

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 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 Illumina NovaSeq 6000 Sequencing System and Next-Generation Sequencing workflows as well as *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 high-throughput Next Generation Sequencing (NGS) 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 QIAamp Viral RNA Mini Kit.
- ▶ **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 flow cell workflow. 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. 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 test has not been FDA cleared or approved.
- ▶ For *in vitro* diagnostic use only.
- ▶ For prescription use only.
- ▶ This test has been authorized by FDA under an EUA for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, to perform high complexity tests.
- ▶ This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- ▶ This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or 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.
- ▶ Laboratories are required to report all positive 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 8*.
- ▶ Detection of SARS-CoV-2 RNA may be affected by sample collection methods, patient factors (eg, 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 and/or probe 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

The Illumina COVIDSeq Test Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: <https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd>.

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

- ▶ Authorized laboratories* using Illumina COVIDSeq Test will 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 will 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 will notify the relevant public health authorities of their intent to run Illumina COVIDSeq Test prior to initiating testing.
- ▶ Authorized laboratories using Illumina COVIDSeq Test will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- ▶ Authorized laboratories will 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 will 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:

- ▶ Illumina COVIDSeq Test (3072 Samples), part # 20043675
- ▶ 8 IDT for Illumina Nextera UD Indexes Sets A–D (384 Indexes, 384 Samples), part # 20027217

Reagents

Reagents Provided



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.

Illumina COVIDSeq Test

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

Table 1 Illumina COVIDSeq Test Box 1 – 3072 Samples, Part # 20043645

Quantity	Label Volume	Reagent	Description	Storage
1	233 ml	ITB	Illumina Tune Beads	Room temperature

Table 2 Illumina COVIDSeq Test Box 2 – 3072 Samples, Part # 20043434

Quantity	Label Volume	Reagent	Description	Storage
1	55.6 ml	ST2 HT	Stop Tagment Buffer 2 HT	Room temperature, post-amp environment

Table 3 Illumina COVIDSeqTest Box 3 – 3072 Samples, Part # 20043646

Quantity	Label Volume (ml)	Reagent	Description	Storage
2	6.15	EBLTS HT	Enrichment BLT HT	2°C to 8°C, post-amp environment
1	10	RSB HT	Resuspension Buffer HT	2°C to 8°C, post-amp environment

Table 4 Illumina COVIDSeqTest Box 4 – 3072 Samples, Part # 20043436

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

Table 5 Illumina COVIDSeq Test Box 5 – 3072 Samples, Part # 20043648

Quantity	Label Volume (ml)	Reagent	Description	Storage
1	45.1	EPH3 HT	Elution Prime Fragment 3HC Mix HT	-25°C to -15°C pre-amp environment
1	100.6	IPM HT	Illumina PCR Mix HT	-25°C to -15°C, pre-amp environment
1	78.9	EPM HT	Enhanced PCR Mix HT	-25°C to -15°C, pre-amp environment

Table 6 Illumina COVIDSeq Test Box 6 – 3072 Samples, Part # 20043647

Quantity	Label Volume (ml)	Reagent	Description	Storage
1	4.6	RVT HT	Reverse Transcriptase HT	-25°C to -15°C, pre-amp environment
1	41.1	FSM HT	First Strand Mix HT	-25°C to -15°C, pre-amp environment

Table 7 Illumina COVIDSEQ Test Box 7 – 3072 Samples, Part # 20043439

Quantity	Label Volume (ml)	Reagent	Description	Storage
1	14.4	CPP1 HT	COVIDSeq Primer Pool 1 HT	-25°C to -15°C, pre-amp environment
1	14.4	CPP2 HT	COVIDSeq Primer Pool 2 HT	-25°C to -15°C, pre-amp environment
1	37.6	TB1 HT	Tagmentation Buffer 1 HT	-25°C to -15°C, post-amp environment

Table 8 Illumina COVIDSeq Positive Control HT, Part # 20043401

Quantity	Label Volume	Reagent	Description	Storage
1	100 µl	COVIDSeq Positive Control HT	COVIDSeq Positive Control HT	-85°C to -65°C, post-amp environment

IDT for Illumina Nextera DNA Unique Dual Indexes, Store at -25°C to -15°C

The Illumina COVIDSeq Test requires 8 IDT for Illumina Nextera DNA Unique Dual Indexes Sets A–D (384 Indexes, 384 Samples) for a total 32 96 indexes, 96 sample index adapter plates.

Quantity	Description	Part Number
8	IDT for Illumina Nextera DNA Unique Dual Indexes Set A (96 Indexes, 96 Samples)	20026121
8	IDT for Illumina Nextera DNA Unique Dual Indexes Set B (96 Indexes, 96 Samples)	20026930
8	IDT for Illumina Nextera DNA Unique Dual Indexes Set C (96 Indexes, 96 Samples)	20026934
8	IDT for Illumina Nextera DNA Unique Dual Indexes Set D (96 Indexes, 96 Samples)	20026933

Reagents Not Provided

Reagents Required, Not Provided

- ▶ 13 QIAamp Viral RNA Mini Kit, Qiagen, # 52906

- ▶ QIAamp Viral RNA Mini Kit reagents. See *QIAamp Viral RNA Mini Handbook (document # #HB-0354-006)*.
- ▶ Qubit dsDNA HS Assay Kit, Thermo Fisher Scientific, # Q32851 or Q32854
- ▶ The following NovaSeq 6000 Sequencing System reagents for 3072 samples:
 - ▶ 2 NovaSeq 6000 Sequencing System S4 Reagent Kit (200 cycles), Illumina, # 20027466
 - ▶ 2 NovaSeq Xp 4-Lane Kit, Illumina, # 20021665
- ▶ 2 N NaOH
- ▶ 400 mM Tris-HCl, pH 8.0
- ▶ 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 -75°C.
- 3 Do not allow more than 3 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 7 days at -25°C to -15°C.

Equipment and Materials

Equipment and Materials Required, Not Provided

Equipment Required, Not Provided

Equipment	Supplier
10 µl single-channel pipettes	General lab supplier
20 µl single-channel pipettes	General lab supplier
200 µl single-channel pipettes	General lab supplier
1000 µl single-channel pipettes	General lab supplier
10 µl 8-channel pipettes	General lab supplier
20 µl 8-channel pipettes	General lab supplier
200 µl 8-channel pipettes	General lab supplier

Equipment	Supplier
1000 µl 8-channel pipettes	General lab supplier
20 µl 12-channel pipettes	General lab supplier
200 µl 12-channel pipettes	General lab supplier
10 ml serological pipettes	General lab supplier
25 ml serological pipettes	General lab supplier
50 ml serological pipettes	General lab supplier
BioShake iQ	QInstruments, part # 1808-0506
DRAGEN Bio-IT Platform	Illumina
QIAamp Viral RNA Mini Kit equipment	See <i>QIAamp Viral RNA Mini Handbook (document # #HB-0354-006)</i>
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: <ul style="list-style-type: none"> • Dynabeads MPC-S (Magnetic Particle Concentrator) • MagnaRack Magnetic Separation Rack 	<ul style="list-style-type: none"> • Thermo Fisher Scientific, catalog #A13346 • Thermo Fisher Scientific, catalog # CS15000
Microcentrifuge	General lab supplier
Microplate Centrifuge	General lab supplier
NovaSeq 6000 Sequencing System	Illumina
NovaSeq Xp Flow Cell Dock	Illumina, # 20021663
Pipette Aid	General lab supplier
Quibit Fluorometer 3.0	Thermo Fisher, catalog # Q33216, Q33217, or Q33218
Refrigerator, 2°C to 8°C	General lab supplier
Bio-Rad C-1000 Touch thermal cycler	Bio-Rad, Part # 1851197
Sealing wedge or roller	General lab supplier
Vortexer	General lab supplier

Materials Required, Not Provided

Consumable	Supplier
10 µl pipette tips	General lab supplier
20 µl pipette tips	General lab supplier
200 µl pipette tips	General lab supplier
200 µl pipette tips	General lab supplier
1000 µl pipette tips	General lab supplier
Hard-Shell 96-Well PCR Plates	Bio-Rad, catalog # HSP-9601
1.7 ml LoBind microcentrifuge tubes	Eppendorf, catalog # 022431021
5 ml LoBind microcentrifuge tube	Eppendorf, catalog # 0030122348
15 ml tubes	General lab supplier
Microseal 'B' adhesive seals	Bio-Rad, part # MSB-1001
RNase/DNase-free Disposable Pipetting Reservoirs	VWR, part # 89094-658

Consumable	Supplier
Qubit dsDNA HS Assay Kit	One of the following, depending on kit size: <ul style="list-style-type: none"> • ThermoFisher Scientific, part # Q32851 • ThermoFisher Scientific, part # Q32854
Qubit Assay Tubes	ThermoFisher Scientific, catalog # Q32856

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 # 20043436—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 # 20043401—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 # 20043439—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 × g for 1 minute. For bead steps, centrifuge at 500 × 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.

Consumables

- ▶ ELB HT (Elution Buffer HT)
- ▶ CPC HT (COVIDSeq Positive Control HT)
- ▶ 1.7 ml LoBind tubes
- ▶ 5 ml LoBind tubes
- ▶ 15 ml tubes

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:

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

- 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 15 ml tube Dilution 3.
 - b Add the following volumes to the tube ***in the order listed.***
 - ▶ Dilution 2 (200 μl)
 - ▶ ELB HT (3.8 ml)
 These volumes produce 5 copies per μl .
 - c Pulse vortex to mix.

Procedure

- 1 For each sample, add 140 μ l 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)*.

Use the following protocol options:

- ▶ Purify viral RNA using the spin protocol.
- ▶ Incubate elution for at least 1 minute.
- ▶ Elute in 30 μ l Buffer AVE instead of 60 μ l.

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:

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

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

Procedure

- 1 Label new PCR plate CDNA1.
- 2 Add 8.5 μ l EPH3 HT to each well.
- 3 Add 8.5 μ l 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.

After first strand synthesis is complete, the sample is inactive.

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:

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

- 2 Save the following COVIDSeq FSS program on the thermal cycler:
 - ▶ Choose the preheat lid option
 - ▶ Set the reaction volume to 25 μ l
 - ▶ 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 μ l)
 - ▶ RVT HT (1 μ l)
 Reagent overage is included to account for small pipetting errors.
- 2 Add 8 μ l 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

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 runs.

Reagent	COVIDSeq PCR 1 Master Mix (µl)	COVIDSeq PCR 2 Master Mix (µl)
IPM HT	15	15
CPP1 HT	4.3	N/A
CPP2 HT	N/A	4.3
Nuclease-free water	4.7	4.7

Reagent overage is included to account for small pipetting errors.

- Add 20 µl COVIDSeq PCR 1 Master Mix to each well of the COV1 plate corresponding to each well of the CDNA1 plate.
- Add 5 µl first strand cDNA synthesis from each well of the CDNA1 plate to the corresponding well of the COV1 plate.
- Add 20 µl COVIDSeq PCR 2 Master Mix to each well of the COV2 plate corresponding to each well of the CDNA1 plate.
- Add 5 µl first strand cDNA synthesis from each well of the CDNA1 plate to the corresponding well of the COV2 plate.
- Seal and shake at 1600 rpm for 1 minute.
- Centrifuge at 1000 x g for 1 minute.
- 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 x g for 1 minute, and then pipette to resuspend.

Preparation

- Prepare the following consumables:

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

- 2 If COV1 and COV2 plates were stored frozen, prepare as follows.
 - a Thaw at room temperature.
 - b Check seals, and then shake at 1600 rpm for 1 minute.
 - c Centrifuge at 1000 x g for 1 minute.
- 3 Save the following COVIDSeq TAG program on the thermal cycler:
 - ▶ Choose the preheat lid option
 - ▶ Set the reaction volume to 50 μ l
 - ▶ 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 μ l from each well of the COV1 plate to the corresponding well of the TAG1 plate.
 - b Transfer 10 μ l 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 μ l)
 - ▶ EBLTS HT (4 μ l)
 - ▶ Nuclease-free water (20 μ l)
- 4 Add 30 μ l 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:

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

Procedure

- 1 Centrifuge the TAG1 plate at 500 x g for 1 minute.
- 2 Add 10 μ l 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 × 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 × 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 × 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 Nextera UD Indexes Set A, B, C, D)
- ▶ 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:

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

- 2 Open each prepared index adapter plate seal as follows. Use a new PCR plate for each different index set.
 - a Align a new 96-well PCR plate above the index adapter plate, and then press down to puncture the foil seal.
 - b Discard the PCR plate.
- 3 Save the following COVIDSeq TAG PCR program on the thermal cycler:
 - ▶ Choose the preheat lid option and set to 100°C
 - ▶ Set the reaction volume to 50 µl

- ▶ 72°C for 3 minutes
- ▶ 98°C for 3 minutes
- ▶ 7 cycles of:
 - ▶ 98°C for 20 seconds
 - ▶ 60°C for 30 seconds
 - ▶ 72°C for 1 minute
- ▶ 72°C for 3 minutes
- ▶ Hold at 10°C

Procedure

- 1 In a 15 ml tube, combine the following volumes to prepare PCR Master Mix. Multiply each volume by the number of samples.
 - ▶ EPM 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.
- 11 Place on the preprogrammed thermal cycler and run the COVIDSeq TAG PCR program.

Pool and Clean Up Libraries

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

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:

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

- 2 Prepare 80% EtOH from absolute EtOH.

Procedure

- 1 Centrifuge 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 or new PCR plate. 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 or PCR plate 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.

Quantify and Normalize Libraries

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

$$\frac{\text{Library concentration } ng/\mu l}{660 \frac{g}{mol} \times \text{average library size } (bp)} = \text{Molarity } (nM)$$

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

Pool and Dilute Libraries

- 1 For each set of 384 samples, combine 25 µl of each normalized pool containing index adapter set A, B, C, D in a new microcentrifuge tube. Do not combine pools with the same index adapter set.
This step produces a final pool of 384 samples for one flow cell lane.
- 2 For each flow cell lane, dilute each 384 sample pool with RSB HT as follows.
These steps produce 30 µl 0.5 nM pooled library.
 - a Combine 3.75 µl library from the 384-sample pool with 26.25 µl RSB Ht.
 - b Vortex, and then centrifuge briefly.

The resulting final loading concentration is 100 pM.

Prepare for Sequencing

This section includes information for preparing a sequencing run using the NovaSeq 6000 Sequencing System. For more information on sequencing, troubleshooting, and maintenance, see the *NovaSeq 6000 Sequencing System Guide (document # 1000000019358)*.

Prepare

- 1 Prepare the following NovaSeq 6000 system Xp consumables.

Reagent	Storage	Instructions
NovaSeq 6000 system Xp S4 Flow Cell	2°C to 8°C	Set aside flow cell package for 10–15 minutes to allow the flow cell to reach room temperature.
NovaSeq 6000 system Xp S4 SBS cartridge	-25°C to -15°C	Thaw a room temperature water bath for 4 hours. Use the following thaw instructions.
NovaSeq 6000 system Xp S4 cluster cartridge	-25°C to -15°C	Thaw in a room temperature water bath for up to 4 hours. Use the following thaw instructions.
ExAmp Reagents	-25°C to -15°C	Remove one tube each of DPX1, DPX2, and DPX3 from storage. Thaw at room temperature for 10 minutes. Set aside on ice.
NovaSeq 6000 system Xp Manifold	Room temperature	Set aside until use.

- 2 Thaw SBS and cluster cartridges as follows.
 - a Remove the SBS and cluster cartridges from -25°C to -15°C storage.
 - b Place each cartridge into a wire thaw rack.
The racks are provided with the instrument and prevent capsizing in the water bath.
 - c Thaw in a room temperature water bath (19°C to 25°C) for 2.5–3.5 hours.
 - d Thoroughly dry the cartridge bases using paper towels. Dry between the wells so that all water is removed.
 - e Inspect the foil seals for water. If present, blot dry with a lint-free tissue.
 - f Inspect the underside of each cartridge to make sure that the reservoirs are free of ice, which indicates that the reagents are thawed.
 - g Invert each cartridge 10 times to mix reagents.
 - h Gently tap the bottom of each cartridge on the bench to reduce air bubbles.
 - i If reagents cannot be loaded onto the instrument within 4 hours, store at 2°C to 8°C.
- 3 Prepare a fresh dilution of 0.2 N NaOH.
- 4 Prepare 400 mM Tris-HCl, pH 8.0.

Create a Sample Sheet.

The Illumina DRAGEN COVIDSeq Test Pipeline requires a sample sheet for each run analysis. Use the `samplesheet.csv` file included in the installer package or available on the Illumina COVIDSeq Test support site as a template to create the sample sheet.

Create a sample sheet as follows.

- 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.

```
CTGTCTCTTATACACATCT
```

- 3 In the **Data** section, enter the following required parameters.
Make sure that there no empty rows between samples.

Field	Description	Requirements
Sample_ID	The ID used to identify the samples in the test reports and included in the output file names.	Sample IDs are not case-sensitive. Make sure Sample IDs contain the following: <ul style="list-style-type: none"> • 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.
Index_ID	The IDT for Illumina Nextera UD Indexes index name associated with the sample.	See <i>Illumina Adapter Sequences (document # 1000000002694)</i> 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.
Index	IDT for Illumina Nextera UD Indexes i7 index sample sheet bases	See <i>Illumina Adapter Sequences (document # 1000000002694)</i> for sample sheet bases and additional information.
Index2	IDT for Illumina Nextera UD Indexes i5 index sample sheet bases.	See <i>Illumina Adapter Sequences (document # 1000000002694)</i> for sample sheet bases and additional information.
Lane	The flow cell lane for the sample.	Enter one of the following values: 1, 2, 3, or 4.
Sample_Type	The sample type for each sample.	Enter one of the following values: <code>PatientSample</code> , <code>NTC</code> , <code>PositiveControl</code> . For each Index Set/Lane combination in the sample sheet, there must be one NTC sample and one <code>PositiveControl</code> sample.

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

Empty Used Reagent Bottles

Use the following instructions to empty the used reagent bottles with **every** sequencing run. If your system is configured to route used reagents externally, the small bottle collects used reagents and must be emptied for each sequencing run. The large bottle must be in place.

**WARNING**

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, see the SDS at support.illumina.com/sds.html.

- 1 Remove and empty the small used reagent bottle as follows.
 - a Raise the lever and remove the small used reagent bottle from the alcove. Grasp the bottle by the sides.
 - b Remove the threaded cap from the cap holder on the front of the bottle.
 - c Seal the bottle opening with the cap to prevent spills.
 - d Keeping the contents separate from the contents of the other bottle, discard in accordance with applicable standards for your region.
 - e Return the uncapped bottle to the alcove, and then lower the lever. Store the cap on the cap holder.
- 2 Remove and empty the large used reagent bottle as follows.
 - a Using the top handle, remove the large used reagent bottle from the left side of the buffer drawer.
 - b Remove the threaded cap from the cap holder on the front of the bottle.
 - c Seal the bottle opening with the threaded cap to prevent spills.
 - d Discard the contents in accordance with applicable standards for your region. Grip both handles when emptying.
 - e Return the uncapped bottle to the buffer drawer. Store the cap on the cap holder.
- 3 Put on a new pair of powder-free gloves to avoid contaminating the surface of the instrument.
- 4 Close the buffer drawer, and then close the liquid compartment doors.

**WARNING**

Failure to empty the used reagent bottles can result in a terminated run and overflow, which damages the instrument and poses a safety risk.

Denature and Neutralize Library Pool

- 1 Add 7 μ l 0.2 N NaOH to each tube of the 30 μ l nondenatured library pool.
- 2 Cap and then vortex briefly.
- 3 Centrifuge at a maximum of 280 \times g for up to 1 minute.
- 4 Incubate at room temperature for 8 minutes to denature.
- 5 Add 8 μ l 400 mM Tris-HCl, pH 8.0 to neutralize.
- 6 Cap and then vortex briefly.
- 7 Centrifuge at a maximum for 280 \times g for up to 1 minute.
- 8 Keep denatured libraries on ice until ready to add the ExAmp master mix.

Prepare the Flow Cell and Dock

- 1 Place the NovaSeq 6000 system Xp flow cell dock on a flat surface. Keep the flow cell level until loaded onto the instrument.
- 2 Inspect the dock and make sure that it is free from particulate.
- 3 Put on a new pair of powder-free gloves to avoid contaminating the glass surface of the flow cell.
- 4 With the flow cell foil package over a flat surface, peel open the foil from the corner tab.
- 5 Remove the clear plastic retainer covering the flow cell.
- 6 Remove the flow cell from the packaging. Grasp the flow cell by the sides to avoid touching the glass or the underside gaskets.

- 7 If particulate is visible on either of the glass surfaces, clean the applicable surface with a lint-free alcohol wipe and dry with a low-lint lab tissue.
- 8 Discard the package appropriately.

**NOTE**

Some scratches and other minor cosmetic defects on the flow cell are normal and do not affect data quality.

- 9 Invert the flow cell so that the top surface faces **downward**.
- 10 Slip the outlet end of the flow cell under the bracket and place it onto the dock.
- 11 With the wells facing up, load the NovaSeq 6000 system Xp manifold over the inlet end of the flow cell. Make sure that the NovaSeq 6000 system Xp manifold arms fit securely into the dock cutouts.
- 12 Close the clamp to secure the flow cell and NovaSeq 6000 system Xp manifold and seal the gaskets.

**NOTE**

The NovaSeq 6000 system Xp manifold is single use only and discarded after loading the flow cell onto the instrument.

Prepare the ExAmp Master Mix

- 1 Briefly vortex and then centrifuge DPX1, DPX2, and DPX3 tubes.
- 2 Combine the following volumes in a 1.7 ml tube in the order specified.

Addition Order	Reagent	Volume per Lane (µl)
1	DPX1	315
2	DPX2	45
3	DPX3	165

- 3 Vortex 20–30 seconds to mix, and then centrifuge briefly.
- 4 For best sequencing performance, immediately proceed to the next step. If necessary, ideal storage of the master mix is up to 1 hour on ice. Use within 30 minutes if storing at room temperature.

Load Libraries Onto the Flow Cell

For best results, do the following:

- ▶ Maintain the loaded flow cell at room temperature. Do not refrigerate or place on ice.
 - ▶ Prolonged incubation of libraries on the flow cell might reduce the percentage of clusters passing filter (%PF).
 - ▶ Start the run within 30 minutes after loading library pools on the flow cell.
 - ▶ Immediate use of the ExAmp/library mix yields best results.
- 1 Add 105 µl ExAmp Master Mix to each denatured library pool, and then vortex for 20–30 seconds to mix.
 - 2 Centrifuge at up to 280 × g for up to 1 minute.
 - 3 For each library, use a 200 µl pipette to load 130 µl ExAmp/library mixture to a new flow cell lane. Avoid contact with the center of the manifold well.
 - 4 After adding the ExAmp/library mixture to all manifold wells, wait approximately 2 minutes for the mixture to reach the opposite end of each lane.
A small air bubble at the outlet end of the lane is normal. A small volume of the mixture may remain in the manifold wells after the lane is filled.
 - 5 **Start the sequencing run within 30 minutes of loading libraries onto the flow cell.**

**CAUTION**

Do not tilt the flow cell when trying to determine if lanes are filled or bubbles are present. Tilting can cause the ExAmp/library mixture to leak from the flow cell. If a lane does not fill completely, do not attempt to correct it. Data yield from the partially filled lane might be reduced. Do not attempt to retrieve the sample from the flow cell.

**NOTE**

Do not tilt the flow cell when transporting it.

Set Up a Sequencing Run

- 1 Remove any items from the surface of the instrument.
Keep the surface clear during the sequencing run and avoid leaning on the instrument. Pressure to the flow cell door can cause it to open, which stops the run. Stopped runs cannot be resumed.
- 2 From the NovaSeq Control Software (NVCS) Main Menu, select **Sequence**.
- 3 If processing 3072 samples on two flow cells, select **A + B**.
- 4 Select **OK** to acknowledge the warning and open the flow cell door.

Load the Flow Cell Onto the Instrument

- 1 If particulate is visible on the flow cell stage, clean the entire stage, including the fluidic interface and the glass surface of the optical alignment target, with an alcohol wipe. Dry with a lint-free tissue.
- 2 Unload the flow cell from the dock as follows.
 - a Open the clamp that secures the flow cell and manifold.
 - b Without allowing liquid to drip onto the flow cell, carefully remove and discard the manifold.
 - c If liquid drips onto the flow cell, clean with a lint-free alcohol wipes and dry with a lint-free lab tissue.
 - d Grasp the sides of the flow cell to remove it from the dock. Keep the flow cell level.
 - e If there is residual material on the gaskets, blot the four flow cell gaskets with a lint-free tissue to dry. Do not touch the gaskets.
 - f Invert the flow cell around the long axis so that the top surface faces up.
- 3 Align the flow cell over the four raised clamps and place it on the flow cell stage.
- 4 Select **Close Flow Cell Door**.
The flow cell door closes, the sensors and RFID are checked, and the flow cell ID appears on the screen.
- 5 Before returning the dock to storage, inspect it to make sure that it is free from particulate.

Load the SBS and Cluster Cartridges

- 1 Open the liquid compartment doors, and then open the reagent chiller door.
- 2 Remove the used SBS and cluster cartridges.
The used cartridges have pierced foil seals.
- 3 Dispose of unused contents in accordance with applicable standards.
- 4 Place an uncapped, empty template tube in position #8 of each cluster cartridge.
- 5 Load the prepared cartridges into the reagent chiller drawer so that the **Insert** labels face the back of the instrument:
 - ▶ Place the SBS cartridge (gray label) into the left position.
 - ▶ Place the cluster cartridge (orange label) containing the uncapped library tube into the right position.
- 6 Slide the drawer into the chiller, and then close the reagent chiller door.
The sensors and RFIDs are checked. The IDs for the library tube and the two cartridges appear on the screen.

Load the Buffer Cartridge

- 1 Pull the metal handle to open the buffer drawer.
- 2 Remove the used buffer cartridge from the right side of the buffer drawer.
The used buffer cartridge has pierced foil seals.
- 3 Place a new buffer cartridge into the buffer drawer so that the **Illumina** label faces the front of the drawer. Align the cartridge with the raised guides on the drawer floor and sides.
When properly loaded, the buffer cartridge is evenly seated and the drawer can close.
- 4 If both used reagents bottles have been emptied, select the checkbox acknowledging that both used reagent bottles are empty.

**WARNING**

Failure to empty the used reagent bottles can result in a terminated run and overflow, which damages the instrument and poses a safety risk.

- 5 Select the available button:
 - **Log In**—Opens the Log In screen to sign in to BaseSpace Sequence Hub.
 - **Run Setup**—Skips BaseSpace Sequence Hub and opens the Run Setup screen to enter run parameters. Which button is available depends on whether the system is configured for BaseSpace Sequence Hub.

Enter Run Parameters

- 1 Select the NovaSeq 6000 system Xp workflow.
- 2 In the Run Name field, enter a name of your preference to identify the current run. The run name can contain alphanumeric characters, hyphens, and underscores.
- 3 Enter the number of cycles for each read and index length in the sequencing run.
 - ▶ **Read 1**—Enter 36 cycles.
 - ▶ **Index 1**—Enter 10 cycles for the Index 1 (i7) primer.
 - ▶ **Index 2**—Enter 10 cycles for the Index 2 (i5) primer.
 - ▶ **Read 2**—Enter 0. Runs are single read.
- 4 Expand **Advanced Options** to apply settings for the current run. These settings are optional unless otherwise indicated.
 - ▶ **Output Folder**—Select **Browse** to change the output folder for the current run. An output folder is required when the run is not connected to BaseSpace Sequence Hub for storage.
 - ▶ **Samplesheet**—Select **Browse** to upload a sample sheet, which is required when using Illumina DRAGEN COVIDSeq Test Pipeline. The CSV file is copied to the output folder and does not affect run parameters. For more information on sample sheets, see [Create a Sample Sheet. on page 20](#).
- 5 Select **Review**.
The software confirms that the specified parameters are appropriate for the recipe.

Confirm Run Parameters

- 1 Confirm the run parameters displayed on the Review screen.
- 2 **[Optional]** Select **Back** to return to the Run Setup screen and edit run parameters.
- 3 Select **Start Run**.
The pre-run checks are started automatically.

Review Pre-Run Checks

- 1 Wait about 5 minutes for the pre-run check to complete.
The run starts automatically after successful completion.

**NOTE**

To avoid overfilling the hard drive, do not copy any data to C:\ after the run starts.

- 2 If pre-run checks fail due to a sensor error, such as flow cell not detected, you must exit and restart the workflow.
- 3 For other pre-run check failures, select **Retry** to restart the failed check or **Retry All** to restart all checks.
- 4 Select the **Error** icon to see error details.
- 5 If the alignment check fails, resolve the error as follows.
 - a Select **Reload**, and then select **OK** to confirm returning to the Load screen.
 - b Remove any items from the top of the instrument, and then select **OK**.
 - c Reload the flow cell, and then select **Run Setup**.
 - d Proceed through each screen to reread each RFID and return to the Pre-Run Checks screen.
 - e Redo the check.

Data Analysis

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 [Create a Sample Sheet](#) on page 20.

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.

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.

One NovaSeq 6000 system Xp sequencing run can produce up to 390 GB BCL data for 36 bp single-end read output.

The Illumina DRAGEN COVIDSeq Test Pipeline can produce an additional 650 GB FASTQ data from BCL conversion and 210 GB data from secondary analysis and reporting.

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.

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 72 hours.
- 5 Store the install script in the `/staging` directory.
- 6 To update the run script permissions, enter the following command:


```
chmod +x /staging/install_covidseq-EUA-1.1.0.run
```
- 7 To run the installation script, enter the following command:


```
/staging/install_covidseq-EUA-1.1.0.run
```

The script removes any previously installed DRAGEN software,

Running the System Check

Make sure that the system is functioning properly by running the `check_covidseq-1.1.0.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.

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 To run the Illumina DRAGEN COVIDSeq Test Pipeline, enter the following command-line argument:


```
covidseq.sh --runFolder <FULL_PATH_TO_RUN_FOLDER>
```
- 2 **[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.
 - ▶ **--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

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.**
The 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 aligns FASTQ files to the SARS-CoV-2 reference genome.**
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 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 30](#) 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 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 30](#) for more information.**
This step generates the `ConsensusFasta` subfolder.
- 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 lane, index set, and sample using the internal control, positive control, and NTC. Only the lanes and index set currently existing in the sample sheet are assessed.

Lane Quality Control

Quality control is performed for each flow cell lane based on whether quality metrics pass predefined thresholds.

If a flow cell lane has a %Q30 < 85% or a total yield < 70 Gb, then the lane fails QC. All index sets within the failed lane display a `N/A` QC status and all patient samples display `Invalid`.

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 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 `COVID-Seq_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 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:

Field	Description
Sample ID	The sample ID in the sample sheet.
Internal control	The status of the internal control in a patient sample. Possible values include <code>Pass</code> , <code>Fail</code> , or <code>N/A</code> .
Result	The result for the patient sample. Possible values include the following: <code>SARS-CoV-2 Detected</code> —The sample lane and index set passed quality control and the SARS-CoV-2 virus is detected in the sample. <code>SARS-CoV-2 Not Detected</code> —The sample lane and index set passed quality control, the internal control was detected in the sample, and the SARS-CoV-2 virus is not detected. <code>Invalid</code> —The sample lane 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.
Consensus Sequence	Indicates if the consensus SARS-CoV-2 sequence in FASTA format was generated for the sample.
Lane	The flow cell lane associated with the sample. Values can include 1, 2, 3, or 4.
Index Set	The index set/adaptor plate associated with the sample using the values from the <code>Index_ID</code> or <code>Index/Index 2</code> columns in the sample sheet. Values can be A, B, C, and D.
Index ID	The index ID associated with the sample. If the <code>Index_ID</code> column is specified in the sample sheet, the <code>Index ID</code> field displays the same value. If not specified, <code>Index ID</code> is derived from the <code>Index</code> and <code>Index2</code> columns from the sample sheet.

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

Field	Description
Sample ID	The sample ID specified in the sample sheet.
Control Type	The control sample type. Values can include <code>Positive</code> or <code>NTC</code> .
Human Control	Indicates if the internal (human) control is detected in a control sample. Values can include <code>Detected</code> or <code>Not Detected</code> .
SARS-CoV-2	Indicates if SARS-CoV-2 is detected in the control sample. Values can include <code>Detected</code> or <code>Not Detected</code> .
Lane	The flow cell lane associated with the same. Values can include 1, 2, 3, or 4.
Index Set	The index set/adaptor plate associated with the control sample using the values from the <code>Index_ID</code> or <code>Index/Index 2</code> columns in the sample sheet. Values can be A, B, C, and D.
Index ID	The index ID specified in the sample sheet. If the <code>Index_ID</code> column is specified in the sample sheet, the Index ID field displays the same value. If not specified, Index ID is derived from the <code>Index</code> and <code>Index2</code> columns from the sample sheet.

PDF Report

The `COVID-Seq_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.

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

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

Field	Description
Lane 1, Lane 2, Lane 3, Lane 4	The QC result for each lane. Values can include <code>PASS</code> or <code>FAIL</code> .
Index Set A, Index Set B, Index Set C, Index Set D	The QC result for each index set within the associated lane. Values can include <code>PASS</code> , <code>FAIL</code> , or <code>N/A</code> .

- ▶ **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
Sample ID	The sample ID in the sample sheet.
Internal control	The quality control result for the internal (human) control in a patient sample. Values include <code>Pass</code> , <code>Fail</code> , or <code>N/A</code> .

Field	Description
Result	<p>The result for the patient sample. Possible values include the following:</p> <p>SARS-CoV-2 Detected—The sample lane and index set passed quality control and the SARS-CoV-2 virus is detected in the sample.</p> <p>SARS-CoV-2 Not Detected—The sample lane and index set passed quality control, the internal (human) control was detected in the sample, and the SARS-CoV-2 virus is not detected.</p> <p>Invalid—The sample lane 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.</p>
Consensus Sequence	Indicates if the consensus SARS-CoV-2 sequence was generated for the sample.
Lane / Index Set	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 A, Index Set B, Index Set C, or Index Set D. If the lane or index set failed quality control, Fail is included at the end of the field value.

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 Logs_Intermediates/VariantCalling/<SAMPLE ID>/<SAMPLE ID>.hard-filtered.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 0.7.
- ▶ Depth is greater than 15.

Regions of sequence with coverage below 15 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 Logs_Intermediates/ConsensusFasta/<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-1.1.0.sh`.

The uninstall script removes the following assets:

- ▶ All scripts (`covidseq.sh`, `check_covidseq-1.1.0.sh`, `uninstall_covidseq-1.1.0.sh`).
- ▶ The Illumina DRAGEN COVIDSeq Test Pipeline Docker image.
- ▶ Test 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-1.1.0.sh
```

Interpretation of Results

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

*If the positive control fails for a given index set, all patient samples associated with that index set are reported as invalid.

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. All sequencing data for this section were generated on a NovaSeq 6000 Sequencing System.

Analytical Sensitivity

The analytical sensitivity (Limit of Detection (LOD)) of the Illumina COVIDSeq Test was determined by a serial dilution of a SARS-CoV-2 *in vitro* transcript with known concentration (Twist Synthetic SARS-CoV-2 RNA Control 1, part # 102019) into pooled negative clinical matrix (nasopharyngeal swab specimen) to 1000 copies per ml, 500 copies per ml, 250 copies per ml, and 125 copies per ml. For each serial dilution 22–24 replicates (extraction replicates) were prepared by two operators and evaluated using the Illumina COVIDSeq Test. The LOD was determined to be 1000 copies per ml.

SARS-CoV-2 (copies per ml)	Replicate Breakdown				Hit Rate Results		
	Extraction Reps	Library Prep Operators	Hits	Total Reps	Percent Positive	Lower CI	Upper CI
125	22	2	8	44	18.2%	9.5%	32.0%
250	24	2	13	48	27.1%	16.6%	41.0%

SARS-CoV-2 (copies per ml)	Replicate Breakdown			Hit Rate Results			
	Extraction Reps	Library Prep Operators	Hits	Total Reps	Percent Positive	Lower CI	Upper CI
500	24	2	31	48	64.6%	50.4%	76.6%
1000	24	2	47	48	97.9%	89.1%	99.6%

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.

Percent of Primer Pairs Homology	Mean	Median	5th percentile	95th percentile
100% homology	97.1%	98.0%	93.9%	100%
>80% homology	97.8%	98.0%	94.9%	100%

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.

Pathogen Name	NCBI Accession Number	Number of Primer Pairs With Homology Above 80%
Adenovirus (e.g. C1 Ad. 71)*	AC 000017 (1)	0
	AC 000007 (2)	
	AC_000008 (5)	
	AC 000018 (7)	
	AC 000019 (35)	
	NC_012959(54)	
Human Metapneumovirus (hMPV)	NC_039199	0
Parainfluenza virus 1	NC_003461	0
Parainfluenza virus 2	NC_003443	0
Parainfluenza virus 3	NC_001796	0
Parainfluenza virus 4	NC_021928	0
Influenza virus A	NC_026438	0
Influenza virus B	NC_002204	0
Respiratory syncytial virus	NC_001803	0
Enterovirus (e.g. EV68)	NC_038308	0

Pathogen Name	NCBI Accession Number	Number of Primer Pairs With Homology Above 80%
Rhinovirus*	NC_038311 (1) NC_038312 (B3) NC_001490 (B14) NC_009996 (C)	0
Human coronavirus 229E	NC_002645	0
Human coronavirus OC43	NC_006213	0
Human coronavirus HKU1	NC_006577	0
Human coronavirus NL63	NC_005831	0
SARS-coronavirus	NC_004718	8
MERS coronavirus	NC_019843	0
Chlamydia pneumoniae	NC_005043	0
Haemophilus influenzae	NZ_LN831035	0
Legionella pneumophila	NZ_LR134380	0
Mycobacterium tuberculosis	NC_000962	0
Streptococcus pneumoniae	NZ_LN831051	0
Streptococcus pyogenes	NZ_CP007593	0
Bordetella pertussis	NC_018518	0
Mycoplasma pneumoniae	NZ_CP010546	0
Pneumocystis jirovecii (PJP)	NJFV01000001 - NJFV01000219	0
Candida albicans	NC_032089 - NC_032096	0
Pseudomonas aeruginosa	NC_002516	0
Staphylococcus epidermis	NZ_CP035288 - NZ_CP035290	0
Streptococcus salivarius	NZ_LR134274	0

* 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 Test with the respiratory pathogens tested.

RefSeq Record Name (Accession Number)	Number of ARTIC Amplicons Detected	COVID-19 Detected
Streptococcus pneumoniae (NZ_LN831051)	0	NO
Human respirovirus 3 (NC_001796)	0	NO
Human adenovirus 1 (AC_000017)	0	NO
Haemophilus influenzae (NZ_LN831035)	0	NO
Streptococcus salivarius (NZ_LR134274)	0	NO
Human adenovirus 5 (AC_000008)	0	NO
Human adenovirus 54 (NC_012959)	0	NO
Chlamydia pneumoniae TW-183 (NC_005043)	0	NO

RefSeq Record Name (Accession Number)	Number of ARTIC Amplicons Detected	COVID-19 Detected
Staphylococcus epidermidis (NZ_CP035288 - NZ_CP035290)	0	NO
Human adenovirus 2 (AC 000007)	0	NO
Rhinovirus C (NC_009996)	0	NO
Influenza B virus (B/Lee/1940) (NC_002204)	0	NO
Human respirovirus 1 (NC_003461)	0	NO
Mycobacterium tuberculosis H37Rv (NC_000962)	0	NO
Human rhinovirus A1 (NC_038311)	0	NO
Rhinovirus B14 (NC_001490)	0	NO
Bordetella pertussis 18323 (NC_018518)	0	NO
Candida albicans SC5314 (NC_032089 - NC_032096)	0	NO
Human coronavirus 229E (NC_002645)	0	NO
Influenza A virus (A/California/07/2009(H1N1)) (NC_026438)	0	NO
Human coronavirus OC43 (NC_006213)	0	NO
Pneumocystis jirovecii (NJFV01000001 - NJFV01000219)	0	NO
Streptococcus pyogenes (NZ_CP007593)	0	NO
Human rhinovirus B3 (NC_038312)	0	NO
Middle East respiratory syndrome-related coronavirus (NC_019843)	0	NO
Human parainfluenza virus 4a (NC_021928)	0	NO
Human metapneumovirus (NC_039199)	0	NO
Enterovirus D68 (NC_038308)	0	NO
Pseudomonas aeruginosa PAO1 (NC_002516)	0	NO
Mycoplasma pneumoniae (NZ_CP010546)	0	NO
Respiratory syncytial virus (NC_001803)	0	NO
Human coronavirus NL63 (NC_005831)	0	NO
Human adenovirus 35 (AC 000019)	0	NO
Human rubulavirus 2 (NC_003443)	0	NO
Severe acute respiratory syndrome-related coronavirus (NC_004718)	0	NO
Human adenovirus 7 (AC 000018)	0	NO
Legionella pneumophila (NZ_LR134380)	0	NO
Human coronavirus HKU1 (NC_006577)	0	NO

Clinical Evaluation

Clinical performance of the COVIDSeq Assay was evaluated in comparison with an RT-PCR assay authorized by the FDA for use under Emergency User Authorization (EUA RT-PCR). 94 clinical nasopharyngeal swab (NP) specimens were tested, including 54 SARS-CoV-2 positive and 40 SARS-CoV-2 negative specimens. The positive and negative percent agreement was calculated in relation to the EUA RT-PCR comparator method and indicated in the following table.

Illumina COVIDSeq Test Results (NP Swabs)				
EUA RT-PCR Comparator	Positive	Negative	Invalid	% agreement (95% CI)
Positive	53	1 ¹	0	98.1% (90.2-99.7%)
Negative	1 ¹	38	1 ²	97.4% (86.8-99.5%)

¹ Root cause analysis revealed labeling error.

² One invalid specimen was excluded from analysis.

References

- 1 Coronavirus. World Health Organization. <https://www.who.int/health-topics/coronavirus/coronavirus>. Accessed May 25, 2020.
- 2 Testing for COVID-19. Centers for Disease Control and Prevention. <https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/testing.html>. Published May 1, 2020. Accessed May 25, 2020

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