Illumina COVIDSeq Test Instructions for Use

FOR IN VITRO DIAGNOSTIC USE

FOR USE UNDER AN EMERGENCY USE AUTHORIZATION (EUA) ONLY

FOR PRESCRIPTION USE ONLY

Intended Use

The Illumina® COVIDSeq™ Test is a Next-Generation Sequencing (NGS) in vitro diagnostic test on the Illumina NovaSeq 6000 Sequencing System, NextSeq 2000 Sequencing System, NextSeq 500 Sequencing System, NextSeq 550 Sequencing System, or NextSeq 550Dx Instrument intended for the qualitative detection of SARS-CoV-2 RNA from nasopharyngeal (NP) swabs, oropharyngeal (OP) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasopharyngeal wash/aspirates, nasal aspirates, and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. Laboratories within the United States and its territories are required to report all positive results to the appropriate health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information. The Illumina® COVIDSeq™ Test is intended for use by qualified and trained clinical laboratory personnel specifically trained in the use of the NovaSeq 6000 Sequencing System, the NextSeq 500 Sequencing System, the NextSeq 550 Sequencing System, the NextSeq 2000 Sequencing System, or the NextSeq 550Dx Instrument, as well as Next-Generation Sequencing workflows and in vitro diagnostic procedures. The Illumina® COVIDSeq™ Test is only for use under the Food and Drug Administration’s Emergency Use Authorization.

Summary and Explanation of the Assay

SARS-CoV-2 belongs to a large family of coronaviruses that lead to respiratory tract diseases in humans ranging from seasonal cold to severe infections, including Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS).¹ SARS-CoV-2 leads to the COVID-19 disease, which is associated in the majority of infections with mild respiratory symptoms. However, for patients with underlying medical conditions and advanced age COVID-19 may lead to severe illness. ¹ The primary route of SARS-CoV-2 transmission between humans is via respiratory route, including droplets of saliva or discharge from infected patients. ¹ Confirmation of COVID-19 relies on detection of SARS-CoV-2 RNA from a patient during an ongoing, current infection. ²

The Illumina COVIDSeq Test is intended for detection of SARS-CoV-2 virus RNA under FDA Emergency Use Authorization and virus genome analysis for research use. Insight into the SARS-CoV-2 strain present in the sample enables tracking of virus strains. This test has been designed to sequence up to 3072 samples simultaneously using the NovaSeq 6000 system or up to 384 samples using the NextSeq 500/550 systems, NextSeq 550Dx instrument, or NextSeq 2000 system to detect and sequence SARS-CoV-2 RNA and internal controls.
Principles of Procedure

The workflow consists of the following procedures: RNA extraction, cDNA synthesis, target amplification, library preparation, library pooling, sequencing, and analysis, which are outlined in more detail:

- **RNA Extraction** — RNA is extracted from decontaminated nasopharyngeal swabs using the Quick-DNA/RNA Viral MagBead Kit (Zymo Research, # R2141) or QIAamp Viral RNA Mini Kit (Qiagen, part # 52906).

- **cDNA Synthesis** — Generates DNA complementary to the RNA by reverse transcriptase with random hexamers.

- **Target Amplification** — The virus genome present in the sample is amplified using two separate PCR reactions that are then pooled together.

- **Library Preparation** — The pooled amplified fragments undergo tagmentation to further fragment and tag amplicons with adapter sequences. Post-tagmentation yield is normalized due to saturation of the bead-linked transposome by typical amplicon inputs. The adapter-tagged amplicons undergo a second round of PCR amplification using a PCR master mix and unique index adapters. After amplification, indexed libraries are pooled and cleaned using purification beads.

- **Quantification** — The pooled library product is quantified using a fluorescent dye with concentration determined by comparison to a DNA standard curve.

- **Sequencing** — Pooled libraries are clustered onto a flow cell, and then sequenced using sequencing by synthesis (SBS) chemistry on the NovaSeq 6000 Sequencing System using the NovaSeq Xp S4 and SP flow cells, NextSeq 500 System, NextSeq 550 System, NextSeq 550Dx Instrument in RUO mode, or NextSeq 2000 System. SBS chemistry uses a reversible-terminator method to detect single, fluorescently labeled deoxynucleotide triphosphate (dNTP) bases as they are incorporated into growing DNA strands. During each sequencing cycle, a single dNTP is added to the nucleic acid chain. The dNTP label serves as a terminator for polymerization. After each dNTP incorporation, the fluorescent dye is imaged to identify the base, and then cleaved to allow incorporation of the next nucleotide. Four reversible terminator-bound dNTPs (A, G, T, and C) are present as single, separate molecules. As a result, natural competition minimizes incorporation bias. During the primary analysis, base calls are made directly from signal intensity measurements during each sequencing cycle, resulting in base by base sequencing. A quality score is assigned to each base call.

- **Analysis** — The Illumina DRAGEN COVIDSeq Test Pipeline analyzes sequencing results to detect the presence of SARS-CoV-2 RNA in each sample for diagnostic use under the FDA Emergency Use Authorization. Analysis can be performed locally using the Illumina DRAGEN COVIDSeq Test Pipeline or on BaseSpace Sequence Hub using the Illumina DRAGEN COVIDSeq Test app.

  For each result with at least 90 SARS-CoV-2 virus targets, the Illumina DRAGEN COVIDSeq Test Pipeline performs small variant calling and generates a consensus sequence in FASTA format for research use.

Warnings and Limitations of the Procedure

- This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under EUAs for use by authorized laboratories.

- This product has been authorized only for the detection of nucleic acid of SARS-CoV-2, not for any other viruses or pathogens.

- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

- Anterior nasal swabs, mid-turbinate swabs, oropharyngeal swabs, and bronchoalveolar lavage specimens are additional acceptable respiratory specimens that can be tested with the Illumina COVIDSeq Test; however, performance with these specimen types has not been determined.
Use of the Illumina COVIDSeq Test is limited to personnel who have been trained in the procedures of molecular diagnostic assays including RT-PCR and the NovaSeq 6000 Sequencing System, NextSeq 2000 System, NextSeq 500 System, NextSeq 550 System, or NextSeq 550Dx Instrument.

Laboratories are required to report all results to the appropriate public health authorities.

The instruments and assay procedures reduce the risk of contamination by amplification product. However, nucleic acid contamination from the positive controls or specimens must be controlled by good laboratory practices and careful adherence to the procedures specified in this package insert.

Optimal performance of this test requires appropriate specimen collection, storage, and transport to the test site. For more information, see Specimen Collection, Transport, and Storage on page 9.

Detection of SARS-CoV-2 RNA may be affected by sample collection methods, patient factors (e.g., presence of symptoms), and/or stage of infection.

False-negative results may arise from degradation of the viral RNA during shipping and storage.

The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.

As with any molecular test, mutations within the target regions of the Illumina COVIDSeq Test could affect primer binding resulting in failure to detect the presence of virus.

Results should be interpreted by a trained professional in conjunction with the patient’s history, clinical signs, and symptoms, and epidemiological risk factors.

Negative results do not preclude infection with the SARS-CoV-2 virus and should not be the sole basis of a patient treatment and management or public health decision. Follow up testing should be performed according to the current CDC recommendations.

Variant calls and consensus sequences performed by the Illumina DRAGEN COVIDSeq Test Pipeline are for information purposes only and should not be used for patient reporting.

Conditions of Authorization for the Laboratory


However, to assist clinical laboratories using the Illumina COVIDSeq Test, the relevant Conditions of Authorization are listed below:

- Authorized laboratories* using Illumina COVIDSeq Test must include with test result reports all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

- Authorized laboratories using Illumina COVIDSeq Test must use Illumina COVIDSeq Test as outlined in the Illumina COVIDSeq Test Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use Illumina COVIDSeq Test are not permitted.

- Authorized laboratories that receive Illumina COVIDSeq Test must notify the relevant public health authorities of their intent to run Illumina COVIDSeq Test prior to initiating testing.

- Authorized laboratories using Illumina COVIDSeq Test must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

- Authorized laboratories must collect information on the performance of Illumina COVIDSeq Test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Illumina Tech Support (via
email: techsupport@illumina.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of Illumina COVIDSeq Test of which they become aware.

- All laboratory personnel using Illumina COVIDSeq Test must be appropriately trained in next generation sequencing and PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use Illumina COVIDSeq Test in accordance with the authorized labeling.

- Illumina, authorized distributors, and authorized laboratories using Illumina COVIDSeq Test must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

* The letter of authorization refers to, “Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests” as “authorized laboratories.”

**Product Components**

The Illumina COVIDSeq Test requires the following components:

- COVIDSeq Test (3072 Samples), part # 20044461
- 8 IDT for Illumina-PCR Indexes Sets 1-4 (384 Indexes), part # 20043137

**Reagents**

**Reagents Provided**

**Illumina COVIDSeq Test**

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

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Illumina COVIDSeq Test Box 1 – 3072 Samples, Part # 20044408</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantity</td>
<td>Label Volume (ml)</td>
</tr>
<tr>
<td></td>
<td>Reagent</td>
</tr>
<tr>
<td>1</td>
<td>233</td>
</tr>
<tr>
<td>1</td>
<td>56</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Illumina COVIDSeq Test Box 2 – 3072 Samples, Part # 20044409</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantity</td>
<td>Label Volume (ml)</td>
</tr>
<tr>
<td></td>
<td>Reagent</td>
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<tr>
<td>2</td>
<td>6.1</td>
</tr>
<tr>
<td>1</td>
<td>114</td>
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<tr>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>1</td>
<td>845</td>
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</table>
Table 3  Illumina COVIDSeq Test Box 3 – 3072 Samples, Part # 20044410

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Label Volume (ml)</th>
<th>Reagent</th>
<th>Description</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.4</td>
<td>CPP1 HT</td>
<td>COVIDSeq Primer Pool 1 HT</td>
<td>-25°C to -15°C, pre-amp environment</td>
</tr>
<tr>
<td>1</td>
<td>14.4</td>
<td>CPP2 HT</td>
<td>COVIDSeq Primer Pool 2 HT</td>
<td>-25°C to -15°C, pre-amp environment</td>
</tr>
<tr>
<td>1</td>
<td>45</td>
<td>EPH3 HT</td>
<td>Elution Prime Fragment 3HC Mix HT</td>
<td>-25°C to -15°C pre-amp environment</td>
</tr>
<tr>
<td>1</td>
<td>79</td>
<td>EPM HT</td>
<td>Enhanced PCR Mix HT</td>
<td>-25°C to -15°C, pre-amp environment</td>
</tr>
<tr>
<td>1</td>
<td>41</td>
<td>FSM HT</td>
<td>First Strand Mix HT</td>
<td>-25°C to -15°C, pre-amp environment</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>IPM HT</td>
<td>Illumina PCR Mix HT</td>
<td>-25°C to -15°C, pre-amp environment</td>
</tr>
<tr>
<td>1</td>
<td>4.6</td>
<td>RVT HT</td>
<td>Reverse Transcriptase HT</td>
<td>-25°C to -15°C, pre-amp environment</td>
</tr>
<tr>
<td>1</td>
<td>38</td>
<td>TB1 HT</td>
<td>Tagmentation Buffer 1 HT</td>
<td>-25°C to -15°C, post-amp environment</td>
</tr>
</tbody>
</table>

Table 4  Illumina COVIDSeq Positive Control HT, Part # 20044883

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Label Volume</th>
<th>Reagent</th>
<th>Description</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100 µl</td>
<td>COVIDSeq Positive Control HT</td>
<td>COVIDSeq Positive Control HT</td>
<td>-85°C to -65°C, pre-amp environment</td>
</tr>
</tbody>
</table>

IDT for Illumina- PCR Indexes, Store at -25°C to -15°C
The Illumina COVIDSeq Test requires 8 IDT for Illumina PCR Indexes Sets 1–4 (384 Indexes).

NOTE  Individual reagent tubes contain a label with the following language “For Research Use Only, not for use in diagnostic procedures”. These RUO labeled reagents have been authorized for use as IVD under the Emergency Use Authorization granted to this product.

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Description</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>IDT for Illumina- PCR Indexes Set 1 (96 Indexes)</td>
<td>20043132</td>
</tr>
<tr>
<td>8</td>
<td>IDT for Illumina- PCR Indexes Set 2 (96 Indexes)</td>
<td>20043133</td>
</tr>
<tr>
<td>8</td>
<td>IDT for Illumina- PCR Indexes Set 3 (96 Indexes)</td>
<td>20043134</td>
</tr>
<tr>
<td>8</td>
<td>IDT for Illumina- PCR Indexes Set 4 (96 Indexes)</td>
<td>20043135</td>
</tr>
</tbody>
</table>

Reagents Not Provided

Reagents Required, Not Provided

- If using the QIAamp Viral RNA Mini Kit RNA extraction method:
  - 13 QIAamp Viral RNA Mini Kit, Qiagen, # 52906
  - QIAamp Viral RNA Mini Kit reagents. See QIAmp Viral RNA Mini Handbook (document # #HB-0354-006).
- If using the Quick- DNA/RNA Viral MagBead extraction method:
  - 8 Quick- DNA/RNA Viral MagBead, Zymo Research, # R2141
Quick-DNA/RNA Viral MagBead reagents. See Quick-DNA/RNA Viral MagBead Instruction Manual.

Qubit dsDNA HS Assay Kit, Thermo Fisher Scientific, # Q32851 or Q32854

If using the NovaSeq 6000 Sequencing System S4 flow cell, the following reagents for 3072 samples:

- 2 NovaSeq 6000 Sequencing System S4 Reagent Kit v1.5 (35 cycles), Illumina, # 20044417
- 2 NovaSeq Xp 4-Lane Kit v1.5, Illumina, # 20043131

If using the NovaSeq 6000 Sequencing System SP flow cell, the following NovaSeq 6000 Sequencing System reagents for 3072 samples:

- 4 NovaSeq 6000 Sequencing System SP Reagent Kit v1.5 (100 cycles), Illumina, # 20028401
- 4 NovaSeq Xp 2-Lane Kit v1.5, Illumina, # 20043130

If using the NextSeq 500/550 System or the NextSeq 550Dx instrument, the following reagents for 3072 samples:

- 8 NextSeq 500/550 High Output Kit v2.5 (75 Cycles), Illumina, # 20024906

If using the NextSeq 2000 System, the following reagents for 3072 samples:

- 8 NextSeq 1000/2000 P2 Reagents (100 cycles), Illumina, #20046811

- 2 N NaOH
- Nuclease-free water
- Ethanol, 100% (200 proof) of molecular biology grade, Sigma-Aldrich, # E7023

NOTE Non-molecular biology grade ethanol can potentially negatively impact performance of the assay

Reagents Optional, Not Provided

- DNAZap
- RNaseZap

Storage and Handling

1. Room temperature is defined as 15°C to 30°C.
2. Do not allow multiple freeze-thaw cycles for CPC HT. If performing library prep multiple times, aliquot CPC HT into low-bind tubes, and then store at -85°C to -65°C.
3. Do not allow more than 8 freeze-thaw cycles for all reagents, excluding CPC HT.
4. Reagents are stable when stored as indicated until the specified expiration date on the kit labels. For storage conditions, see the Storage column in the tables in Reagents Provided on page 4. Do not use expired reagents.
5. Changes in the physical appearance of the reagents provided can indicate deterioration of the materials. If changes in the physical appearance occur (eg, obvious changes in reagent color or cloudiness apparent with microbial contamination), do not use the reagents.
6. Sequence libraries as soon as possible after pooling. Pooled libraries are stable for up to 30 days at -25°C to -15°C.
## Equipment and Materials

### Equipment and Materials Required, Not Provided

#### Equipment Required, Not Provided

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 µl single-channel pipettes</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>20 µl single-channel pipettes</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>200 µl single-channel pipettes</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>1000 µl single-channel pipettes</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>10 µl 8-channel pipettes</td>
<td>General lab supplier</td>
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<tr>
<td>20 µl 8-channel pipettes</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>200 µl 8-channel pipettes</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>1000 µl 8-channel pipettes</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>20 µl 12-channel pipettes</td>
<td>General lab supplier</td>
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<tr>
<td>200 µl 12-channel pipettes</td>
<td>General lab supplier</td>
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<tr>
<td>25 ml serological pipettes</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>50 ml serological pipettes</td>
<td>General lab supplier</td>
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<tr>
<td>BioShake iQ</td>
<td>QiNstruments, part # 1808-0506</td>
</tr>
<tr>
<td>DRAGEN Bio-IT Platform or BaseSpace Sequence Hub</td>
<td>Illumina</td>
</tr>
</tbody>
</table>

Required equipment for one the following extraction methods:

- **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 | 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:

- NextSeq 500
- NextSeq 550
- NextSeq 550Dx
- NextSeq 2000
- NovaSeq 6000

NovaSeq Xp Flow Cell Dock | Illumina, # 20021663 |

Pipette Aid | General lab supplier |

Quibit Fluorometer 3.0 | Thermo Fisher, catalog # Q33216, Q33217, or Q33218 |
**Equipment**

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refrigerator, 2°C to 8°C</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>One of the following thermal cyclers:</td>
<td></td>
</tr>
<tr>
<td>• C1000 Touch™ Thermal Cycler with 96-Well Fast Reaction Module</td>
<td>• Bio-Rad, part # 1851196</td>
</tr>
<tr>
<td>• C1000 Touch™ Thermal Cycler with 96-Deep Well Reaction Module</td>
<td>• Bio-Rad, part # 1851197</td>
</tr>
<tr>
<td>• Veriti 96-well Thermal Cycler</td>
<td>• Thermo Fisher, catalog # 4375786</td>
</tr>
<tr>
<td>• GeneAmp PCR System 9700 Fast Thermal Cycler</td>
<td>• Thermo Fisher, catalog # 4339386</td>
</tr>
<tr>
<td>• Thermal cycler that meets the minimum specification requirements. See Recommended Thermal Cycler Specifications on page 8</td>
<td></td>
</tr>
<tr>
<td>Sealing wedge or roller</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>Vortexer</td>
<td>General lab supplier</td>
</tr>
</tbody>
</table>

**Recommended Thermal Cycler Specifications**

The following are the recommended minimum requirements for a thermal cycler used in the Illumina COVIDSeq Test.

<table>
<thead>
<tr>
<th>Specification</th>
<th>Minimum Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lid type</td>
<td>Heated</td>
</tr>
<tr>
<td>Temperature range</td>
<td>4°C to 99°C</td>
</tr>
<tr>
<td>Format</td>
<td>0.2 mL tubes, 96-well plate</td>
</tr>
<tr>
<td>Temperature accuracy</td>
<td>±0.25°C (35°C to 99.9°C)</td>
</tr>
<tr>
<td>Temperature uniformity</td>
<td>±0.5°C well-to-well within 30 seconds of arrival at target temperature</td>
</tr>
<tr>
<td>Peak ramp rate</td>
<td>At least 1.5°C</td>
</tr>
<tr>
<td>Sample ramp rate</td>
<td>± 1.25°C</td>
</tr>
</tbody>
</table>

**Materials Required, Not Provided**

<table>
<thead>
<tr>
<th>Consumable</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 µl pipette tips</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>20 µl pipette tips</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>200 µl pipette tips</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>200 µl pipette tips</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>1000 µl pipette tips</td>
<td>General lab supplier</td>
</tr>
<tr>
<td>Hard-Shell 96-Well PCR Plates</td>
<td>Bio-Rad, catalog # HSP-9601 or equivalent</td>
</tr>
<tr>
<td>1.7 ml LoBind microcentrifuge tubes</td>
<td>Eppendorf, catalog # 022431021</td>
</tr>
<tr>
<td>5 ml LoBind microcentrifuge tube</td>
<td>Eppendorf, catalog # 0030122348</td>
</tr>
<tr>
<td>15 ml tubes</td>
<td>General lab supplier</td>
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<tr>
<td>Microseal ‘B’ adhesive seals</td>
<td>Bio-Rad, part # MSB-1001</td>
</tr>
<tr>
<td>RNase/DNase-free Disposable Pipetting Reservoirs</td>
<td>VWR, part # 89094-658</td>
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<tr>
<td>Qubit dsDNA HS Assay Kit</td>
<td>One of the following, depending on kit size:</td>
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<tr>
<td></td>
<td>• ThermoFisher Scientific, part # Q32851</td>
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<tr>
<td></td>
<td>• ThermoFisher Scientific, part # Q32854</td>
</tr>
<tr>
<td>Qubit Assay Tubes</td>
<td>ThermoFisher Scientific, catalog # Q32856</td>
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</table>
Optional Materials, Not Provided

<table>
<thead>
<tr>
<th>Consumable</th>
<th>Supplier</th>
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</thead>
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<tr>
<td>96 deep well plate, 2000 µl (for use with Quick-DNA/RNA Viral MagBead)</td>
<td>Eppendorf, catalog # 951033707</td>
</tr>
</tbody>
</table>

Specimen Collection, Transport, and Storage

⚠️ CAUTION
Handle all specimens as infectious agents.

1 Nasopharyngeal swab samples collected in viral transport tubes are acceptable. In addition, anterior nasal swabs, mid-turbinate swabs, oropharyngeal swabs, and bronchoalveolar lavage specimens are acceptable respiratory specimens that can be tested with the Illumina COVIDSeq Test. However, performance with these specimen types has not been determined.

2 Specimens should be transported and tested as soon as possible after collection. Specimens are stable for up to 24 hours at room temperature or up to 72 hours when stored at 2°C to 8°C. If specimens cannot be tested within 72 hours of collection, they should be frozen at -70°C or colder until tested.

3 Transportation of patient samples must comply with all applicable governing regulations for the transport of etiologic agents.

⚠️ CAUTION
Exceeding the specified storage times can negatively impact test results.

Warnings and Precautions

- This assay contains potentially hazardous chemicals. For health and safety information, review Safety Data Sheets (SDS) available on the Illumina website prior to handling any chemical materials.

- Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Use in a well-ventilated area, wear protective clothing, and dispose of any containers and unused contents in accordance with applicable local governmental safety standards.

- This assay contains a flammable chemical. Keep away from heat and open flames. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Use in a well-ventilated area, wear protective clothing, and dispose of any containers and unused contents in accordance with applicable local governmental safety standards.

- Handle in accordance with good industrial hygiene and safety practices. Wash after handling this material and before eating, drinking, and/or smoking. Regular cleaning of equipment, work area, and clothing is recommended.

- Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink, or smoke in designated work areas. Wear disposable gloves and laboratory coats when handling specimens and assay reagents. Wash hands thoroughly after handling specimens and assay reagents.

- Do not reuse container. Dispose containers in accordance with applicable regional, national, and local laws and regulations.

- Do not use any assay components beyond their stated expiration date on the assay box label. Do not interchange assay components from different assay lots. Assay lots are identified on the assay box label. Store the assay components at the specified temperature.

- To prevent sample or reagent degradation, make sure that all sodium hypochlorite vapors from cleaning have fully dissipated before starting the protocol.

- Failure to follow the procedures as outlined can result in erroneous results or significant reduction in sample quality.
For environmental, health, and safety information, see the safety data sheets (SDS) at support.illumina.com/sds.html.

Procedural Notes

Avoiding Contamination

- Use proper laboratory practices to prevent nucleases and PCR products 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.

Quality Control

Control material with known performance characteristics may be evaluated to detect differences in processing and technical procedures in the laboratory. Include a no template control (NTC) and positive control with every sample prep batch. The Illumina COVIDSeq Test includes COVIDSeq Positive Control HT to use as a positive control. COVIDSeq Positive Control HT consists of ssRNA fragments that provide coverage of greater than 99.9% of the SARS-CoV-2 viral genome bases. The Illumina COVIDSeq Test also includes internal human target controls for each sample in the assay as a process control to prepare libraries for sequencing.

The following external controls are used in the Illumina COVIDSeq Test:

- **ELB HT (Elution Buffer HT)**, Illumina part # 20044409—No Template Control (NTC) used in the Illumina COVIDSeq Test for 94 unique test samples and to dilute the COVIDSeq Positive Control HT at 5000 copies per ml. One control is required for 94 samples. The ELB HT monitors the following actions:
  - Cross-contamination during RNA extraction and downstream library prep steps.
  - Reagent integrity.

- **COVIDSeq Positive Control HT**, Illumina part # 20044883—SARS-CoV-2 positive control used in the Illumina COVIDSeq Test for 94 samples. One control is required for 94 samples. COVIDSeq Positive Control HT monitors the following actions:
  - Positive control used to detect SARS-CoV-2.
  - Reagent integrity.

The following internal controls are used in the Illumina COVIDSeq Test:

- **CCP1 HT (COVIDSeq Primer Pool 1 HT) and CCP2 HT (COVIDSeq Primer Pool 2 HT)** Illumina, part # 20044410—A total of two pools that include SARS-CoV-2 specific primers and a set of primer pairs targeting human transcripts. The targeted human transcripts are commonly prevalent in nasopharyngeal swabs, oropharyngeal swabs, nasal swab, and mid-turbinate specimens. Therefore, these human target primers act as internal process control for each sample that will be used in the Illumina COVIDSeq Test. CCP1 and CCP2 monitor the following actions:
  - Successful completion of each step from extraction through DNA tagmentation, library prep, and sequencing to validate negative samples when the SARS-CoV-2 virus is not detected and "PASS" is displayed as the internal control result.
NOTE: Illumina requires using one NTC and one positive control per 96-well plate. For library preparation of each sample, the internal process control is included in the Illumina COVIDSeq Test.

Instructions for Use

Tips and Techniques

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

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 x 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.

Consumables

- ELB HT (Elution Buffer HT)
- CPC HT (COVIDSeq Positive Control HT)
- 1.7 ml LoBind tubes
- 5 ml LoBind tubes
- [Quick-DNA/RNA Viral MagBead] 2000 µl 96 deep well plate

About Reagents

- Aliquot CPC HT into low-bind tubes. Store at -85°C to -65°C
- Vortex before each use

Preparation

1. Prepare the following consumables:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Storage</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELB HT</td>
<td>2°C to 8°C</td>
<td>Thaw at room temperature, and then invert to mix. Keep on ice until use.</td>
</tr>
<tr>
<td>CPC HT</td>
<td>-85°C to -65°C</td>
<td>Dilute to 5 copies per µl using the following instructions. Keep diluted positive control on ice.</td>
</tr>
</tbody>
</table>

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

3. Dilute CPC HT 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 HT 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.
Quick-DNA/RNA Viral MagBead Procedure

1. For each sample, add 400 µl patient sample to a new deep-well plate. For every 94 samples, include one tube of dilution 3 CPC HT (positive control) and ELB HT (no template control).

2. To extract RNA, use the Quick-DNA/RNA Viral MagBead. For information, see Quick-DNA/RNA Viral MagBead Instruction Manual available 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 sample, add 140 µl patient sample to new 1.7 ml microcentrifuge tube. For every 94 samples, include one tube of dilution 3 CPC HT (positive control) and ELB HT (no template control).

2. To extract RNA, use the QIAamp Viral RNA Mini Kit. For information, see QIAamp Viral RNA Mini Handbook (document # HB-0354-006) 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.

Consumables

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

About Reagents

- Vortex before each use

Preparation

1. Prepare the following consumables:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Storage</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPH3 HT</td>
<td>-25°C to -15°C</td>
<td>Thaw at room temperature, and then invert to mix.</td>
</tr>
</tbody>
</table>

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

**Preparation**

1 Prepare the following consumables:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Storage</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSM HT</td>
<td>-25°C to -15°C</td>
<td>Thaw and bring to room temperature. Invert to mix, and then keep on ice.</td>
</tr>
<tr>
<td>RVT HT</td>
<td>-25°C to -15°C</td>
<td>Invert to mix before use. Keep on ice.</td>
</tr>
</tbody>
</table>

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 HT (9 µl)
   - RVT HT (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 \times 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 HT (Illumina PCR Mix HT)
- CPP1 HT (COVIDSeq Primer Pool 1 HT)
- CPP2 HT (COVIDSeq Primer Pool 2 HT)
Nuclease-free water
15 ml tube (2 for four 96-well sample plates)
96-well PCR plates (3)
Microseal 'B' adhesive seal

Preparation
1 Prepare the following consumables:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Storage</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPP1 HT</td>
<td>-25°C to -15°C</td>
<td>Thaw at room temperature. Keep on ice until use.</td>
</tr>
<tr>
<td>CPP2 HT</td>
<td>-25°C to -15°C</td>
<td>Thaw at room temperature. Keep on ice until use.</td>
</tr>
<tr>
<td>IPM HT</td>
<td>-25°C to -15°C</td>
<td>Thaw at room temperature, and then invert to mix. Keep on ice until use.</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>COVIDSeq PCR 1 Master Mix (µl)</th>
<th>COVIDSeq PCR 2 Master Mix (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPM HT</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>CPP1 HT</td>
<td>4.3</td>
<td>N/A</td>
</tr>
<tr>
<td>CPP2 HT</td>
<td>N/A</td>
<td>4.3</td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td>4.7</td>
<td>4.7</td>
</tr>
</tbody>
</table>

Reagent overage is included to account for small pipetting errors.
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 HT to tagment PCR amplicons, which is a process that fragments and tags the PCR amplicons with adapter sequences.

Consumables

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

About Reagents

- Store EBLTS HT 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:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Storage</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBLTS HT</td>
<td>2°C to 8°C</td>
<td>Bring to room temperature. Vortex thoroughly before use.</td>
</tr>
<tr>
<td>TB1 HT</td>
<td>-25°C to -15°C</td>
<td>Bring to room temperature. Vortex thoroughly before use.</td>
</tr>
</tbody>
</table>

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 HT (12 µl)
   - EBLTS HT (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 HT (Stop Tagment Buffer 2 HT)
- TWB HT (Tagmentation Wash Buffer HT)
- Microseal 'B' adhesive seal

**About Reagents**
- Dispense ST2 HT and TWB HT slowly to minimize foaming.
- Dispense TWB HT directly onto beads.

**Preparation**
1 Prepare the following consumables:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Storage</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST2 HT</td>
<td>Room temp</td>
<td>Vortex before use.</td>
</tr>
<tr>
<td>TWB HT</td>
<td>2°C to 8°C</td>
<td>Vortex before use.</td>
</tr>
</tbody>
</table>

**Procedure**
1 Centrifuge the TAG1 plate at 500 x g for 1 minute.
2 Add 10 µl ST2 HT 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 x 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 HT to each well.
   c Seal and shake at 1600 rpm for 1 minute.
   d Centrifuge 500 x 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 prepared 10 base pair Index 1 (i7) adapters, Index 2 (i5) adapters, and sequences required for sequencing cluster generation.

**Consumables**
- EPM HT (Enhanced PCR Mix HT)
- 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 cannot be reused.

Preparation

1. Prepare the following consumables:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Storage</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPM HT</td>
<td>-25°C to -15°C</td>
<td>Invert to mix. Keep on ice until use.</td>
</tr>
<tr>
<td>Index adapters</td>
<td>-25°C to -15°C</td>
<td>Thaw at room temperature. Vortex to mix, and then centrifuge at 1000 × g for 1 minute.</td>
</tr>
</tbody>
</table>

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 HT (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 HT.
4. Use a 20 µl pipette to remove any remaining TWB HT.
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.
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

- ITB (Illumina Tune Beads)
- RSB HT (Resuspension Buffer HT)
- Freshly prepared 80% ethanol (EtOH)
- 1.7 ml tube (2 per 96-well sample plate)
- PCR 8-tube strip

About Reagents

- ITB
  - 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:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Storage</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITB</td>
<td>Room temp.</td>
<td>Vortex thoroughly to mix.</td>
</tr>
<tr>
<td>RSB HT</td>
<td>2°C to 8°C</td>
<td>Let stand for 30 minutes to bring to room temp. Vortex and invert to mix.</td>
</tr>
</tbody>
</table>

2 Prepare 80% EtOH from absolute EtOH.

Procedure

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 PCR plate to a PCR 8-tube strip. Change tips after each column. These volumes result in 60 µl pooled library per row.
   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 HT.
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 tubes 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.
   a Use 400 bp as the average library size.
   \[
   \text{Library concentration (ng/µl)} \times \frac{600}{\text{average library size (bp)}} \times 10^6 = \text{Molarity (nM)}
   \]
3 Dilute each library pool to a minimum of 30 µl at a normalized concentration 4 nM using RSB HT.

Pool and Dilute Libraries
This step pools and dilutes libraries to the starting concentration for your sequencing system. After diluting to the starting concentration, libraries are ready to be denatured and diluted to the final loading concentration.
1 For each set of 384 samples, combine 25 µl of each normalized pool containing index adapter set 1, 2, 3, 4 in a new microcentrifuge tube. Do not combine pools with the same index adapter set.
   This step produces a final pool of 384 samples diluted to a starting concentration of 4 nM. For each sequencing system, the following are the number of samples required per flow cell.
   a NextSeq 500/550 HO flow cell, NextSeq 550Dx HO flow cell, or NextSeq 1000/2000 P2 flow cell: 384 samples per flow cell.
   b Noveq 6000 system SP flow cell: 384 samples per lane and 768 total samples per flow cell.
   c Noveq 6000 system S4 flow cell: 384 samples per lane and 1536 total samples per flow cell.
2 Follow the denature and dilute instructions for your system to dilute to the final loading concentration.
   a For the NextSeq 500/550 Sequencing System and NextSeq 550Dx Sequencing System, see the NextSeq System Denature and Dilute Libraries Guide (document # 15048776).
   b For the Noveq 6000 Sequencing System, see the Novaq 6000 Denature and Dilute Libraries Guide (document # 1000000106351).
   c 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>
<thead>
<tr>
<th>Sequencing System</th>
<th>Starting Concentration (nM)</th>
<th>Final Loading Concentration (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NextSeq 500/550 or 550Dx HO flow cell</td>
<td>4</td>
<td>1.4</td>
</tr>
<tr>
<td>Noveq 6000 SP Flow Cell</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>Noveq 6000 S4 Flow Cell</td>
<td>4</td>
<td>100</td>
</tr>
</tbody>
</table>
Prepare for Sequencing

Consumables

- If using the NovaSeq 6000 Sequencing System S4 flow cell:
  - 2 NovaSeq 6000 Sequencing System S4 Reagent Kit v1.5 (35 cycles), Illumina, # 20044417
  - 2 NovaSeq Xp 4-Lane Kit v1.5, Illumina, # 20043131

- If using the NovaSeq 6000 Sequencing System SP flow cell:
  - 4 NovaSeq 6000 Sequencing System SP Reagent Kit v1.5 (100 cycles), Illumina, # 20028401
  - 4 NovaSeq Xp 2-Lane Kit v1.5, Illumina, # 20043130

- If using the NextSeq 500/550 System or NextSeq 550Dx Instrument:
  - 8 NextSeq 500/550 High Output Kit v2.5 (75 Cycles), Illumina, # 20024906

- If using the NextSeq 2000 Sequencing System
  - 8 NextSeq 1000/2000 P2 Reagents (100 Cycles), Illumina, # 20046811

Sample Sheet Requirements

The Illumina DRAGEN COVIDSeq Test Pipeline requires a sample sheet for each run analysis. This requirement does not apply to the NextSeq 2000, which uses the Illumina DRAGEN COVIDSeq Test in BaseSpace Sequence Hub.

Use the samplesheet.csv file for your sequencing system included in the installer packager or available on the Illumina COVIDSeq Test support site as a template to create the sample sheet.

Make sure your sample sheet meets the following requirements.

1. Save the sample sheet with the name SampleSheet.csv in the sequencing run folder.
2. In Settings, enter the following value for the AdapterRead1 parameter.
   
   CTGTCTCTTTATACATCT

3. In the Data section, enter the following required parameters.

   Make sure that there are no empty rows between samples.

<table>
<thead>
<tr>
<th>Field</th>
<th>Description</th>
<th>Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample_ID</td>
<td>The ID used to identify the samples in the test reports and included in the output file names.</td>
<td>Sample IDs are not case-sensitive. Make sure Sample IDs contain the following: * Unique for the run. * ≤ 100 characters with no spaces. * Alphanumeric characters, underscores, and dashes only. An alphanumeric character must be added before and after an underscore or dash.</td>
</tr>
<tr>
<td>Index_ID</td>
<td>The IDT for Illumina-PCR Indexes index name associated with the sample.</td>
<td>See Illumina Adapter Sequences (document # 100000002694) for index names and additional information. The name must be unique for each flow cell lane. If the Index_ID is not specified, the Index Set field is derived from Index and Index2. If specifying all three, the index names and associated sequences must match.</td>
</tr>
</tbody>
</table>

Adjustments to final loading concentration should follow the denature and dilute instructions for your sequencing system.
<table>
<thead>
<tr>
<th>Field</th>
<th>Description</th>
<th>Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Index</td>
<td>IDT for Illumina-PCR Indexes i7 index sample sheet bases</td>
<td>See <a href="document#1000000002694">Illumina Adapter Sequences</a> for sample sheet bases for your sequencing system and additional information. If Index_ID is specified, Index is not required.</td>
</tr>
<tr>
<td>Index2</td>
<td>IDT for Illumina-PCR Indexes i5 index sample sheet bases</td>
<td>See <a href="document#1000000002694">Illumina Adapter Sequences</a> for sample sheet bases for your sequencing system and additional information. If Index_ID is specified, Index2 is not required.</td>
</tr>
<tr>
<td>Lane</td>
<td>The flow cell lane for the sample.</td>
<td>If using the NovaSeq 6000 System, enter one of the following values: 1, 2, 3, or 4. If using the NextSeq 500/550, NextSeq 500Dx, or NextSeq 2000, this field is not included.</td>
</tr>
<tr>
<td>Sample_Type</td>
<td>The sample type for each sample.</td>
<td>Enter one of the following case-sensitive values: PatientSample, NTC, PositiveControl. If using the NovaSeq 6000 System, there must be one NTC sample and one PositiveControl sample for each Index Set/Lane combination in the sample sheet. If using the NextSeq 500/550, NextSeq 500Dx, or NextSeq 2000 there must be one NTC sample and one PositiveControl sample for each Index Set combination in the sample sheet.</td>
</tr>
</tbody>
</table>

4 [Optional] Enter any additional data parameters, such as Sample_Name.

Set Up Sequencing Run

1 If using the NovaSeq 6000 system, refer to the [NovaSeq 6000 Sequencing System Guide](document#1000000019358) for sequencing instructions.
   ▶ Use v1.7 of the NovaSeq Control Software (NVCS).
   ▶ If using the Illumina DRAGEN COVIDSeq Test BaseSpace Sequence Hub app, select Run Monitoring and Storage as the Configuration option.
   ▶ Use the following number of cycles and index lengths:
     ▶ Read 1 — Enter 36 as the value.
     ▶ Index 1 and Index 2 — Enter 10 as the value.
     ▶ Read 2 — Enter 0 as the value.

2 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).
   ▶ Use v4.0 of the NextSeq Control Software (NCS).
   ▶ If using the NextSeq 550Dx, use RUO mode.
   ▶ Set up your sequencing run in manual mode.
   ▶ If using the Illumina DRAGEN COVIDSeq Test BaseSpace Sequence Hub app, select Run Monitoring and Storage as the Configuration option.
   ▶ Enter Single-Read as the Read Type.
   ▶ Use the following number of cycles and index lengths:
     ▶ Read 1 — Enter 36 as the value.
     ▶ Index 1 and Index 2 — Enter 10 as the value.

3 If using the NextSeq 2000, refer to the [NextSeq 1000/2000 Sequencing System Guide](document#1000000109376).
   ▶ When creating a run in BaseSpace Sequence Hub, make sure to do the following:
     ▶ Select BaseSpace for analysis location.
     ▶ Select Illumina DRAGEN COVIDSeq Test for analysis type.
If Illumina DRAGEN COVIDSeq Test does not appear as an analysis type, contact Illumina Technical Support.

Set up the analysis as described in the following Set Up Analysis in BaseSpace Sequence Hub for NextSeq 2000 section.

Use v1.2 of the NextSeq 1000/2000 Control Software.

Make sure Online Run Setup and Proactive, Run Monitoring, and Storage are selected in the Settings screen to enable Cloud mode.

After sequencing is complete, analysis either takes place on your system using the Illumina DRAGEN COVIDSeq Test Pipeline or in BaseSpace Sequence Hub using the Illumina DRAGEN COVIDSeq. For information on performing analysis locally, see the Illumina DRAGEN COVIDSeq Test Pipeline Software Guide (document # 1000000128119). For information on performing analysis on BaseSpace Sequence Hub, see Illumina DRAGEN COVIDSeq Test App Guide (document # 1000000129048).

Set Up Analysis in BaseSpace Sequence Hub for NextSeq 2000

1. To enable fast mode, set the Fast Mode option to True. Fast mode turns off alignment, variant calling, and consensus sequence FASTA generation to analyze results.

2. To exclude run logs, QC metric files, and other file types, set the Metrics and Logs Datasets option to False. Setting this option to false improves analysis speed, but the Logs_Intermediates_Lane_* folder is not generated.

3. Identify the location for your positive and no template controls using either the sample ID or well position.

4. Enter the positive control and no template control for each index set.
   - If you used the index set during library preparation, enter the sample ID or well position for the positive and no template controls.
   - If you did not use the index set, enter NA.

5. Select Submit Run.

Interpretation of Results

<table>
<thead>
<tr>
<th>SARS-CoV-2 Detected in Sample ID</th>
<th>Internal Control Sample ID</th>
<th>NTC</th>
<th>Positive Control</th>
<th>Sample Result</th>
<th>Interpretation</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>Pass or N/A</td>
<td>Pass</td>
<td>Pass</td>
<td>SARS-CoV-2 Detected</td>
<td>Positive for SARS-CoV-2 for the Sample ID.</td>
<td>Report results to physician, patient, and appropriate public health authorities.</td>
</tr>
<tr>
<td>-</td>
<td>Pass</td>
<td>Pass</td>
<td>Pass</td>
<td>SARS-CoV-2 Not Detected</td>
<td>Negative for SARS-CoV-2 for the Sample ID.</td>
<td>Report results to physician, patient, and appropriate public health authorities.</td>
</tr>
<tr>
<td>-</td>
<td>Fail</td>
<td>Pass</td>
<td>Pass</td>
<td>Invalid</td>
<td>Invalid for the Sample ID.</td>
<td>Quality control for the Sample ID is FAIL. Re-extract sample and repeat Illumina COVIDSeq Test for the sample</td>
</tr>
<tr>
<td>+ or -</td>
<td>Pass or Fail</td>
<td>Fail</td>
<td>Pass</td>
<td>Invalid*</td>
<td>All samples in the Index Set are Invalid.</td>
<td>Quality control for the Index set is FAIL. Repeat Illumina COVIDSeq Test for all Invalid samples of failed Index Set.</td>
</tr>
<tr>
<td>+ or -</td>
<td>Pass or Fail</td>
<td>Pass</td>
<td>Fail</td>
<td>Invalid*</td>
<td>All samples in the Index Set are Invalid.</td>
<td>Quality control for the Index set is FAIL. Repeat Illumina COVIDSeq Test for all Invalid samples of failed Index Set.</td>
</tr>
<tr>
<td>+ or -</td>
<td>Pass or Fail</td>
<td>Fail</td>
<td>Fail</td>
<td>Invalid*</td>
<td>All samples in the Index Set are Invalid.</td>
<td>Quality control for the Index set is FAIL. Repeat Illumina COVIDSeq Test for all Invalid samples of failed Index Set.</td>
</tr>
</tbody>
</table>
Performance Characteristics

The following data outlined in the clinical performance and analytical performance sections were generated by using the protocols and materials outlined in the Instructions for Use starting with nasopharyngeal (NP) swab samples.

Analytical Sensitivity

The analytical sensitivity (Limit of Detection (LOD)) of the Illumina COVIDSeq Test using the Zymo extraction method was determined by serial dilution of heat inactivated SARS-CoV-2 virus with known concentration (ATCC VR-1986HK) into pooled negative clinical matrix (nasopharyngeal swab specimen) to 1000 copies per ml, 750 copies per ml, 500 copies per ml, and 250 copies per ml. For each serial dilution, 22–24 extraction replicates were evaluated using the Illumina COVIDSeq Test. The LOD using Zymo extraction was ≤ 500 copies per ml for all sequencing platforms and reagent kits tested.

The LOD of the Illumina COVIDSeq Test using the Qiagen extraction method was determined by serial dilution of heat inactivated SARS-CoV-2 virus with known concentration (ATCC VR-1986HK) into pooled negative clinical matrix (nasopharyngeal swab specimen) to 1500 copies per ml, 1000 copies per ml, 750 copies per ml, and 500 copies per ml. The LOD using Qiagen extraction was determined to be 1000 copies per ml for all sequencing platforms and reagent kits tested.
### Inclusivity

*In silico* primer analysis and synthetic short read analysis were performed to evaluate the inclusivity of the Illumina COVIDSeq Test. For primer analysis, SARS-CoV-2 sequences available on GISAID (1382 sequences) and NCBI (162 sequences) were evaluated. A BLASTn (NCBI) analysis was performed to quantify the level of primer homology across these sequences by querying each individual SARS-CoV-2 primer sequence against the downloaded SARS-CoV-2 sequences.

The following table below summarizes the homology analysis for all 1544 SARS-CoV-2 sequences.

<table>
<thead>
<tr>
<th>Percent of Primer Pairs Homology</th>
<th>Mean</th>
<th>Median</th>
<th>5th percentile</th>
<th>95th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% homology</td>
<td>97.1%</td>
<td>98.0%</td>
<td>93.9%</td>
<td>100%</td>
</tr>
<tr>
<td>&gt;80% homology</td>
<td>97.8%</td>
<td>98.0%</td>
<td>94.9%</td>
<td>100%</td>
</tr>
</tbody>
</table>

In summary, the Illumina COVIDSeq Test has excellent coverage across known strains of SARS-CoV-2.

### Cross-Reactivity

*In silico* analyzes were performed to evaluate the cross-reactivity of the Illumina COVIDSeq Test with representative common respiratory pathogens. For the primer analysis, 38 non SARS-CoV-2 consensus genomes were downloaded from NCBI as the negative sample cohort. A BLASTn (NCBI) analysis was then performed to quantify the number of primer pairs with more than 80% homology with each of the genomes in the cohort.

The following table shows the results from the analysis.
<table>
<thead>
<tr>
<th>Pathogen Name</th>
<th>NCBI Accession Number</th>
<th>Number of Primer Pairs With Homology Above 80%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus (e.g. C1 Ad. 71)*</td>
<td>AC 000017 (1)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>AC 000007 (2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AC 000008 (5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AC 000018 (7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AC 000019 (35)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NC_012959(54)</td>
<td></td>
</tr>
<tr>
<td>Human Metapneumovirus (hMPV)</td>
<td>NC_039199</td>
<td>0</td>
</tr>
<tr>
<td>Parainfluenza virus 1</td>
<td>NC_003461</td>
<td>0</td>
</tr>
<tr>
<td>Parainfluenza virus 2</td>
<td>NC_003443</td>
<td>0</td>
</tr>
<tr>
<td>Parainfluenza virus 3</td>
<td>NC_001796</td>
<td>0</td>
</tr>
<tr>
<td>Parainfluenza virus 4</td>
<td>NC_021928</td>
<td>0</td>
</tr>
<tr>
<td>Influenza virus A</td>
<td>NC_026438</td>
<td>0</td>
</tr>
<tr>
<td>Influenza virus B</td>
<td>NC_002204</td>
<td>0</td>
</tr>
<tr>
<td>Respiratory syncytial virus</td>
<td>NC_001803</td>
<td>0</td>
</tr>
<tr>
<td>Enterovirus (e.g. EV68)</td>
<td>NC_038308</td>
<td>0</td>
</tr>
<tr>
<td>Rhinovirus*</td>
<td>NC_038311 (1)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>NC_038312 (B3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NC_001490 (B14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NC_009996 (C)</td>
<td></td>
</tr>
<tr>
<td>Human coronavirus 229E</td>
<td>NC_002645</td>
<td>0</td>
</tr>
<tr>
<td>Human coronavirus OC43</td>
<td>NC_006213</td>
<td>0</td>
</tr>
<tr>
<td>Human coronavirus HKU1</td>
<td>NC_006577</td>
<td>0</td>
</tr>
<tr>
<td>Human coronavirus NL63</td>
<td>NC_005831</td>
<td>0</td>
</tr>
<tr>
<td>SARS-coronavirus</td>
<td>NC_004718</td>
<td>8</td>
</tr>
<tr>
<td>MERS coronavirus</td>
<td>NC_019843</td>
<td>0</td>
</tr>
<tr>
<td>Chlamydia pneumoniae</td>
<td>NC_005043</td>
<td>0</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>NZ_LN831035</td>
<td>0</td>
</tr>
<tr>
<td>Legionella pneumophilia</td>
<td>NZ_LR134380</td>
<td>0</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>NC_000962</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>NZ_LN831051</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>NZ_CP007593</td>
<td>0</td>
</tr>
<tr>
<td>Bordetella pertussis</td>
<td>NC_018518</td>
<td>0</td>
</tr>
<tr>
<td>Mycoplasma pneumoniae</td>
<td>NZ_CP010546</td>
<td>0</td>
</tr>
<tr>
<td>Pneumocystis jirovecii (PJP)</td>
<td>NJFV010000001 - NJFV010000219</td>
<td>0</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>NC_032089 - NC_032096</td>
<td>0</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>NC_002516</td>
<td>0</td>
</tr>
<tr>
<td>Staphylococcus epidermis</td>
<td>NZ_CP035288 - NZ_CP035290</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus salivarius</td>
<td>NZ_LR134274</td>
<td>0</td>
</tr>
</tbody>
</table>

* Aggregated results for testing of six different human adenovirus (1, 2, 5, 7, 35, 54) and four different rhinoviruses (1, B3, B14, C).

All pathogens were determined to have no cross-reactivity with the primers used except for SARS-coronavirus (SARS-2003), which has a small portion of primer pairs (8/98) that are potentially cross-reactive. However, these eight potentially cross-reactive primer pairs would not lead to false positive detection of SARS-2003 due to the exclusion of ambiguous k-mer(s) in the detection algorithm.
An analysis of simulated short reads was also performed on the same negative cohort. The following table shows the results of this analysis and indicates no cross-reactivity of the Illumina COVIDSeq Testt with the respiratory pathogens tested.

<table>
<thead>
<tr>
<th>RefSeq Record Name (Accession Number)</th>
<th>Number of ARTIC Amplicons Detected</th>
<th>COVID-19 Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus pneumoniae (NZ_LN831051)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Human respiro virus 3 (NC_001796)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Human adenovirus 1 (AC 000017)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Haemophilus influenzae (NZ_LN831035)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Streptococcus salivarius (NZ_LR134274)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Human adenovirus 5 (AC_000008)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Human adenovirus 54 (NC_012959)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Chlamydia pneumoniae TW-183 (NC_005043)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Staphylococcus epidermidis (NZ_CP035288 - NZ_CP035290)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Human adenovirus 2 (AC 000007)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Rhinovirus C (NC_009996)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Influenza B virus (B/Lee/1940) (NC_002204)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Human respiro virus 1 (NC_003461)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis H37Rv (NC_000962)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Human rhinovirus A1 (NC_038311)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Rhinovirus B14 (NC_001490)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Bordetella pertussis 18323 (NC_018518)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Candida albicans SC5314 (NC_032089 - NC_032098)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Human coronavirus 229E (NC_002645)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Influenza A virus (A/California/07/2009[H1N1]) (NC_026438)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Human coronavirus OC43 (NC_006213)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Pneumocystis jirovecii (NJFV01000001 - NJFV010000219)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Streptococcus pyogenes (NZ_CP007593)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Human rhinovirus B3 (NC_038312)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Middle East respiratory syndrome-related coronavirus (NC_019843)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Human parainfluenza virus 4a (NC_021928)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Human metapneumovirus (NC_039199)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Enterovirus D68 (NC_038308)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa PAO1 (NC_002516)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Mycoplasma pneumoniae (NZ_CP010546)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Respiratory syncytial virus (NC_001803)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Human coronavirus NL63 (NC_005831)</td>
<td>0</td>
<td>NO</td>
</tr>
<tr>
<td>Human adenovirus 35 (AC 000019)</td>
<td>0</td>
<td>NO</td>
</tr>
</tbody>
</table>
Clinical Evaluation

Clinical performance of the Illumina COVIDSeq Test with the Zymo extraction method was evaluated in comparison with an RT-PCR assay authorized by the FDA for use under Emergency Use Authorization (EUA RT-PCR). 96 clinical nasopharyngeal swab (NP) specimens were evaluated including 44 SARS-CoV-2 positive and 52 SARS-CoV-2 negative specimens (based on the EUA RT-PCR). The positive and negative percent agreement for the COVIDSeq Test are indicated in the following table.

### Zymo Extraction Method

<table>
<thead>
<tr>
<th>Sequencing Platform (Reagent Kit)</th>
<th>EUA RT-PCR Result</th>
<th>COVIDSeq Result (Zymo)</th>
<th>Positive % Agreement (CI)*</th>
<th>Negative % Agreement (CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>NextSeq 550 (High Output v2.5)</td>
<td>Positive</td>
<td>42</td>
<td>95.5% (84.5-99.4%)</td>
<td>100% (93.2-100.0%)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
<td>97.7% (88.0-99.9%)</td>
<td>100% (93.2-100.0%)</td>
</tr>
<tr>
<td>NextSeq 2000 (P2 v3)</td>
<td>Positive</td>
<td>42</td>
<td>95.5% (84.5-99.4%)</td>
<td>100% (93.2-100.0%)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
<td>97.7% (88.0-99.9%)</td>
<td>100% (93.2-100.0%)</td>
</tr>
<tr>
<td>NovaSeq 6000 (SP v1.5)</td>
<td>Positive</td>
<td>42</td>
<td>95.5% (84.5-99.4%)</td>
<td>100% (93.2-100.0%)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
<td>97.7% (88.0-99.9%)</td>
<td>100% (93.2-100.0%)</td>
</tr>
<tr>
<td>NovaSeq 6000 (S4 v1.5)</td>
<td>Positive</td>
<td>43</td>
<td>97.7% (88.0-99.9%)</td>
<td>100% (93.2-100.0%)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
<td>97.7% (88.0-99.9%)</td>
<td>100% (93.2-100.0%)</td>
</tr>
</tbody>
</table>

* Excludes all specimens invalid with the COVIDSeq Test or indeterminant with the EUA RT-PCR assay.

Clinical performance of the with the Qiagen extraction method was evaluated in comparison with an EUA RT-PCR assay. 84 clinical nasopharyngeal swab (NP) specimens were evaluated including 40 SARS-CoV-2 positive and 44 SARS-CoV-2 negative specimens (based on the EUA RT-PCR). The positive and negative percent agreement for the COVIDSeq Test are indicated in the following table.

### Qiagen Extraction Method

<table>
<thead>
<tr>
<th>Sequencing Platform (Reagent Kit)</th>
<th>EUA RT-PCR Result</th>
<th>COVIDSeq Result (Qiagen)</th>
<th>Positive % Agreement (CI)*</th>
<th>Negative % Agreement (CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>NextSeq 550 (High Output v2.5)</td>
<td>Positive</td>
<td>39</td>
<td>97.5% (86.8-99.9%)</td>
<td>97.7% (88.0-99.9%)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>1</td>
<td>97.7% (88.0-99.9%)</td>
<td>97.7% (88.0-99.9%)</td>
</tr>
<tr>
<td>NextSeq 2000 (P2 v3)</td>
<td>Positive</td>
<td>39</td>
<td>97.5% (86.8-99.9%)</td>
<td>97.7% (88.0-99.9%)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>1</td>
<td>97.7% (88.0-99.9%)</td>
<td>97.7% (88.0-99.9%)</td>
</tr>
<tr>
<td>NovaSeq 6000 (SP v1.5)</td>
<td>Positive</td>
<td>39</td>
<td>97.5% (86.8-99.9%)</td>
<td>97.7% (88.0-99.9%)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>1</td>
<td>97.7% (88.0-99.9%)</td>
<td>97.7% (88.0-99.9%)</td>
</tr>
</tbody>
</table>
Sequencing Platform (Reagent Kit) EUA RT-PCR Result COVIDSeq Result (Qiagen) Positive % Agreement (CI)* Negative % Agreement (CI)*

<table>
<thead>
<tr>
<th>NovaSeq 6000 (S4 v1.5)</th>
<th>Positive</th>
<th>Negative</th>
<th>97.5% (86.8-99.9%)</th>
<th>97.7% (88.0-99.9%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>39</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>43</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Excludes all specimens invalid with the COVIDSeq Test or indeterminant with the EUA RT-PCR assay.

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples, and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method and instrument used were QIAamp Viral RNA Mini Kit and NextSeq 550Dx (in RUO mode). The results are summarized in the following table.

Table 5  Summary of LoD Confirmation Result Using the FDA SARS-CoV-2 Reference Panel

<table>
<thead>
<tr>
<th>Reference Materials Provided by FDA</th>
<th>Specimen Type</th>
<th>Product LoD</th>
<th>Cross-Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>SARS-CoV-2</td>
<td>Nasopharyngeal Swab</td>
<td>5400 NDU/ml</td>
<td>N/A</td>
</tr>
<tr>
<td>MERS-CoV</td>
<td></td>
<td>N/A</td>
<td>ND</td>
</tr>
</tbody>
</table>

- NDU/ml—RNA NAAT detectable units/ml.
- N/A—Not applicable.
- ND—Not detected.

References


Additional Label

Print and attach the following Emergency Use Only label to the front of the sequencing instrument. If an instrument includes a label indicating For Research Use Only, cover it with the following label. The instrument should retain this label throughout the EUA use of the Illumina COVIDSeq Test.

Emergency Use Only

This instrument is authorized for use with the Illumina COVIDSeq Test.
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For Emergency Use Only

Prescription Use Only

Product Labeling

For a complete reference to symbols that may appear on product packaging and labeling, refer to the symbol key for your kit at support.illumina.com.
Illumina DRAGEN COVIDSeq Test Pipeline
Software Guide
## Revision History

<table>
<thead>
<tr>
<th>Document #</th>
<th>Date</th>
<th>Description of Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000000128119 v01</td>
<td>April 2021</td>
<td>Added new Detection Algorithm section. AddNextSeq 2000 Sequencing System as a compatible sequencing system in the Intended Use. Added and updated quality control metrics for different instruments to the Lane Quality Control and Flow Cell Quality Control sections. Added statement of output folder size variation to Storage Requirements. Added information for detaching from the screen process in the Running the System Check and Running the Illumina DRAGEN COVIDSeq Test Pipeline sections. Added information about the analysisFolder command to the Running the Illumina DRAGEN COVIDSeq Test Pipeline section. Updated software version numbers throughout from 1.2 to 1.3. Updated the Install section for additional command steps and uninstalling of previous software versions. Updated the Output Folder Structure to reflect changes in the Sample_Analysis folders. Updated folder names and variant and region criteria in the Variant Calling and Consensus Sequence Generation for Research Use Only section. Updated Warnings and Limitations to reflect rephrased regulatory statements.</td>
</tr>
<tr>
<td>1000000128119 v00</td>
<td>August 2020</td>
<td>Initial release.</td>
</tr>
</tbody>
</table>

FOR IN VITRO DIAGNOSTIC USE

FOR USE UNDER AN EMERGENCY USE AUTHORIZATION (EUA) ONLY
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Intended Use

The Illumina® COVIDSeq™ Test is a Next-Generation Sequencing (NGS) in vitro diagnostic test on the Illumina NovaSeq 6000 Sequencing System, NextSeq 2000 Sequencing System, NextSeq 500 Sequencing System, NextSeq 550 Sequencing System, or NextSeq 550Dx Instrument intended for the qualitative detection of SARS-CoV-2 RNA from nasopharyngeal (NP) swabs, oropharyngeal (OP) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasopharyngeal wash/aspirates, nasal aspirates, and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. Laboratories within the United States and its territories are required to report all results to the appropriate health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information. The Illumina® COVIDSeq™ Test is intended for use by qualified and trained clinical laboratory personnel specifically trained in the use of the NovaSeq 6000 Sequencing System, the NextSeq 500 Sequencing System, the NextSeq 550 Sequencing System, the NextSeq 2000 Sequencing System, or the NextSeq 550Dx Instrument, as well as Next-Generation Sequencing workflows and in vitro diagnostic procedures. The Illumina® COVIDSeq™ Test is only for use under the Food and Drug Administration’s Emergency Use Authorization.

Warnings and Limitations

- This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under EUAs for use by authorized laboratories.
- This product has been authorized only for the detection of nucleic acid of SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
Overview

The Illumina DRAGEN COVIDSeq Test Pipeline analyzes sequencing reads of RNA libraries prepared using the Illumina COVIDSeq Test. The Illumina DRAGEN COVIDSeq Test Pipeline uses the Illumina DRAGEN Bio-IT Platform to perform analysis to determine the presence of SARS-CoV-2 as the diagnostic EUA output and generates results in PDF and tab-delimited formats.

Additionally, Illumina DRAGEN COVIDSeq Test Pipeline performs small variant calling for samples with at least 90 SARS-CoV-2 virus targets detected using the SARS-CoV-2 reference genome and generates a consensus sequence in FASTA format. Variant calls and consensus sequences are generated for informational purposes as research use only and not for patient reporting.

The Illumina DRAGEN COVIDSeq Test Pipeline requires a sample sheet. See the Illumina COVIDSeq Test Instructions for Use (document # 1000000128490) for information on creating a sample sheet.

Installation Requirements

Illumina DRAGEN COVIDSeq Test Pipeline contains the following minimum operating requirements.

The Illumina DRAGEN COVIDSeq Test Pipeline is compatible with a DRAGEN Server v2 and v3.

By default, the software includes the following items:

- Linux CentOS 7.3 operating system, or later.

The following additional software is required before installing Illumina DRAGEN COVIDSeq Test Pipeline.

- Docker version 18.09, or later.

Storage Requirements

The DRAGEN Server provides NVMe SSD located in /staging directory to use as the software output directory.

If using the DRAGEN Server v2, store sequencing run data in a network-attached folder to make sure the required disk space is available on the NVMe SSD drives for analysis output. Network-attached storage is required for long-term storage for both DRAGEN Server v2 and v3.

Analysis output is automatically written to the /staging/covidseq_analysis_<timestamp> to make sure the DRAGEN Server processes read and write data on the NVMe SSD. You can modify this location using the command-line.

Before beginning analysis, develop a strategy to copy data from the DRAGEN Server to a network-attached storage. Delete output data on the DRAGEN Server as soon as possible.

The following are the run and analysis output sizes for each sequencing system per 36 bp. Output folder size can vary based on the number of positive samples. The following table are recommended storage requirements.

<table>
<thead>
<tr>
<th>Sequencing System</th>
<th>Run Folder Output (GB)</th>
<th>Analysis Output (GB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NovaSeq 6000 SP flow cell</td>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td>NovaSeq 6000 S4 flow cell</td>
<td>225–240</td>
<td>860</td>
</tr>
<tr>
<td>NextSeq 500/550 and 550Dx HO flow cell</td>
<td>12</td>
<td>30</td>
</tr>
</tbody>
</table>

Document # 1000000128119 v01

FOR IN VITRO DIAGNOSTIC USE
FOR USE UNDER AN EMERGENCY USE AUTHORIZATION (EUA) ONLY
FOR PRESCRIPTION USE ONLY
Install the Illumina DRAGEN COVIDSeq Test Pipeline

Use the instructions in this section to install the Illumina DRAGEN COVIDSeq Test Pipeline.

Illumina recommends running Docker as a non-root user by adding the user to the docker group. It is possible to run the Illumina DRAGEN COVIDSeq Test Pipeline as root but not recommended. For more information, see the Docker website.

The Illumina DRAGEN COVIDSeq Test Pipeline installation script uninstalls any existing DRAGEN software on the server. If you would like to use a different DRAGEN pipeline, you will need to uninstall the Illumina DRAGEN COVIDSeq Test Pipeline, and download a DRAGEN software installation package from the DRAGEN support page.

1. Contact your local Illumina Field Application Scientist to obtain the Illumina DRAGEN COVIDSeq Test Pipeline installer package.

2. Install Docker 18.09 or later using the install instructions for CentOS provided in the Docker documentation.

3. Install the DRAGEN Server license using the instructions provided in the Illumina DRAGEN Server Site Prep & Installation Guide.

4. Download the Illumina DRAGEN COVIDSeq Test Pipeline installation script provided in the email from Illumina. The link expires after 1 week.

5. Store the install script in the /staging directory.

6. Enter the following command to operate the using the third-party virtual terminal tool, screen.

   ```bash
   screen -S <name>
   ```

7. To update the run script permissions, enter the following command:

   ```bash
   chmod +x /staging/install_covidseq-EUA-1.3.0.run
   ```

8. To uninstall previous versions of the Illumina DRAGEN COVIDSeq Test Pipeline, enter the following command:

   ```bash
   /staging/uninstall_covidseq-1.1.0.sh
   ```

9. To run the installation script, enter the following command:

   ```bash
   /staging/install_covidseq-EUA-1.3.0.run
   ```

   The script removes any previously installed DRAGEN software, Depending on your previous version of DRAGEN, you might need to restart your server after install.

Running the System Check

Make sure that the system is functioning properly by running the `check_covidseq-1.3.0.sh` script. The self-test script checks the following functions:

- If all required services are running.
- If the proper Docker image is installed.
- If the Illumina DRAGEN COVIDSeq Test Pipeline successfully runs on a test data set.

The self-test runs for approximately five minutes. If the self-test prints a failure message, contact Illumina Technical Support and provide the `/staging/check_covidseq_<timestamp>.tgz` output file.

To detach from the screen process at anytime, enter `crtl-a d`.
Running the Illumina DRAGEN COVIDSeq Test Pipeline

The Illumina DRAGEN COVIDSeq Test Pipeline is started by selecting the shell script using the command line, and then running the software with Docker. Analysis outputs are located in the /staging/covidseq_analysis_<timestamp> directory.

This location ensures that the server is on an optimized NVMe SSD.

Do not move files or press CTRL+C when the app is running. Moving files during the analysis can cause the analysis to fail or provide incorrect results. Pressing CTRL+C stops the analysis and might cause an error. If an error does occur, restart the server.

1. If detached from the screen process, enter the following command to reattach to screen:
   ```bash
   screen -r name
   ```

2. To run the Illumina DRAGEN COVIDSeq Test Pipeline, enter the following command-line argument:
   ```bash
   covidseq.sh --runFolder <FULL_PATH_TO_RUN_FOLDER>
   ```

3. [Optional] Enter any of the other following available commands:
   - `--analysisFolder` — Full path to the alternative analysis folder. For high performance, this folder must be on an NVMe SSD partition. Make sure to use a different folder than the test data folder /staging/illumina/covidseq. If the Illumina DRAGEN COVIDSeq Test Pipeline is uninstalled, the test data folder is deleted.
   - `--sampleSheet` — Full path to the sample sheet. This command is required if your sample sheet is not named SampleSheet.csv.
   - `--version` — Displays the version of the software, and then exits.
   - `--fastMode` — Turns off alignment, variant calling, and consensus sequence FASTA generation to improve speed.
   - `--help` — Displays a help screen, and then exits.

Process Lane Subsets or Multiple Flow Cells

If using the NovaSeq 6000 Sequencing System, Illumina DRAGEN COVIDSeq Test Pipeline supports processing subsets of lanes in a flow cell because quality control is performed at the lane-level.

To analyze a subset of lanes, create a copy of the sample sheet, and then remove all samples that are not in the lanes to process. Specify this new sample sheet on the command line.

To analyze multiple flow cells, perform multiple, serial executions of the software. Only initiate a new analysis after the previous is completed. Running multiple executions of the software concurrently on the same server can cause the analysis to fail or produce incorrect results.

Each flow cell includes a separate run folder.

Analysis Methods

The Illumina DRAGEN COVIDSeq Test Pipeline performs analysis using the following steps. Each step creates a subfolder in Logs_intermediates subfolder under the analysis folder.

1. Validates the sample sheet fields.
   - This step generates the SampleSheetValidation subfolder.

2. Performs run quality checks on the BCL data from the run folder.
   - This step generates the RunQC subfolder.
3 Converts BCL data in the run folder to FASTQ sample data. All samples from the run are available as FASTQ files compressed in a gzip.
This step generates the FastqGeneration subfolder.

4 For each sample, Illumina DRAGEN COVIDSeq Test Pipeline determines the presence of SARS-CoV-2 and an internal (human) control. The read coverage per target is compared to a fixed target threshold to determine covered targets. The number of covered targets is then used to detect SARS-CoV-2 ($\geq$ virusThreshold) and the internal control ($\geq$ humanThreshold). The result is preliminary and undergoes quality control in later steps.
This step generates the VirusDetection subfolder.

5 For each sample with a result of "SARS-CoV-2 Detected" and at least 90 SARS-CoV-2 targets detected, Illumina DRAGEN COVIDSeq Test Pipeline performs variant calling to determine any variants present in the sample with respect to the SARS-CoV-2 reference genome (NC_045512.2) and the human control amplicon sequences.
This step generates the MapAlign subfolder.

6 For each sample with a result of "SARS-CoV-2 Detected" and at least 90 SARS-CoV-2 targets detected, Illumina DRAGEN COVIDSeq Test Pipeline generates a consensus genome in FASTA format using variant calls and coverage metrics as input. See Variant Calling and Consensus Sequence Generation for Research Use Only on page 11 for more information.
This step generates the VariantCalling subfolder.

7 For each sample with a result of "SARS-CoV-2 Detected" and at least 90 SARS-CoV-2 targets detected, Illumina DRAGEN COVIDSeq Test Pipeline performs quality control of each sample and generates a report in TSV format. Quality control is performed at the lane, plate, and sample-level and incorporates information from NTC and positive controls before determining patient results.
This step generates the JsonTSVReport subfolder.

8 Generates a PDF report that contains the summarized information.

Detection Algorithm

The Illumina DRAGEN COVIDSeq Test Pipeline uses a kmer based algorithm to detect SARS-CoV-2 and any external controls. The algorithm uses a kmer reference database to match kmers from the sequencing read to kmers from the SARS-CoV-2 reference genome (NC_0455). To create the kmer reference list, the SARS-CoV-2 reference genome is split in 32 bp kmers, and then any kmers that contain cross-reactivity are removed. To measure cross-reactivity, the kmer reference list uses the NCBI database of 100k human and animal pathogens. Bat and pangolin viruses are not included because of the similarity to the SARS-CoV-2 genome.

The kmer algorithm is performed using the following process:

1 The sequencing read is split into 32 bp kmers.
2 The kmers are matched to the kmer reference list.
3 Each of the reference kmers is labeled with a corresponding amplicon from either SARS-CoV-2 or external control.
4 If an amplicon contains at least 150 matched read and reference kmers, the amplicon is detected.
5 If the following number of amplicons are present, the algorithm detects SARS-CoV-2 or the external control.
   ▶ SARS-CoV-2 is detected if there are at least 5 SARS-CoV-2 amplicons.
   ▶ External control is detected if there are at least 3 external control amplicons.

6 For each run QC, a positive and negative control are added with the following requirements:
   ▶ SARS-CoV-2 should be detected in the positive control.
   ▶ SARS-CoV-2 or external controls should not be detected in the negative control.
Output Structure

The Illumina DRAGEN COVIDSeq Test Pipeline outputs results in the following folder structure. Key output files are shown below.

**Results**
- COVID-Seq_RunReport.pdf
- COVID-Seq_RunReport.tsv
- Errors.tsv

**Logs_Intermediates**
- ConsensusFasta
  - <sampleID>.consensus_metrics.csv
- FastqGeneration
- FindSampleValidity
- JsonTsvReport
- MapAlign
- VariantCalling
- VirusDetection

**Sample_Analysis**
- <sampleID1>
  - <sampleID1>.fasta
  - <sampleID1>.fasta.md5sum
  - <sampleID1>.bam
  - <sampleID1>.bam.bai
  - <sampleID1>.bam.md5sum
  - <sampleID1>.hard-filtered.vcf.gz
  - <sampleID1>.hard-filtered.vcf.gz.md5sum
  - <sampleID1>.hard-filtered.vcf.gz.tbi
  - <sampleID1>.consensus_filtered_variants.vcf.gz
  - <sampleID1>.consensus_filtered_variants.vcf.gz.md5sum
  - <sampleID1>.consensus_filtered_variants.vcf.gz.tbi
- <sampleID2>
Quality Control

Quality control is performed on each flow cell or flow cell lane, depending on your sequencing system, each index set, and each patient sample using the internal control, positive control, and NTC. If using the NovaSeq 6000, only the lanes and index set currently existing in the sample sheet are assessed.

Lane Quality Control

If using the NovaSeq 6000, quality control is performed for each flow cell lane based on whether quality metrics pass predefined thresholds. If using the NextSeq 500/550 or NextSeq 550Dx, quality is performed on each flow cell. See Lane Quality Control on page 8 for more information.

The flow cell lane must meet the following requirements to pass quality control. If the lane fails quality control, all index sets display a N/A QC status and all patient samples display Invalid.

<table>
<thead>
<tr>
<th>Flow Cell</th>
<th>% Q30</th>
<th>Yield (Gb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NovaSeq S4</td>
<td>≥ 90%</td>
<td>≥ 88</td>
</tr>
<tr>
<td>NovaSeq SP</td>
<td>≥ 90%</td>
<td>≥ 14</td>
</tr>
</tbody>
</table>

Flow Cell Quality Control

If using the NextSeq 500/550, NextSeq 550Dx, or NextSeq 2000, quality is performed on each flow cell. If using the NovaSeq 6000, quality control is performed on each flow cell lane. See Lane Quality Control on page 8 for more information.

The flow cell must meet the following requirements to pass quality control. If the flow cell fails quality control, all index sets within the failed flow cell display a N/A QC status and all patient samples display Invalid.

<table>
<thead>
<tr>
<th>Flow Cell</th>
<th>% Q30</th>
<th>Yield (Gb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NextSeq 550/550 Dx HO</td>
<td>≥ 80.0</td>
<td>≥ 12.0</td>
</tr>
<tr>
<td>NextSeq 2000</td>
<td>≥ 85.0</td>
<td>≥ 14.0</td>
</tr>
</tbody>
</table>

Index Set Quality Control

Quality control is performed on each index set based on the NTC and positive control samples. Each index set is required to have one NTC and one positive control sample. If the associate lane or flow cell failed QC, the index set is not assessed.

The index set fails QC if one of the following events occurs:

- The SARS-CoV-2 virus or internal control is detected in the NTC.
- The SARS-CoV-2 virus is not detected in the positive control.
- A software error occurs in either the NTC or positive control.

If an index set fails QC, all patient samples in the index set display Invalid.
Internal Control

An internal control is assessed for each patient sample. If the SARS-CoV-2 virus and the internal control are not detected in the patient sample, then the sample displays an Invalid result and the internal control is reported as Fail.

If the internal control is detected in the patient sample, then the internal control is reported as Pass.

If the SARS-CoV-2 virus is detected in the patient sample, but the internal control is not detected, the internal control is reported as N/A. The N/A internal control does not impact patient sample validity when the SARS-CoV-2 virus is detected.

Analysis Outputs

The Illumina DRAGEN COVIDSeq Test Pipeline generates the tab-separated values (TSV) and PDF report. The TSV report contains test results for both patient and control samples. The PDF report contains only results for patient samples.

TSV Run Report

The COVIDSeq_RunReport.tsv run report is located in the Results subfolder in the analysis folder. The report contains the following sections:

- **Header**—Contains information on the test name, run ID, run date, report date/time, instrument serial number, flow cell ID, and software version.

- **Quality Control**—Contains information about the quality control status for each lane or flow cell and each index set. Lane values can be PASS or FAIL. Index set can be PASS, FAIL, or N/A.

- **Patient Sample Results**—The patient sample results include the following fields:

<table>
<thead>
<tr>
<th>Field</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample ID</td>
<td>The sample ID in the sample sheet.</td>
</tr>
<tr>
<td>Internal control</td>
<td>The status of the internal control in a patient sample. Possible values include Pass, Fail, or N/A.</td>
</tr>
<tr>
<td>Result</td>
<td>The result for the patient sample. Possible values include the following: SARS-CoV-2 Detected—The sample lane or flow cell and index set passed quality control and the SARS-CoV-2 virus is detected in the sample. SARS-CoV-2 Not Detected—The sample lane or flow cell and index set passed quality control, the internal control was detected in the sample, and the SARS-CoV-2 virus is not detected. Invalid—The sample lane or flow cell index set failed quality control, a software error occurred for the sample, or neither the internal control or the SARS-CoV-2 virus was detected.</td>
</tr>
<tr>
<td>Consensus Sequence</td>
<td>Indicates if the consensus SARS-CoV-2 sequence in FASTA format was generated for the sample.</td>
</tr>
<tr>
<td>Lane</td>
<td>The flow cell lane associated with the sample. If using the NovaSeq 6000, values can include 1, 2, 3, or 4. If using the NextSeq 500/550 or NextSeq 550Dx, the value is 1, 2, 3, 4 all together. If using the NextSeq 2000, the value is 1.</td>
</tr>
<tr>
<td>Index Set</td>
<td>The index set/adapter plate associated with the sample using the values from the Index_ID or Index/Index 2 columns in the sample sheet. Values can be 1, 2, 3, or 4.</td>
</tr>
<tr>
<td>Index ID</td>
<td>The index ID associated with the sample. If the Index_ID column is specified in the sample sheet, the Index ID field displays the same value. If not specified, Index ID is derived from the Index and Index2 columns from the sample sheet.</td>
</tr>
</tbody>
</table>
Control Sample Results—The control sample results include the following fields:

<table>
<thead>
<tr>
<th>Field</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample ID</td>
<td>The sample ID specified in the sample sheet.</td>
</tr>
<tr>
<td>Control Type</td>
<td>The control sample type. Values can include Positive or NTC.</td>
</tr>
<tr>
<td>Human Control</td>
<td>Indicates if the internal (human) control is detected in a control sample. Values can include Detected or Not Detected.</td>
</tr>
<tr>
<td>SARS-CoV-2</td>
<td>Indicates if SARS-CoV-2 is detected in the control sample. Values can include Detected or Not Detected.</td>
</tr>
<tr>
<td>Lane</td>
<td>The flow cell lane associated with the same. If using the NovaSeq 6000, values can include 1, 2, 3, or 4. If using the NextSeq 500/550 or NextSeq550Dx, the value is 1, 2, 3, 4 all together. If using the NextSeq 2000, the value is 1.</td>
</tr>
<tr>
<td>Index Set</td>
<td>The index set/adapter plate associated with the control sample using the values from the Index_ID or Index/Index 2 columns in the sample sheet. Values can be 1, 2, 3, or 4.</td>
</tr>
<tr>
<td>Index ID</td>
<td>The index ID specified in the sample sheet. If the Index_ID column is specified in the sample sheet, the Index ID field displays the same value. If not specified, Index ID is derived from the Index and Index2 columns from the sample sheet.</td>
</tr>
</tbody>
</table>

PDF Report

The COVIDSeq_RunReport.pdf run report is located in the Results subfolder in the analysis folder. The report contains the following sections:

Run Information—Includes information on the following fields.

<table>
<thead>
<tr>
<th>Field</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run ID</td>
<td>The unique ID associated with the sequencing run.</td>
</tr>
<tr>
<td>Run Date</td>
<td>The date of the sequencing run.</td>
</tr>
<tr>
<td>Instrument Serial</td>
<td>The unique serial number associated with the sequencing system.</td>
</tr>
<tr>
<td>Flow Cell ID</td>
<td>Unique ID for the sequenced flow cell.</td>
</tr>
<tr>
<td>Software Version</td>
<td>The software version used to perform analysis and generate reports.</td>
</tr>
</tbody>
</table>

Quality control—Includes information on the following fields.

<table>
<thead>
<tr>
<th>Field</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lane 1, Lane 2, Lane 3, Lane 4</td>
<td>The QC result for each lane. If using the NovaSeq 6000, values can include PASS or FAIL. If using the NextSeq 500/550 or NextSeq550Dx, the value is Lane 1, 2, 3, 4. If using the NextSeq 2000, the value is Lane 1.</td>
</tr>
<tr>
<td>Index Set 1, Index Set 2, Index Set 3, Index Set 4</td>
<td>The QC result for each index set within the associated lane. Values can include PASS, FAIL, or N/A.</td>
</tr>
</tbody>
</table>

Invalid Results, SARS-CoV-2 Detected, SARS-CoV-2 Not Detected—List of all patient samples with Invalid, SARS-CoV-2 Detected, or SARS-CoV-2 Not Detected results. The number of samples is displayed in each section’s header.
### Field Description

<table>
<thead>
<tr>
<th>Field</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample ID</td>
<td>The sample ID in the sample sheet.</td>
</tr>
<tr>
<td>Internal control</td>
<td>The quality control result for the internal (human) control in a patient sample. Values include Pass, Fail, or N/A.</td>
</tr>
<tr>
<td>Result</td>
<td>The result for the patient sample. Possible values include the following:</td>
</tr>
<tr>
<td></td>
<td>SARS-CoV-2 Detected—The sample lane or flow cell and index set passed quality control and the SARS-CoV-2 virus is detected in the sample.</td>
</tr>
<tr>
<td></td>
<td>SARS-CoV-2 Not Detected—The sample lane or flow cell and index set passed quality control, the internal (human) control was detected in the sample, and the SARS-CoV-2 virus is not detected.</td>
</tr>
<tr>
<td></td>
<td>Invalid—The sample lane or flow cell or index set failed quality control, a software error occurred for the sample, or neither the internal control or the SARS-CoV-2 virus was detected.</td>
</tr>
<tr>
<td>Consensus Sequence</td>
<td>Indicates if the consensus SARS-CoV-2 sequence was generated for the sample.</td>
</tr>
<tr>
<td>Lane / Index Set</td>
<td>The lane and index set associated with the sample. For Lane, values can include Lane 1, Lane 2, Lane 3, or Lane 4. For Index Set, values can include Index Set 1, Index Set 2, Index Set 3, or Index Set 4. If the lane or index set failed quality control, Fail is included at the end of the field value.</td>
</tr>
</tbody>
</table>

### Variant Calling and Consensus Sequence Generation for Research Use Only

**Illumina DRAGEN COVIDSeq Test Pipeline** performs variant and consensus sequence generation for each sample with a result of "SARS-CoV-2 Detected" and at least 90 SARS-CoV-2 virus targets detected. Variant calls and consensus sequences are for information purposes only and should not be used for patient reporting.

Variant calling and consensus sequence generation is not performed for invalid samples.

The variant calling output file is generated in VCF 4.2 file format and located in `Sample_Analysis/<Sample ID>/<Sample ID>.hard-filtered.vcf.gz`.

The consensus filtered variant calling output file is located in `Sample_Analysis/<Sample ID>/<Sample ID>.consensus_filtered_variants.vcf.gz`.

To generate a consensus sequence in FASTA format, detected sequence variants that meet the following criteria are applied to the SARS-CoV-2 reference sequence (NCBI Accession NC_045512.2).

- All DRAGEN quality filters pass.
- Allele frequency is greater than or equal to 0.5.
- Depth is greater than 10.

Regions of sequence with coverage below 10 are masked as low-confidence. Hard-masking is applied, and all bases in low-confidence regions are converted to "N". A soft-masked sequence is also provided and indicates all low-confidence regions with lower case characters.

The hard-masked consensus FASTA is available in `Sample_Analysis/<Sample ID>/<Sample ID>.fasta`.

### Uninstall Illumina DRAGEN COVIDSeq Test Pipeline

The Illumina DRAGEN COVIDSeq Test Pipeline includes an uninstall script located in the `/usr/local/bin` called `uninstall_covidseq-1.3.0.sh`.

The uninstall script removes the following assets:

- All scripts (covidseq.sh, check_covidseq-1.3.0.sh, uninstall_covidseq-1.3.0.sh).
- The Illumina DRAGEN COVIDSeq Test Pipeline Docker image.
- Data stored in /staging/illumina/covidseq.

The script does not uninstall Docker.

To uninstall the Illumina DRAGEN COVIDSeq Test Pipeline, enter the following command as root.

```
/usr/local/bin/uninstall_covidseq-1.3.0.sh
```
Technical Assistance

For technical assistance, contact Illumina Technical Support.

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

Illumina Customer Support Telephone Numbers

<table>
<thead>
<tr>
<th>Region</th>
<th>Toll Free</th>
<th>Regional</th>
</tr>
</thead>
<tbody>
<tr>
<td>North America</td>
<td>+1.800.809.4566</td>
<td></td>
</tr>
<tr>
<td>Australia</td>
<td>+1.800.775.688</td>
<td></td>
</tr>
<tr>
<td>Austria</td>
<td>+43 800006249</td>
<td>+43 19286540</td>
</tr>
<tr>
<td>Belgium</td>
<td>+32 80077160</td>
<td>+32 34002973</td>
</tr>
<tr>
<td>China</td>
<td>400.066.5835</td>
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<tr>
<td>Denmark</td>
<td>+45 80820183</td>
<td>+45 89871156</td>
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<tr>
<td>Finland</td>
<td>+358 800918363</td>
<td>+358 974790110</td>
</tr>
<tr>
<td>France</td>
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<td>+33 170770446</td>
</tr>
<tr>
<td>Germany</td>
<td>+49 8001014940</td>
<td>+49 8938035677</td>
</tr>
<tr>
<td>Hong Kong, China</td>
<td>800960230</td>
<td></td>
</tr>
<tr>
<td>Ireland</td>
<td>+353 1800936608</td>
<td>+353 016950506</td>
</tr>
<tr>
<td>Italy</td>
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<td>+39 236003759</td>
</tr>
<tr>
<td>Japan</td>
<td>0800.111.5011</td>
<td></td>
</tr>
<tr>
<td>Netherlands</td>
<td>+31 8000222493</td>
<td>+31 207132960</td>
</tr>
<tr>
<td>New Zealand</td>
<td>0800.451.650</td>
<td></td>
</tr>
<tr>
<td>Norway</td>
<td>+47 800 16836</td>
<td>+47 21939693</td>
</tr>
<tr>
<td>Singapore</td>
<td>+1.800.579.2745</td>
<td></td>
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<tr>
<td>South Korea</td>
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<tr>
<td>Spain</td>
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<td>+34 800300143</td>
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<tr>
<td>Sweden</td>
<td>+46 850619671</td>
<td>+46 200883979</td>
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<tr>
<td>Switzerland</td>
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<td>+41 800200442</td>
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<tr>
<td>Taiwan, China</td>
<td>00806651752</td>
<td></td>
</tr>
<tr>
<td>United Kingdom</td>
<td>+44 8000126019</td>
<td>+44 2073057197</td>
</tr>
<tr>
<td>Other countries</td>
<td>+44.1799.534000</td>
<td></td>
</tr>
</tbody>
</table>

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

Product documentation — Available for download from support.illumina.com.
FOR IN VITRO DIAGNOSTIC USE
FOR USE UNDER AN EMERGENCY USE AUTHORIZATION (EUA) ONLY
FOR PRESCRIPTION USE ONLY

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## Revision History

<table>
<thead>
<tr>
<th>Document</th>
<th>Date</th>
<th>Description of Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Document # 1000000129048 v01</td>
<td>April 2021</td>
<td>Added NextSeq 2000 Sequencing System as a compatible sequencing system in the Intended Use. Added guidance for sample sheet to the Workflow Requirements section. Added information about FAST mode and the Metrics and Logs Datasets options to the Set Parameters and Analysis Methods sections. Added and updated quality control metrics for different instruments to the Lane Quality Control and Flow Cell Quality Control sections. Updated software version numbers throughout the document from 1.2 to 1.3. Updated the Output Folder Structure to reflect changes in the Sample_Analysis folders. Updated folder names and variant and region criteria in the Variant Calling and Consensus Sequence Generation for Research Use Only section. Corrected and standardized the software naming convention throughout the document for the DRAGEN COVIDSeq Test (EUA) App. Updated Warnings and Limitations to reflect rephrased regulatory statements.</td>
</tr>
<tr>
<td>Document # 1000000129048 v00</td>
<td>August 2020</td>
<td>Initial release.</td>
</tr>
</tbody>
</table>
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Intended Use

The Illumina® COVIDSeq™ Test is a Next-Generation Sequencing (NGS) in vitro diagnostic test on the Illumina NovaSeq 6000 Sequencing System, NextSeq 2000 Sequencing System, NextSeq 500 Sequencing System, NextSeq 550 Sequencing System, or NextSeq 550Dx Instrument intended for the qualitative detection of SARS-CoV-2 RNA from nasopharyngeal (NP) swabs, oropharyngeal (OP) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasopharyngeal wash/aspirates, nasal aspirates, and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. Laboratories within the United States and its territories are required to report all results to the appropriate health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information. The Illumina® COVIDSeq™ Test is intended for use by qualified and trained clinical laboratory personnel specifically trained in the use of the NovaSeq 6000 Sequencing System, the NextSeq 500 Sequencing System, the NextSeq 550 Sequencing System, the NextSeq 2000 Sequencing System, or the NextSeq 550Dx Instrument, as well as Next-Generation Sequencing workflows and in vitro diagnostic procedures. The Illumina® COVIDSeq™ Test is only for use under the Food and Drug Administration’s Emergency Use Authorization.

Warnings and Limitations

▶ This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under EUAs for use by authorized laboratories.

▶ This product has been authorized only for the detection of nucleic acid of SARS-CoV-2, not for any other viruses or pathogens.

▶ The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
Overview

The DRAGEN COVIDSeq Test (EUA) app analyzes sequencing reads of RNA libraries prepared using the Illumina COVIDSeq Test. The DRAGEN COVIDSeq Test (EUA) uses the Illumina DRAGEN Bio-IT Platform to perform analysis to determine the presence of SARS-CoV-2 as the diagnostic EUA output and generates results in PDF and tab-delimited formats. Additionally, DRAGEN COVIDSeq Test (EUA) performs small variant calling for samples with at least 90 SARS-CoV-2 virus targets detected using the SARS-CoV-2 reference genome and generates a consensus sequence in FASTA format. Variant calls and consensus sequences are generated for informational purposes as research use only and not for patient reporting.

Workflow Requirements

- A valid sample sheet (CSV) in BaseSpace Sequence Hub that was either:
  - Used in a completed run. The completed run must use Run Monitoring and Storage as the Configuration option.
  - Uploaded to a project folder in BaseSpace Sequence Hub via the GUI or BaseSpace CLI prior to running the app.

See the Illumina COVIDSeq Test Instructions for Use (document #1000000128490) for information on setting up your sample sheet.

Set Parameters

1. Open DRAGEN COVIDSeq Test (EUA) from BaseSpace Sequence Hub as follows.
   a. Select the Apps tab, and then select DRAGEN COVIDSeq Test (EUA).
   b. From the Version drop-down list, select 1.3.0.
   c. Select Launch Application.

2. To override the default name, enter a preferred name in the Analysis Name field. The default is the app name with the date and time the session started.

3. From the Save Results To field, select Select Project, and then select a project for the storage of app results.
   You can also create a new project by selecting New and then entering a name and description.

4. From the COVIDSeq Run to Analyze field, select Select Run(s) to select a completed COVIDSeq run to analyze.
   The completed run must contain a valid sample sheet.

5. [Optional] From the Override Sample Sheet field, select a sample sheet.
   Overriding the run’s sample sheet allows you to fix any errors in the original sample sheet or adjust the scope of the analysis.

6. Set the Fast Mode option by selecting the Enable Fast Mode checkbox.
   In Fast Mode, only virus detection is performed. Alignment, variant calling and consensus generation is disabled.

7. To exclude run logs, QC metric files, and other file types, deselect the Create Metrics and Logs Datasets checkbox.
Deselecting this option improves analysis speed, but only the results and sample analysis are generated under the **Logs_Intermediates_Lane_<Lane number or all>** folder. See *Analysis Methods on page 3* for more information on the subfolders excluded.

8 Select **Launch Application** to start the analysis.

   When the analysis is complete, the status of the app session is automatically updated and you receive a confirmation email. When the app is launched through a workgroup account, the confirmation email is sent to the workgroup owner.

### Analysis Methods

The DRAGEN COVIDSeq Test (EUA) App performs analysis using the following steps. Each step creates a subfolder in **Logs_Intermediates_Lane_<Lane number or all>** subfolder under the analysis folder.

1. Validates the sample sheet fields.
   
   This step generates the **SampleSheetValidation** subfolder. If the Metrics & Logs option is disabled, the subfolder is not available.

2. Performs run quality checks on the BCL data from the run folder.
   
   This step generates the **RunQC** subfolder. If the Metrics & Logs option is disabled, the subfolder is not available.

3. Converts BCL data in the run folder to FASTQ sample data. All samples from the run are available as FASTQ files compressed in a gzip.
   
   This step generates the **FastqGeneration** subfolder. If the Metrics & Logs option is disabled, the subfolder is not available.

4. For each sample, DRAGEN COVIDSeq Test (EUA) App determines the presence of SARS-CoV-2 and an internal (human) control. The read coverage per target is compared to a fixed target threshold to determine covered targets. The number of covered targets is then used to detect SARS-CoV-2 ($\geq$ virusThreshold) and the internal control ($\geq$ humanThreshold). The result is preliminary and undergoes quality control in later steps.
   
   The step generates the **VirusDetection** subfolder. If the Metrics & Logs option is disabled, the subfolder is not available.

5. For each sample with a result of "SARS-CoV-2 Detected" and at least 90 SARS-CoV-2 targets detected, DRAGEN COVIDSeq Test (EUA) App aligns FASTQ files to the SARS-CoV-2 reference genome (NC_045512.2) and the human control amplicon sequences.
   
   This step generates the **MapAlign** subfolder. If the FAST mode is enabled or Metrics & Logs option is disabled, the subfolder is not available.

6. For each sample with a result of "SARS-CoV-2 Detected" and at least 90 SARS-CoV-2 targets detected, DRAGEN COVIDSeq Test (EUA) App performs variant calling to determine any variants present in the sample with respect to the SARS-CoV-2 reference genome. This step produces VCF files containing detected variants for each processed sample. See *Variant Calling and Consensus Sequence Generation for Research Use Only on page 9* for more information.
   
   This step generates the **VariantCalling** subfolder. If the FAST mode is enabled or Metrics & Logs option is disabled, the subfolder is not available.

7. For each sample with a result of "SARS-CoV-2 Detected" and at least 90 SARS-CoV-2 targets detected, DRAGEN COVIDSeq Test (EUA) App generates a consensus genome in FASTA format using variant calls and coverage metrics as input. See *Variant Calling and Consensus Sequence Generation for Research Use Only on page 9* for more information.
This step generates the ConsensusFasta subfolder. If the FAST mode is enabled or Metrics & Logs option is disabled, the subfolder is not available.

8 For all samples, the TSV Run Report Generator performs quality control of each sample and generates a report in TSV format. Quality control is performed at the lane, plate, and sample-level and incorporates information from NTC and positive controls before determining patient results. This step generates the JsonTSVReport subfolder.

9 Generates a PDF report that contains the summarized information.

Quality Control

Quality control is performed on each flow cell or flow cell lane, depending on your sequencing system, each index set, and each patient sample using the internal control, positive control, and NTC. If using the NovaSeq 6000, only the lanes and index set currently existing in the sample sheet are assessed.

Lane Quality Control

If using the NovaSeq 6000, quality control is performed for each flow cell lane based on whether quality metrics pass predefined thresholds. If using the NextSeq 500/550 or NextSeq 550Dx, quality is performed on each flow cell. See Flow Cell Quality Control on page 4 for more information.

The flow cell lane must meet the following requirements to pass quality control. If the lane fails quality control, all index sets display a N/A QC status and all patient samples display Invalid.

<table>
<thead>
<tr>
<th>Flow Cell</th>
<th>% Q30</th>
<th>Yield (Gb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NovaSeq S4</td>
<td>≥ 90%</td>
<td>≥ 88</td>
</tr>
<tr>
<td>NovaSeq SP</td>
<td>≥ 90%</td>
<td>≥ 14</td>
</tr>
</tbody>
</table>

Flow Cell Quality Control

If using the NextSeq 500/550, NextSeq 550Dx, or NextSeq 2000, quality is performed on each flow cell. If using the NovaSeq 6000, quality control is performed on each flow cell lane. See Lane Quality Control on page 4 for more information.

The flow cell must meet the following requirements to pass quality control. If the flow cell fails quality control, all index sets within the failed flow cell display a N/A QC status and all patient samples display Invalid.

<table>
<thead>
<tr>
<th>Flow Cell</th>
<th>% Q30</th>
<th>Yield (Gb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NextSeq 550/550 Dx HO</td>
<td>≥ 80.0</td>
<td>≥ 12.0</td>
</tr>
<tr>
<td>NextSeq 2000</td>
<td>≥ 85.0</td>
<td>≥ 14.0</td>
</tr>
</tbody>
</table>

Index Set Quality Control

Quality control is performed on each index set based on the NTC and positive control samples. Each index set is required to have one NTC and one positive control sample. If the associate lane or flow cell failed QC, the index set is not assessed.

The index set fails QC if one of the following events occurs:

- The SARS-CoV-2 virus or internal control is detected in the NTC.
- The SARS-CoV-2 virus is not detected in the positive control.
A software error occurs in either the NTC or positive control. If an index set fails QC, all patient samples in the index set display Invalid.

**Internal Control**

An internal control is assessed for each patient sample. If the SARS-CoV-2 virus and the internal control are not detected in the patient sample, then the sample displays an Invalid result and the internal control is reported as Fail.

If the internal control is detected in the patient sample, then the internal control is reported as Pass.

If the SARS-CoV-2 virus is detected in the patient sample, but the internal control is not detected, the internal control is reported as N/A. The N/A internal control does not impact patient sample validity when the SARS-CoV-2 virus is detected.

**View Analysis Output**

1. When analysis is complete, select Analyses from the My Data tab in BaseSpace Sequence Hub.
2. Select the analysis name.
3. Select any of the following tabs to view information on the analysis session.
   - **Summary**—General information about the analysis session, including log files.
   - **Reports**—Analysis reports.
   - **Inputs**—The samples and settings specified for the analysis session.
   - **Files**—Output files.

**Summary**

The Summary page provides access to analysis settings, execution details, and log files.

<table>
<thead>
<tr>
<th>Property</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>Name of the analysis session.</td>
</tr>
<tr>
<td>Application</td>
<td>Application that generated the analysis.</td>
</tr>
<tr>
<td>Date Started</td>
<td>Date and time the analysis session started.</td>
</tr>
<tr>
<td>Date Completed</td>
<td>Date and time the analysis session completed.</td>
</tr>
<tr>
<td>Duration</td>
<td>Duration of the analysis.</td>
</tr>
<tr>
<td>Compute Charge</td>
<td>Cost in iCredits to compute the analysis.</td>
</tr>
<tr>
<td>Session Type</td>
<td>Number of nodes used, which can be multinode or single node.</td>
</tr>
<tr>
<td>Node Count</td>
<td>Total number of nodes.</td>
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<td>Status</td>
<td>Status of the analysis session:</td>
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<tr>
<td></td>
<td>• Queued for Analysis</td>
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<tr>
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<td>• Running</td>
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<td>• Complete</td>
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<td>• Aborted</td>
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<tr>
<td>Delivery</td>
<td>Delivery status of the analysis results.</td>
</tr>
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</table>

**Reports**

The DRAGEN COVIDSeq Test (EUA) app provides a summary of key analysis metrics for the analyzed samples.
**Inputs**

The Inputs page lists the samples and settings specified for the analysis session.

**Files**

The Files page provides access to the output files for each sample analysis. The output files are organized in the following folder structure. Key output files are shown below.

- **Results**
  - COVIDSeq_RunReport.pdf — Contains only results for patient samples. See *PDF Report on page 7.*
  - COVIDSeq_RunReport.tsv — Contains test results for both patient and control samples. See *TSV Run Report on page 8*
  - Errors.tsv

- **Logs_Intermediates_Lane_<Lane number>** (on NovaSeq 6000) — See *Variant Calling and Consensus Sequence Generation for Research Use Only on page 9.* On NextSeq 500/550 and NextSeq 550Dx this folder is called Logs_Intermediates_Lane_all.

- **ConsensusFasta**
  - <Sample ID>
    - <Sample ID>.consensus_metrics.csv

- **FastqGeneration**
  - Reports
    - Demultiplex_Stats.csv
    - Top_Unknown_Barcodes.csv

- **FindSampleValidity**

- **JsonTsvReport**

- **MapAlign**

- **RunQc**

- **SampleSheetValidation**

- **VariantCalling**

- **VirusDetection**

- **Sample_Analysis**
  - <Sample ID>
    - <Sample ID>.fasta
    - <Sample ID>.fasta.md5sum
    - <Sample ID>.bam
    - <Sample ID>.bam.bai
    - <Sample ID>.bam.md5sum
    - <Sample ID>.hard-filtered.vcf.gz
    - <Sample ID>.hard-filtered.vcf.gz.md5sum
    - <Sample ID>.hard-filtered.vcf.gz.gz.tbi
PDF Report

The COVIDSeq_RunReport.pdf run report is located in the Results subfolder in the analysis folder. The report contains the following sections:

- **Run Information**—Includes information on the following fields.

<table>
<thead>
<tr>
<th>Field</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run ID</td>
<td>The unique ID associated with the sequencing run.</td>
</tr>
<tr>
<td>Run Date</td>
<td>The date of the sequencing run.</td>
</tr>
<tr>
<td>Instrument Serial</td>
<td>The unique serial number associated with the sequencing system.</td>
</tr>
<tr>
<td>Flow Cell ID</td>
<td>Unique ID for the sequenced flow cell.</td>
</tr>
<tr>
<td>Software Version</td>
<td>The software version used to perform analysis and generate reports.</td>
</tr>
</tbody>
</table>

- **Quality control**—Includes information on the following fields.

<table>
<thead>
<tr>
<th>Field</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lane 1, Lane 2, Lane 3, Lane 4</td>
<td>The QC result for each lane. If using the NovaSeq 6000, values can include PASS or FAIL. If using the NextSeq 500/550 or NextSeq 550Dx, the value is Lane 1,2,3,4.</td>
</tr>
<tr>
<td>Index Set 1, Index Set 2, Index Set 3, Index Set 4</td>
<td>The QC result for each index set within the associated lane. Values can include PASS, FAIL, or N/A.</td>
</tr>
</tbody>
</table>

- **Invalid Results. SARS-CoV-2 Detected. SARS-CoV-2 Not Detected**—List of all patient samples with Invalid, SARS-CoV-2 Detected, or SARS-CoV-2 Not Detected results. The number of samples is displayed in each section’s header.

<table>
<thead>
<tr>
<th>Field</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample ID</td>
<td>The sample ID in the sample sheet.</td>
</tr>
<tr>
<td>Internal control</td>
<td>The quality control result for the internal (human) control in a patient sample. Values include Pass, Fail, or N/A.</td>
</tr>
<tr>
<td>Result</td>
<td>The result for the patient sample. Possible values include the following: SARS-CoV-2 Detected—The sample lane or flow cell and index set passed quality control and the SARS-CoV-2 virus is detected in the sample. SARS-CoV-2 Not Detected—The sample lane or flow cell and index set passed quality control, the internal (human) control was detected in the sample, and the SARS-CoV-2 virus is not detected. Invalid—The sample lane or flow cell or index set failed quality control, a software error occurred for the sample, or neither the internal control or the SARS-CoV-2 virus was detected.</td>
</tr>
<tr>
<td>Consensus Sequence</td>
<td>Indicates if the consensus SARS-CoV-2 sequence was generated for the sample.</td>
</tr>
<tr>
<td>Lane / Index Set</td>
<td>The lane and index set associated with the sample. For Lane, values can include Lane 1, Lane 2, Lane 3, or Lane 4. If using the NextSeq 500/550 or NextSeq 550Dx, the value is Lane 1,2,3,4. For Index Set, values can include Index Set 1, Index Set 2, Index Set 3, or Index Set 4. If the lane or index set failed quality control, Fail is included at the end of the field value.</td>
</tr>
</tbody>
</table>
TSV Run Report

The COVIDSeq_RunReport.tsv run report is located in the Results subfolder in the analysis folder. The report contains the following sections:

- **Header**—Contains information on the test name, run ID, run date, report date/time, instrument serial number, flow cell ID, and software version.

- **Quality Control**—Contains information about the quality control status for each lane or flow cell and each index set. Lane values can be \textit{PASS} or \textit{FAIL}. Index set can be \textit{PASS}, \textit{FAIL}, or \textit{N/A}.

- **Patient Sample Results**—The patient sample results include the following fields:

<table>
<thead>
<tr>
<th>Field</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample ID</td>
<td>The sample ID in the sample sheet.</td>
</tr>
<tr>
<td>Internal control</td>
<td>The status of the internal control in a patient sample. Possible values include \textit{Pass}, \textit{Fail}, or \textit{N/A}.</td>
</tr>
<tr>
<td>Result</td>
<td>The result for the patient sample. Possible values include: \textit{SARS-CoV-2 Detected}—The sample lane or flow cell and index set passed quality control and the SARS-CoV-2 virus is detected in the sample. \textit{SARS-CoV-2 Not Detected}—The sample lane or flow cell and index set passed quality control, the internal control was detected in the sample, and the SARS-CoV-2 virus is not detected. \textit{Invalid}—The sample lane or flow cell index set failed quality control, a software error occurred for the sample, or neither the internal control or the SARS-CoV-2 virus was detected.</td>
</tr>
<tr>
<td>Consensus Sequence</td>
<td>Indicates if the consensus SARS-CoV-2 sequence in FASTA format was generated for the sample.</td>
</tr>
<tr>
<td>Lane</td>
<td>The flow cell lane associated with the sample. If using the NovaSeq 6000, values can include 1, 2, 3, or 4. If using the NextSeq 500/550 or NextSeq 550Dx, the value is 1, 2, 3, or 4 all together. If using the NextSeq 2000, the value is 1.</td>
</tr>
<tr>
<td>Index Set</td>
<td>The index set/adapter plate associated with the sample using the values from the Index_ID or Index/Index 2 columns in the sample sheet. Values can be 1, 2, 3, or 4.</td>
</tr>
<tr>
<td>Index ID</td>
<td>The index ID associated with the sample. If the Index_ID column is specified in the sample sheet, the Index ID field displays the same value. If not specified, Index ID is derived from the Index and Index2 columns from the sample sheet.</td>
</tr>
</tbody>
</table>

- **Control Sample Results**—The control sample results include the following fields:

<table>
<thead>
<tr>
<th>Field</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample ID</td>
<td>The sample ID specified in the sample sheet.</td>
</tr>
<tr>
<td>Control Type</td>
<td>The control sample type. Values can include \textit{Positive} or \textit{NTC}.</td>
</tr>
<tr>
<td>Human Control</td>
<td>Indicates if the internal (human) control is detected in a control sample. Values can include \textit{Detected} or \textit{Not Detected}.</td>
</tr>
<tr>
<td>SARS-CoV-2</td>
<td>Indicates if SARS-CoV-2 is detected in the control sample. Values can include \textit{Detected} or \textit{Not Detected}.</td>
</tr>
<tr>
<td>Lane</td>
<td>The flow cell lane associated with the same. If using the NovaSeq 6000, values can include 1, 2, 3, or 4. If using the NextSeq 500/550 or NextSeq 550Dx, the value is 1, 2, 3, or 4 all together. If using the NextSeq 2000, the value is 1.</td>
</tr>
<tr>
<td>Index Set</td>
<td>The index set/adapter plate associated with the control sample using the values from the Index_ID or Index/Index 2 columns in the sample sheet. Values can be 1, 2, 3, or 4.</td>
</tr>
</tbody>
</table>
### Field Description

<table>
<thead>
<tr>
<th>Field</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Index ID</td>
<td>The index ID specified in the sample sheet. If the Index_ID column is specified in the sample sheet, the Index ID field displays the same value. If not specified, Index ID is derived from the Index and Index2 columns from the sample sheet.</td>
</tr>
</tbody>
</table>

### Variant Calling and Consensus Sequence Generation for Research Use Only

DRAGEN COVIDSeq Test (EUA) App performs variant and consensus sequence generation for each sample with a result of "SARS-CoV-2 Detected" and at least 90 SARS-CoV-2 virus targets detected. Variant calls and consensus sequences are for information purposes only and should not be used for patient reporting. Variant calling and consensus sequence generation is not performed for invalid samples.

The variant calling output file is generated in VCF 4.2 file format and located in `Sample_Analysis/<Sample ID>/<Sample ID>.hard-filtered.vcf.gz`.

The consensus filtered variant calling output file is located in `Sample_Analysis/<Sample ID>/<Sample ID>.consensus_filtered_variants.vcf.gz`.

To generate a consensus sequence in FASTA format, detected sequence variants that meet the following criteria are applied to the SARS-CoV-2 reference sequence (NCBI Accession NC_045512.2).

- All DRAGEN quality filters pass.
- Allele frequency is greater than or equal to 0.5.
- Depth is greater than 10.

Regions of sequence with coverage below 10 are masked as low-confidence. Hard-masking is applied, and all bases in low-confidence regions are converted to "N". A soft-masked sequence is also provided and indicates all low-confidence regions with lowercase characters.

The hard-masked consensus FASTA is available in `Sample_Analysis/<Sample ID>/<Sample ID>.fasta`.

### Download Project Files with BaseSpace CLI

Due to the size and sheer number of files contained in a BaseSpace Sequence Hub project, attempting to download project files via the BaseSpace Sequence Hub GUI may be problematic. It is recommended that you use BaseSpace CLI to download all the files in a project.

Enter the following command in BaseSpace CLI to download all the files in a BaseSpace project.

```bash
bs project download --id <project ID> -o <output directory>
```
**Technical Assistance**

For technical assistance, contact Illumina Technical Support.

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

### Illumina Customer Support Telephone Numbers

<table>
<thead>
<tr>
<th>Region</th>
<th>Toll Free</th>
<th>Regional</th>
</tr>
</thead>
<tbody>
<tr>
<td>North America</td>
<td>+1.800.809.4566</td>
<td></td>
</tr>
<tr>
<td>Australia</td>
<td>+1.800.775.688</td>
<td></td>
</tr>
<tr>
<td>Austria</td>
<td>+43 800006249</td>
<td>+43 19286540</td>
</tr>
<tr>
<td>Belgium</td>
<td>+32 80077160</td>
<td>+32 34002973</td>
</tr>
<tr>
<td>China</td>
<td>400.066.5835</td>
<td></td>
</tr>
<tr>
<td>Denmark</td>
<td>+45 80820183</td>
<td>+45 89871156</td>
</tr>
<tr>
<td>Finland</td>
<td>+358 800918363</td>
<td>+358 974790110</td>
</tr>
<tr>
<td>France</td>
<td>+33 805102193</td>
<td>+33 170770446</td>
</tr>
<tr>
<td>Germany</td>
<td>+49 8001014940</td>
<td>+49 8938035677</td>
</tr>
<tr>
<td>Hong Kong, China</td>
<td>800960230</td>
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</tr>
<tr>
<td>Ireland</td>
<td>+353 1800936608</td>
<td>+353 016950506</td>
</tr>
<tr>
<td>Italy</td>
<td>+39 800985513</td>
<td>+39 236003759</td>
</tr>
<tr>
<td>Japan</td>
<td>0800.111.5011</td>
<td></td>
</tr>
<tr>
<td>Netherlands</td>
<td>+31 8000222493</td>
<td>+31 207132960</td>
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<tr>
<td>New Zealand</td>
<td>0800.451.650</td>
<td></td>
</tr>
<tr>
<td>Norway</td>
<td>+47 800 16836</td>
<td>+47 21939693</td>
</tr>
<tr>
<td>Singapore</td>
<td>+1.800.579.2745</td>
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</tr>
<tr>
<td>South Korea</td>
<td>+82 80 234 5300</td>
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<tr>
<td>Spain</td>
<td>+34 911899417</td>
<td>+34 800300143</td>
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<tr>
<td>Sweden</td>
<td>+46 850619671</td>
<td>+46 200883979</td>
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<tr>
<td>Switzerland</td>
<td>+41 565800000</td>
<td>+41 800200442</td>
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<tr>
<td>Taiwan, China</td>
<td>00806651752</td>
<td></td>
</tr>
<tr>
<td>United Kingdom</td>
<td>+44 8000126019</td>
<td>+44 2073057197</td>
</tr>
<tr>
<td>Other countries</td>
<td>+44.1799.534000</td>
<td></td>
</tr>
</tbody>
</table>

**Safety data sheets (SDSs)** — Available on the Illumina website at support.illumina.com/sds.html.  
**Product documentation** — Available for download from support.illumina.com.
IMPORTANT CUSTOMER INFORMATION

Illumina COVIDSeq Test, part # 20044461

FOR IN VITRO DIAGNOSTIC USE.
FOR USE UNDER AN EMERGENCY USE AUTHORIZATION (EUA) ONLY.
FOR PRESCRIPTION USE ONLY.

This product information card is not the complete instructions for use. To access the Illumina COVIDSeq Test Instructions for Use and other documentation:

1 Open a web browser.
2 Go to https://support.illumina.com/clinical_support/clinical_kits/illumina-covidseq-test-ivd/documentation.html.
3 Select View Options under the document name you want to access.
4 Select the title of the document you want to open.

To access the Fact Sheet for Healthcare Providers and the Fact Sheet for Patients:

1 Open a web browser.
3 Navigate to the Product Literature section on the page.
4 Select the title of the document you want to open.

Contact your local account manager or Illumina Customer Support at +1.800.809.ILMN (4566) or by email at techsupport@illumina.com if you have questions, need technical support to access the Instructions for use, or require a printed copy free of charge.

• This product has not been cleared or approved, but has been authorized for emergency use by FDA under Emergency Use Authorizations (EUAs) for use authorized laboratories.
• This product has been authorized only for the detection of nucleic acid of SARS-CoV-2, not for any other viruses or pathogens.
• The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.