seal. If present, centrifuge at 500 x g for 1 minute, and then place on the magnetic stand (~3 minutes).
8. Remove and discard all supernatant.
9. Wash beads as follows.
   a. Remove from the magnetic stand.
   b. Add 100 µl TWB to each well.
   c. Seal and shake at 1600 rpm for 1 minute.
   d. Centrifuge 500 × g for 1 minute.
   e. Place on the magnetic stand and wait until the liquid is clear (~3 minutes).
   f. For first wash only, remove and discard all supernatant from each well.
10. Wash beads a **second** time.
    Leave supernatant in plate for second wash to prevent beads from overdrying.

Amplify Tagmented Amplicons

This step amplifies the tagmented amplicons using a PCR program. The PCR step adds prepaired 10 base pair Index 1 (i7) adapters, Index 2 (i5) adapters, and sequences required for sequencing cluster generation.

Consumables

- EPM (Enhanced PCR Mix)
- Index adapters (IDT for Illumina-PCR Indexes Set 1, 2, 3, 4)
- Nuclease-free water
- 15 ml tubes (1 per two 96-well sample plates)
- 96-well PCR plate

About Reagents

- Index adapter plates
  - Do not add samples to the index plate wells.
  - Index plate wells are considered single use and should not be reused.

Preparation

1. Prepare the following consumables:

Reagent	Storage	Instructions
EPM	-25°C to -15°C	Invert to mix. Keep on ice until use.
Index adapters	-25°C to -15°C	Thaw at room temperature. Vortex to mix, and then centrifuge at 1000 × g for 1 minute.

2. Open each prepared index adapter plate seal as follows. Use a new PCR plate for each different index set.
   a. Align a new 96-well PCR plate above the index adapter plate, and then press down to puncture the foil seal.
   b. Discard the PCR plate.
3. Save the following COVIDSeq TAG PCR program on the thermal cycler:
   - Choose the preheat lid option and set to 100°C
   - Set the reaction volume to 50 µl
   - 72°C for 3 minutes
   - 98°C for 3 minutes
   - 7 cycles of:
     - 98°C for 20 seconds
     - 60°C for 30 seconds
     - 72°C for 1 minute
   - 72°C for 3 minutes
   - Hold at 10°C

Procedure

1. In a 15 ml tube, combine the following volumes to prepare PCR Master Mix. Multiply each volume by the number of samples.
   - EPM (24 µl)
   - Nuclease-free water (24 µl)
2. Vortex PCR Master Mix to mix.

3. Keep the TAG1 plate on magnetic stand and remove TWB.
4. Use a 20 µl pipette to remove any remaining TWB.
5. Remove the TAG1 plate from the magnetic stand.
6. Add 40 µl PCR Master Mix to each well.
7. Add 10 µl index adapters to each well of the PCR plate.
8. Seal and shake at 1600 rpm for 1 minute.
9. If liquid is visible on the seal, centrifuge at 500 x g for 1 minute.
10. Inspect to make sure beads are resuspended. To resuspend, set your pipette to 35 µl with the plunger down, and then slowly pipette to mix.
11. Place on the preprogrammed thermal cycler and run the COVIDSeq TAG PCR program.

Pool and Clean Up Libraries

This step combines libraries from each 96-well sample plate into one 1.7 ml tube. Libraries of optimal size are then bound to magnetic beads, and fragments that are too small or large are washed away.

Consumables

- **[Illumina COVIDSeq Test (RUO)]** ITB (Illumina Tune Beads)
- **[Illumina COVIDSeq Assay (96 Samples)]** IPB (Illumina Purification Beads)
- RSB (Resuspension Buffer)
- Freshly prepared 80% ethanol (EtOH)
- 1.7 ml tube (2 per 96-well sample plate)
- **[Illumina COVIDSeq Test (RUO)]** PCR 8-tube strip

About Reagents

- ITB or IPB
  - Vortex before each use.
  - Vortex frequently to make sure that beads are evenly distributed.
  - Aspirate and dispense slowly due to the viscosity of the solution.

Preparation

1. Prepare the following consumables:

Reagent	Storage	Instructions
ITB or IPB	Room temperature	Vortex thoroughly to mix.

Reagent	Storage	Instructions
RSB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Vortex and invert to mix.

2. Prepare 2.5 ml 80% EtOH from absolute EtOH for each tube of pooled libraries.

Procedure for Illumina COVIDSeq Assay (96 Samples)

The following steps describe the procedure for the Illumina COVIDSeq Assay (96 Samples) kit. For the Illumina COVIDSeq Test (RUO) kit, refer to *Procedure for Illumina COVIDSeq Test (RUO)* on page 15.

1. Centrifuge the TAG1 plate at 500 × g for 1 minute.
2. Place on the magnetic stand and wait until the liquid is clear (~3 minutes).
3. To pool libraries, complete the following steps appropriate for your kit. Repeat the steps for each additional sample plate.
   a. Label a new 1.7 ml tube Pooled IPB.
   b. Transfer 5 µl library from each well of the TAG1 plate into the Pooled IPB tube.
4. Vortex the Pooled IPB tubes to mix, and then centrifuge briefly.
5. Vortex IPB to resuspend.
6. Add IPB using the resulting volume of Pooled IPB tube volume multiplied by 0.9.
   For example, for 96 samples, add 432 µl IPB to each tube.
7. Vortex to mix.
8. Incubate at room temperature for 5 minutes.
9. Centrifuge briefly.
10. Place on the magnetic stand and wait until the liquid is clear (~5 minutes).
11. Remove and discard all supernatant.
12. Wash beads as follows.
    a. Keep on the magnetic stand and add 1000 µl fresh 80% EtOH to each tube.
    b. Wait 30 seconds.
    c. Remove and discard all supernatant.
13. Wash beads a **second** time.
14. Use a 20 µl pipette to remove all residual EtOH.
15. Add 55 µl RSB.

    Note

    Due to library yield excess, the RSB volume does not impact batches with a small number of samples.

16. Vortex to mix, and then centrifuge briefly.
17. Incubate at room temperature for 2 minutes.

18. Place on the magnetic stand and wait until the liquid is clear (~2 minutes).
19. Transfer 50 µl supernatant from each Pooled IPB tube to a new microcentrifuge tube.

SAFE STOPPING POINT

If you are stopping, cap the tube and store at -25°C to -15°C for up to 30 days.

Procedure for Illumina COVIDSeq Test (RUO)

The following steps describe the procedure for the Illumina COVIDSeq Test (RUO) kit. For the Illumina COVIDSeq Assay (96 Samples) kit, refer to *Procedure for Illumina COVIDSeq Assay (96 Samples)* on page 14.

1. Centrifuge the TAG1 plate at 500 × g for 1 minute.
2. Place on the magnetic stand and wait until the liquid is clear (~3 minutes).
3. To pool libraries, do as follows. Repeat the steps for each additional sample plate.
   a. Use a 20 µl eight-channel pipette to transfer 5 µl library from each well of the TAG1 plate to a PCR 8-tube strip, resulting in 60 µl pooled library per row. Change tips after each column.
   b. Label a new 1.7 ml tube Pooled ITB.
   c. Transfer 55 µl pooled library from each well of the PCR 8-tube strip into the Pooled ITB tube. For each sample plate, these volumes results in 440 µl pools of pooled libraries.

   If processing 3072 samples, these steps result in 32 Pooled ITB tubes.
4. Vortex the Pooled ITB tubes to mix, and then centrifuge briefly.
5. Vortex ITB to resuspend.
6. Add ITB using the resulting volume of Pooled ITB tube volume multiplied by 0.9.
   For example, for 96 samples, add 396 µl ITB to each tube.
7. Vortex to mix.
8. Incubate at room temperature for 5 minutes.
9. Centrifuge briefly.
10. Place on the magnetic stand and wait until the liquid is clear (~5 minutes).
11. Remove and discard all supernatant.
12. Wash beads as follows.
    a. Keep on the magnetic stand and add 1000 µl fresh 80% EtOH to each tube.
    b. Wait 30 seconds.
    c. Remove and discard all supernatant.
13. Wash beads a **second** time.
14. Use a 20 µl pipette to remove all residual EtOH.
15. Add 55 µl RSB.

> **Note**
>
> Due to library yield excess, the RSB volume does not impact batches with a small number of samples.

16. Vortex to mix, and then centrifuge briefly.
17. Incubate at room temperature for 2 minutes.
18. Place on the magnetic stand and wait until the liquid is clear (~2 minutes).
19. Transfer 50 µl supernatant from each Pooled ITB tube to a new microcentrifuge tube.

SAFE STOPPING POINT

If you are stopping, cap the tube and store at -25°C to -15°C for up to 30 days.

Quantify and Normalize Libraries

1. Analyze 2 µl library pool using a Qubit dsDNA HS Assay kit.
   If libraries are outside the standard range, dilute to 1:10 concentration, and analyze again.
2. Calculate the molarity value using the following formula.
   - Use 400 bp as the average library size.

$$\frac{Library\ concentration\ ng/\mu l}{660\ \frac{g}{mol}\ x\ average\ library\ size\ (bp)} x10^6 = Molarity\ (nM)$$

3. Dilute each library pool to a minimum of 30 µl at a normalized concentration 4 nM using RSB.

Pool and Dilute Libraries

After diluting to the starting concentration of 4 nM, libraries are ready to be denatured and diluted to the final loading concentration.

1. Transfer the designated volume of normalized libraries containing the appropriate index adapter sets to a new microcentrifuge tube for each number of samples specified in for v3 primers and Table 1.
   If you have multiple normalized pools, combine the designated volume of each normalized pool in the tube. Doing so produces a final pool of samples diluted to a starting concentration of 4 nM. Do not combine pools with the same index adapter set.

   Table 1 Normalized Pool Volumes and Sample Numbers for Denature and Dilution by Instrument

Sequencing System	Volume of Normalized Libraries Used per Run (µl)	Samples per Final Pool of Normalized Libraries	Samples per Flow Cell
iSeq 100 v1 or v2 Flow Cell	2	8	8

Sequencing System	Volume of Normalized Libraries Used per Run (µl)	Samples per Final Pool of Normalized Libraries	Samples per Flow Cell
MiSeq v2 Flow Cell	5	30	30
MiSeq v3 Flow Cell	5	48	48
MiniSeq HO Flow Cell	25	48	48
NextSeq 500/550 or 550Dx HO Flow Cell	25	384	384
NovaSeq 6000 SP Flow Cell	25	384	384 per lane, 768 per flow cell
NovaSeq 6000 S4 Flow Cell	25	384	384 per lane, 1536 per flow cell
NextSeq 1000/2000 P2 Flow Cell	25	384	384

2. Follow the denature and dilute instructions for your system to dilute to the final loading concentration.
   - For the iSeq Sequencing System, see the *iSeq 100 Sequencing System Guide (document # 1000000036024)*.
   - For the MiSeq Sequencing System, see the *MiSeq System Denature and Dilute Libraries Guide (document # 15039740)*.
   - For the MiniSeq Sequencing System, see the *MiniSeq System Denature and Dilute Libraries Guide (document # 1000000002697)*.
   - For the NextSeq 500/550 Sequencing System and NextSeq 550Dx Sequencing System, see the *NextSeq System Denature and Dilute Libraries Guide (document # 15048776)*.
   - For the NovaSeq 6000 Sequencing System, see the *NovaSeq 6000 Denature and Dilute Libraries Guide (document # 1000000106351)*.
   - For the NextSeq 2000 Sequencing System, see the *NextSeq 1000/2000 Sequencing System Guide (document # 1000000109376)*.
3. Use the following loading concentrations for your system.

   Table 2 Loading Concentrations by Instrument

Sequencing System	Starting Concentration (nM)	Final Loading Concentration (pM)
iSeq 100 v1 or v2 Flow Cell	4	75
MiSeq v2 Flow Cell	4	10

Sequencing System	Starting Concentration (nM)	Final Loading Concentration (pM)
MiSeq v3 Flow Cell	4	12
MiniSeq HO flow cell	4	1.2
NextSeq 500/550 or 550Dx HO flow cell	4	1.4
NovaSeq 6000 SP Flow Cell	4	100
NovaSeq 6000 S4 Flow Cell	4	100
NextSeq 1000/2000 P2 Flow Cell	4	1000

Adjustments to final loading concentration should follow the denature and dilute instructions for your sequencing system.

Prepare for Sequencing

The Illumina COVIDSeq Assay (96 Samples) is compatible with the iSeq 100 i1 reagents v2, the MiSeq reagent kits v2 and v3, and the MiniSeq High Output (HO) reagent kit.

The Illumina COVIDSeq Test (RUO) is compatible with the NovaSeq 6000 Sequencing System SP and S4 flow cells, the NextSeq 2000 Sequencing System, the NextSeq 500/550 Sequencing Systems, and the NextSeq 550DX instrument.

Read Recommendations for Sequencing

Read length and depth can be optimized based on sample type and workflow needs. Viral titer and quality of extracted RNA may impact the read configuration necessary to achieve optimal consensus genome calling. Increasing read depth may improve coverage.

Read length of 2 × 150 is recommended for all runs. Shorter read lengths down to 2 × 75 may be used.

For low complexity high-titer samples (for example nasopharyngeal swabs) a minimum read depth of 0.5M fragments (1M total reads) is recommended.

For high complexity low-titer samples (for example wastewater) a minimum read depth of 4M fragments (8M total reads) is recommended.

Set Up Sequencing Run for Illumina COVIDSeq Assay (96 Samples)

Refer to the documentation for your sequencing system and the following information to set up your run.

- If using the iSeq System, refer to the *iSeq System Guide (document # 1000000036024)*.
- If using the MiSeq, refer to the *MiSeq System Guide (document # 15027617 for Windows 10 instruments, document # 1000000154717 for Windows 7 instruments)*.

- If using the MiniSeq System, refer to the *MiniSeq System Guide (document # 1000000002695)*.
- If using Local Run Manager, refer to the *Local Run Manager Software Guide (document # 1000000111492)*.
- If using a BaseSpace Sequence Hub app, make sure to enable monitoring and storage as needed for your instrument.
    - For the iSeq System, select or enable **Run Analysis, Collaboration, and Storage** in the system settings.
    - For the MiniSeq System, select **Run Monitoring and Storage** as the Configuration option.
- Enter **Paired End** as the Read Type.
- Enter **10** as the value for Index 1 and Index 2.

Set Up Sequencing Run for Illumina COVIDSeq Test (RUO)

Refer to the documentation for your sequencing system and the following information to set up your run.

- If using the NextSeq 500/550 or NexSeq 550Dx, refer to the *NextSeq 500 System Guide (document # 15046563)*, *NextSeq 550 System Guide (document # 15069765)*, or *NextSeq 550Dx Instrument Reference Guide (document # 1000000009513)*.
- If using the NovaSeq 6000 system, refer to the *NovaSeq 6000 Sequencing System Guide (document # 1000000019358)* for sequencing instructions.
- If using the NextSeq 2000, refer to the *NextSeq 1000/2000 Sequencing System Guide (document # 1000000109376)*.
- If using Local Run Manager, refer to the *Local Run Manager Software Guide (document # 1000000111492)*.
- Enter **Paired End** as the Read Type.
- Enter **10** as the value for Index 1 and Index 2.

Analysis Software

After sequencing completes, analysis either takes place locally using installed pipeline software or in BaseSpace Sequence Hub.

Consumables and Equipment

The protocol described in this documentation assumes that you have reviewed the contents of this section, confirmed protocol contents, and obtained all required consumables and equipment.

Illumina COVIDSeq Assay Kit Contents (96 Samples)

The Illumina COVIDSeq Assay (96 Samples) for low throughput sequencing has four different kit options. Each kit option contains a different set of IDT for Illumina-PCR Indexes.

The Illumina COVIDSeq Assay (96 Samples) does not contain the optional COVIDSeq Positive Control which must be purchased separately. See *COVIDSeq Positive Control (Optional)* on page 22.

Table 3 Illumina COVIDSeq Assay Kit Options (96 Samples)

Kit	Catalog #
Illumina COVIDSeq Assay (96 Samples) including Index Set 1	20049393
Illumina COVIDSeq Assay (96 Samples) including Index Set 2	20051772
Illumina COVIDSeq Assay (96 Samples) including Index Set 3	20051773
Illumina COVIDSeq Assay (96 Samples) including Index Set 4	20051774

Illumina COVIDSeq Assay (96 Samples)

Promptly store reagents at the indicated temperature to ensure proper performance.

Table 4 Illumina COVIDSeq Assay Box 1 – 96 Samples, Part # 20051272

Quantity	Label Volume (ml)	Reagent	Description	Storage
1	15	IPB	Illumina Purification Beads	Room temperature, post-amp environment
1	2	ST2	Stop Tagment Buffer 2	Room temperature, post-amp environment

Table 5 Illumina COVIDSeq Assay Box 2 – 96 Samples, Part # 20051273

Quantity	Label Volume (ml)	Reagent	Description	Storage
2	2	EBLTS	Enrichment BLT	2°C to 8°C, post-amp environment
3	2	ELB	Elution Buffer	2°C to 8°C, pre-amp environment
2	2	RSB	Resuspension Buffer	-25°C to -15°C, post-amp environment*
1	50	TWB	Tagmentation Wash Buffer	15°C to 30°C, post-amp environment*

* Storage temperature 2°C to 8°C is also acceptable.

Table 6 Illumina COVIDSeq Assay Box 3 – 96 Samples, Part # 20051274

Quantity	Label Volume (ml)	Reagent	Description	Storage
1	2	C5P1	COVIDSeq v5.4.2 Primer Pool 1	-25°C to -15°C, pre-amp environment
1	2	C5P2	COVIDSeq v5.4.2 Primer Pool 2	-25°C to -15°C, pre-amp environment
4	0.5	EPH3	Elution Prime Fragment 3HC Mix	-25°C to -15°C pre-amp environment
3	2	EPM	Enhanced PCR Mix	-25°C to -15°C, pre-amp environment
3	0.5	FSM	First Strand Mix	-25°C to -15°C, pre-amp environment
4	2	IPM	Illumina PCR Mix	-25°C to -15°C, pre-amp environment
2	0.5	RVT	Reverse Transcriptase	-25°C to -15°C, pre-amp environment
6	0.5	TB1	Tagmentation Buffer 1	-25°C to -15°C, post-amp environment

Table 7 Illumina COVIDSeq Assay Box 4 – 96 Samples, Indexes

Quantity	Description	Storage
1	One of the following sets: • IDT for Illumina- PCR Indexes Set 1 (96 Indexes) • IDT for Illumina- PCR Indexes Set 2 (96 Indexes) • IDT for Illumina- PCR Indexes Set 3 (96 Indexes) • IDT for Illumina- PCR Indexes Set 4 (96 Indexes)	-25°C to -15°C

COVIDSeq Positive Control (Optional)

The COVIDSeq Positive Control (CPC) is optional. It is sold separately from the Illumina COVIDSeq Assay and Illumina COVIDSeq Test.

Table 8 COVIDSeq Positive Control, Catalog #20051775

Quantity	Label Volume (ml)	Reagent	Description	Storage
1	100 µl	CPC	COVIDSeq Positive Control	-85°C to -65°C, pre-amp environment

Illumina COVIDSeq Test (RUO) Kit Contents (3072 Samples)

The Illumina COVIDSeq Test (RUO) for high throughput sequencing requires the Illumina COVIDSeq Test (3072 Samples) and 8 IDT for Illumina-PCR Indexes Sets 1–4.

Component	Kit	Catalog #
Library Preparation	Illumina COVIDSeq Test (3072 Samples)	20043675
Indexes	IDT for Illumina-PCR Indexes Sets 1–4 (384 Indexes)	20043137

Illumina COVIDSeq Test (RUO)

Promptly store reagents at the indicated temperature to ensure proper performance.

Table 9 Illumina COVIDSeq Test Box 1 – 3072 Samples, Part # 20044405

Quantity	Label Volume (ml)	Reagent	Description	Storage
1	233	ITB	Illumina Tune Beads	Room temperature
1	56	ST2 HT	Stop Tagment Buffer 2 HT	Room temperature, post-amp environment

Table 10 Illumina COVIDSeqTest Box 2 – 3072 Samples, Part # 20044406

Quantity	Label Volume (ml)	Reagent	Description	Storage
2	6.1	EBLTS HT	Enrichment BLT HT	2°C to 8°C, post-amp environment
1	114	ELB HT	Elution Buffer HT	2°C to 8°C, pre-amp environment
1	10	RSB HT	Resuspension Buffer HT	2°C to 8°C, post-amp environment
1	845	TWB HT	Tagmentation Wash Buffer HT	2°C to 8°C, post-amp environment

Table 11 Illumina COVIDSeq Test Box 3 – 3072 Samples, Part # 20044407

Quantity	Label Volume (ml)	Reagent	Description	Storage
1	14.4	C5P1 HT	COVIDSeq v5.4.2 Primer Pool 1 HT	-25°C to -15°C, pre-amp environment
1	14.4	C5P2 HT	COVIDSeq v5.4.2 Primer Pool 2 HT	-25°C to -15°C, pre-amp environment
1	45	EPH3 HT	Elution Prime Fragment 3HC Mix HT	-25°C to -15°C pre-amp environment
1	79	EPM HT	Enhanced PCR Mix HT	-25°C to -15°C, pre-amp environment
1	41	FSM HT	First Strand Mix HT	-25°C to -15°C, pre-amp environment

Quantity	Label Volume (ml)	Reagent	Description	Storage
1	100	IPM HT	Illumina PCR Mix HT	-25°C to -15°C, pre-amp environment
1	4.6	RVT HT	Reverse Transcriptase HT	-25°C to -15°C, pre-amp environment
1	38	TB1 HT	Tagmentation Buffer 1 HT	-25°C to -15°C, post-amp environment

IDT for Illumina- PCR Indexes , Store at -25°C to -15°C

The Illumina COVIDSeq Test (RUO) requires 8 IDT for Illumina PCR Indexes Sets 1–4 (32 total 96-well plates).

Quantity	Description	Part Number
8	IDT for Illumina- PCR Indexes Set 1 (96 Indexes)	20043132
8	IDT for Illumina- PCR Indexes Set 2 (96 Indexes)	20043133
8	IDT for Illumina- PCR Indexes Set 3 (96 Indexes)	20043134
8	IDT for Illumina- PCR Indexes Set 4 (96 Indexes)	20043135

Consumables and Equipment

In addition to your kit (Illumina COVIDSeq Test (RUO) or Illumina COVIDSeq Assay (96 Samples)) and IDT for Illumina-PCR Indexes, make sure that you have the required consumables and equipment before starting the protocol.

Consumables

Consumable	Supplier
10 µl pipette tips	General lab supplier
20 µl pipette tips	General lab supplier
200 µl pipette tips	General lab supplier
200 µl pipette tips	General lab supplier
1000 µl pipette tips	General lab supplier

Consumable	Supplier
Hard-Shell 96-Well PCR Plates with the following specifications: • 96 wells with 0.2 ml well capacity. • Polypropylene or equivalent. • Compatible with the specific thermal cycler you use.	General lab supplier
96 deep-well plate, 2000 µl	Eppendorf, catalog # 951033707
8-tube strips	General lab supplier
1.7 ml LoBind microcentrifuge tubes	Eppendorf, catalog # 022431021
5 ml LoBind microcentrifuge tube	Eppendorf, catalog # 0030122348
15 ml tubes	General lab supplier
Lab tissue, low-lint	VWR, catalog # 21905-026, or equivalent
Lint-free alcohol wipe	General lab supplier
Microseal 'B' adhesive seals	Bio-Rad, part # MSB-1001
RNase/DNase-free Disposable Pipetting Resovoirs	VWR, part # 89094-658
One of the following, depending on the extraction method used: • QIAamp Viral RNA Mini Kit • Quick DNA/RNA Viral MagBead Order quantity depending on kit size.	• Qiagen, catalog # 52906 • Zymo Research, catalog # R2141
Qubit dsDNA HS Assay Kit	One of the following, depending on kit size: • ThermoFisher Scientific, part # Q32851 • ThermoFisher Scientific, part # Q32854
Qubit Assay Tubes	ThermoFisher Scientific, catalog # Q32856
If using the iSeq 100 System: • iSeq 100 i1 Reagent v2 (300 Cycles)	• Illumina, catalog # 20031371
If using the MiSeq System v2 reagent kit: • MiSeq Reagent Kit v2 (300 Cycles)	• Illumina, catalog # MS-102-2002
If using the MiSeq System v3 reagent kit: • MiSeq Reagent Kit v3 (600 Cycles)	• Illumina, catalog # MS-102-3003
If using the MiniSeq System: • MiniSeq High Output Reagent Kit (300 Cycles)	• Illumina, catalog # FC-420-1003

Consumable	Supplier
If using the NovaSeq 6000 Sequencing System S4 flow cell: • 2 NovaSeq 6000 Sequencing System S4 Reagent Kit v1.5 (35 cycles) • 2 NovaSeq Xp 4-Lane Kit v1.5	• Illumina, catalog # 20044417 • Illumina, catalog # 20043131
If using the NovaSeq 6000 Sequencing System SP flow cell: • 4 NovaSeq 6000 Sequencing System SP Reagent Kit v1.5 (100 cycles) • 4 NovaSeq Xp 2-Lane Kit v1.5	• Illumina, catalog # 20028401 • Illumina, catalog # 20043130
If using the NextSeq 500/550 System or the NextSeq 550Dx instrument: • 8 NextSeq 500/550 High Output Kit v2.5 (75 Cycles)	• Illumina, catalog # 20024906
If using the NextSeq 2000 System • 8 NextSeq 1000/2000 P2 Reagents (100 cycles)	• Illumina, catalog # 20046811

Equipment Required, Not Provided

Equipment	Supplier
10 µl single-channel pipettes	General lab supplier
20 µl single-channel pipettes	General lab supplier
200 µl single-channel pipettes	General lab supplier
1000 µl single-channel pipettes	General lab supplier
10 µl 8-channel pipettes	General lab supplier
20 µl 8-channel pipettes	General lab supplier
200 µl 8-channel pipettes	General lab supplier
1000 µl 8-channel pipettes	General lab supplier
20 µl 12-channel pipettes	General lab supplier
200 µl 12-channel pipettes	General lab supplier
10 ml serological pipettes	General lab supplier
25 ml serological pipettes	General lab supplier
50 ml serological pipettes	General lab supplier
BioShake iQ	QInstruments, part # 1808-0506

Equipment	Supplier
Equipment for one the following extraction methods (as needed): • Quick-DNA/RNA Viral MagBead equipment	• See *Quick-DNA/RNA Viral MagBead Instruction Manual*, Zymo Research
• QIAamp Viral RNA Mini Kit equipment	• See *QIAamp Viral RNA Mini Handbook (document # HB-0354-006)*, Qiagen
Freezer, -25°C to -15°C	General lab supplier
Freezer, -85°C to -65°C (if using optional positive control)	General lab supplier
Magnetic Stand-96	Thermo Fisher Scientific, catalog # AM10027
One of the following magnetic stands: • Dynabeads MPC-S (Magnetic Particle Concentrator) • MagnaRack Magnetic Separation Rack	• Thermo Fisher Scientific, catalog #A13346 • Thermo Fisher Scientific, catalog # CS15000
Microcentrifuge	General lab supplier
Microplate Centrifuge	General lab supplier
One of the following sequencing systems: • iSeq 100 • MiSeq • MiniSeq • NextSeq 500 • NextSeq 550 • NextSeq 550Dx • NextSeq 2000 • NovaSeq 6000	Illumina
NovaSeq Xp Flow Cell Dock	Illumina, # 20021663
Pipette Aid	General lab supplier
Qubit Fluorometer 3.0	Thermo Fisher, catalog # Q33216, Q33217, or Q33218
Refrigerator, 2°C to 8°C	General lab supplier

Equipment	Supplier
Thermal cycler with the following specifications: • Heated lid • Minimum temperature control range: 4°C to 99°C • Format: 0.2 mL tubes, 96-well plate • Temperature accuracy: ±0.25°C (35°C to 99.9°C) • Temperature uniformity: ±0.5°C well-to-well within 30 seconds of arrival at target temperature • Peak ramp rate: At least 1.5°C • Sample ramp rate: ± 1.25°C	General lab supplier
Sealing wedge or roller	General lab supplier
Vortexer	General lab supplier

COVIDSeq Positive Control Preparation

COVIDSeq Positive Control Preparation

This procedure dilutes the COVIDSeq Positive Control (CPC) and prepares it for use with the Illumina COVIDSeq Assay (96 Samples) and Illumina COVIDSeq Test (RUO) kits.

Consumables

- 1.7 ml LoBind tubes
- 5 ml LoBind tubes
- COVIDSeq Positive Control

About Reagents

- Do not allow multiple freeze-thaws of the CPC. If performing library prep multiple times, aliquot CPC into low-bind tubes. Store at -85°C to -65°C.
- Vortex before each use.

Preparation for Illumina COVIDSeq Assay (96 Samples)

The following steps describe the procedure for the Illumina COVIDSeq Assay (96 Samples) kit. For the Illumina COVIDSeq Test (RUO) kit, refer to *Preparation for Illumina COVIDSeq Test (RUO)* on page 30.

Use of the COVIDSeq Positive Control (CPC) with the Illumina COVIDSeq Assay (96 Samples) is recommended but not required.

1. Prepare the following consumables:

Reagent	Storage	Instructions
ELB	2°C to 8°C	Thaw at room temperature, and then invert to mix. Keep on ice until use.
CPC	-85°C to -65°C	Dilute using the following instructions. Keep diluted positive control on ice.

2. Dilute CPC as follows.
   a. Label a 1.7 ml tube Dilution 1.
   b. Add the following volumes to the tube ***in the order listed***.
      - CPC (1 µl)

- ELB (99 µl)

These volumes produce 10000 copies per µl.

c. Pulse vortex to mix.

3. Dilute CPC a second time as follows.
   a. Label a 1.7 ml tube Dilution 2.
   b. Add the following volumes to the tube ***in the order listed***.
      - Dilution 1 (1 µl)
      - ELB (99 µl)

      These volumes produce 100 copies per µl.
   c. Pulse vortex to mix.

Preparation for Illumina COVIDSeq Test (RUO)

The following steps describe the preparation procedure for the Illumina COVIDSeq Test (RUO) kit. For the Illumina COVIDSeq Assay (96 Samples) kit, refer to *Preparation for Illumina COVIDSeq Assay (96 Samples)* on page 29.

Use of the COVIDSeq Positive Control (CPC) with the Illumina COVIDSeq Test (RUO) is recommended but not required.

1. Prepare the following consumables:

Reagent	Storage	Instructions
ELB HT	2°C to 8°C	Thaw at room temperature, and then invert to mix. Keep on ice until use.
CPC	-85°C to -65°C	Dilute using the following instructions. Keep diluted positive control on ice.

2. Dilute CPC as follows.
   a. Label a 1.7 ml tube Dilution 1.
   b. Add the following volumes to the tube ***in the order listed***.
      - CPC (5 µl)
      - ELB HT (495 µl)

      These volumes produce 10000 copies per µl.
   c. Pulse vortex to mix.
3. Dilute CPC a second time as follows.
   a. Label a 1.7 ml tube Dilution 2.
   b. Add the following volumes to the tube ***in the order listed***.
      - Dilution 1 (5 µl)
      - ELB HT (495 µl)

These volumes produce 100 copies per µl.

c. Pulse vortex to mix.

4. Dilute CPC a third time as follows.
   a. Label a 5 ml tube Dilution 3.
   b. Add the following volumes to the tube ***in the order listed***.
      - Dilution 2 (200 µl)
      - ELB HT (3.8 ml)

      These volumes produce 5 copies per µl.
   c. Pulse vortex to mix.

Revision History

Document	Date	Description of Change
Document # 1000000126053 v09	November 2024	• Updated primer tubes from v3 to v5.4.2. • Removed instructions for optional v4 primer set. • Removed positive control from HT kits (available separately).
Document # 1000000126053 v08	February 2022	• Corrected QIAmp Viral RNA Mini Handbook document number. • Updated Illumina COVIDSeq Assay Box 2 – 96 Samples, RSB and TWB storage temperatures.

Document	Date	Description of Change
Document # 1000000126053 v07	October 2021	• Added information for optional ARTIC v4 primers to the Overview and the Illumina COVIDSeq Assay (96 Samples) Kit Contents. • Added references to new technical note for read length recommendations to the Prepare for Sequencing section. • Reorganized the Pool and Clean Up section to provide separate subsections for the two kit types. • Updated Illumina COVIDSeq Assay (96 Samples) Kit Contents and note in protocol Introduction to account for reagent differences between the Illumina COVIDSeq RUO Kits. • Updated Pool and Dilute Libraries section to create new table for normalized pool volumes and recommended samples for v4 primers. • Updated 96-Well PCR Plate in Consumables list to change from a specific plate or equivalent to a list of specifications, including a specification to confirm compatibility with the chosen thermal cycler. • Corrected MiSeq System Guide document number in Prepare for Sequencing section. • Corrected steps related to dilution of COVIDSeq Positive Control for Illumina COVIDSeq Assay (96 Samples) to confirm that only two dilutions are needed.

Document	Date	Description of Change
Document # 1000000126053 v06	July 2021	• Added information for compatibility with the iSeq 100 System to appropriate topics, including the Introduction, Pool and Dilute Libraries, and the Prepare for Sequencing sections. • Moved sample sheet information for Illumina COVIDSeq Test (RUO) to an appendix. • Updated Illumina COVIDSeq Assay naming conventions and kit configurations to reflect current on-market use. • Reorganized Prepare for Sequencing section for improved ease of use. • Removed redundant sequencing consumables information from Prepare for Sequencing section. Information remains in Consumables and Equipment list in an appendix.
Document # 1000000126053 v05	June 2021	• Renamed document Illumina COVIDSeq RUO Kits Reference Guide to account for use with the 96-sample Illumina COVIDSeq Assay. • Added information for the 96-sample Illumina COVIDSeq Assay to the Introduction, Extract DNA, Pool and Clean Up Libraries, Pool and Dilute Libraries, and Prepare for Sequencing sections. • Added information for compatibility with the MiSeq and MiniSeq Sequencing Systems to the Introduction, Pool and Dilute Libraries, and the Prepare for Sequencing sections. • Added new Kit Contents section to Appendix A for the 96-sample Illumina COVIDSeq Assay. • Moved COVIDSeq Positive Control (CPC) preparation instructions to new Appendix B and updated for Illumina COVIDSeq Assay.

Document	Date	Description of Change
Document # 1000000126053 v04	April 2021	• Added information about variant analysis software options to the Introduction and Prepare for Sequencing section. • Added reference to technical note for performing library prep multiple times for a low throughput batch size. • Updated the Illumina COVIDSeq Test (3072 samples) from an eight box configuration to a four box configuration in the Kit Contents section. • Updated the Read Length recommendations in the Prepare for Sequencing section. • Updated Thermal Cycler recommendations for confirmation of microtiter plate compatibility.
Document # 1000000126053 v03	February 2021	• Added instructions to set up analysis in BaseSpace Sequence Hub for NextSeq 2000 and other NextSeq 2000-specific information throughout the protocol. • Added Thermal Cycler recommendations. • Updated materials, consumables, and equipment part numbers in the Prepare for Sequencing, Product Components, and Consumables and Equipment sections, including new consumables for the NextSeq 2000. • Updated version numbers of flow cells and control software for the NovaSeq 6000 system. • Updated the tube for Dilution 3 in Extract RNA to 5 ml LoBind tube. • Updated the protocol options for Quick-DNA/RNA Viral MagBead Procedure for precision and clarity. • Updated temperature in PCR program on the thermal cycler for Amplify cDNA Preparation from 65°C to 63°C.

Document	Date	Description of Change
Document # 1000000126053 v02	July 2020	• Added instructions for extracting RNA using the Quick-DNA/RNA Viral Magbead kit. • Added safe stopping point after pooling and cleaning up libraries. • Updated index kit configurations to IDT for Illumina-PCR Indexes. • Removed sequencing instructions. • Added dilution and sequencing preparation instructions for the NovaSeq 6000 Sequencing System SP flow cell, NextSeq 500 Sequencing System, NextSeq 550 Sequencing System, and NextSeq 550Dx Instrument. • Moved data analysis information to *Illumina COVIDSeq Test Pipeline Software Guide document # 1000000128122*.
Document # 1000000126053 v01	June 2020	No content changes.
Document # 1000000126053 v00	June 2020	Initial release.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Website:	www.illumina.com
Email:	techsupport@illumina.com

Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download from support.illumina.com.

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