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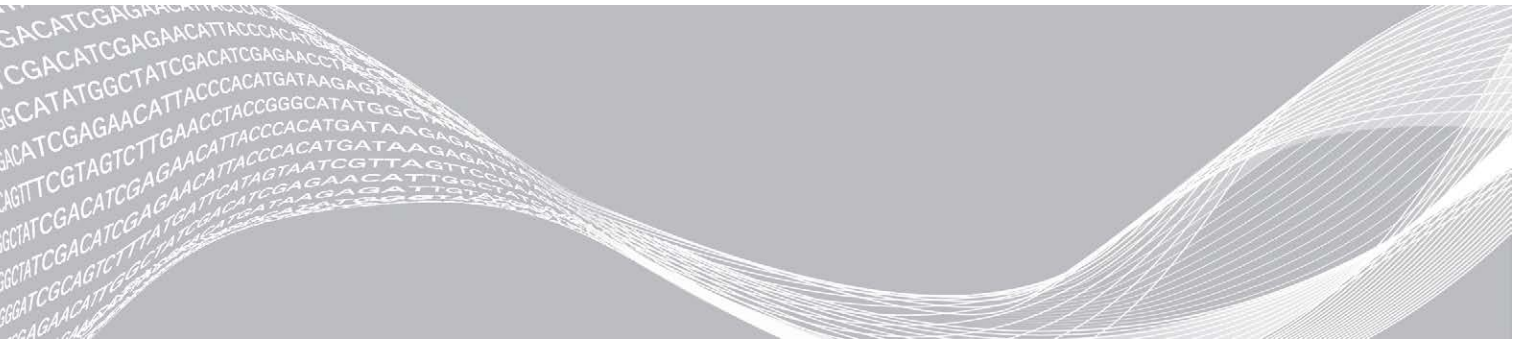
Illumina DRAGEN COVIDSeq Test (EUA) v1.2

App Guide

FOR IN VITRO DIAGNOSTIC USE



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Intended Use

The DRAGEN COVIDSeq Test (EUA) is intended to analyze sequencing reads from the Illumina COVIDSeq Test generated on the NextSeq 550Dx Instrument. The Illumina® COVIDSeq™ Test is a Next-Generation Sequencing (NGS) *in vitro* diagnostic test on the Illumina 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, and nasal aspirates from individuals suspected of COVID-19 by their healthcare provider.

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.

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 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 Interim Order respecting the importation and sale of medical devices for use in relation to COVID-19.

Warnings and Limitations

- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The performance of this device has not been assessed in a population vaccinated against COVID-19.

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 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 can generate information about the genomic sequence of the virus present in a sample for surveillance or other informational purposes only and should not be used 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.

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 **1.2.0**.
   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 to store app results to.
You can also create a new project by selecting **New** and then entering a name and description.

4 From the COVID-Seq Run to Analyze field, select **Select Run(s)** to select a completed COVID-Seq 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 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 subfolder under the analysis folder.

1 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.

2 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 (≥ virusThreshold) and the internal control (≥ humanThreshold). The result is preliminary and undergoes quality control in later steps.
The step generates the `VirusDetection` subfolder.

3 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.

4 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 Informational Use* on page 8 for more information.
This step generates the `VariantCalling` subfolder.

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 generates a consensus genome in FASTA format using variant calls and coverage metrics as input. See *Variant Calling and Consensus Sequence Generation for Informational Use* on page 8 for more information.
This step generates the `ConsensusFasta` subfolder.

6 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.

7 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.

Flow Cell Quality Control

For the NextSeq 550Dx, quality is performed on each flow cell.

The flow cell must have a %Q30 ≥ 85% or a total yield ≥ 12 Gb 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`.

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.

Property	Definition
Name	Name of the analysis session.
Application	Application that generated the analysis.
Date Started	Date and time the analysis session started.
Date Completed	Date and time the analysis session completed.
Duration	Duration of the analysis.
Compute Charge	Cost in iCredits to compute the analysis.
Session Type	Number of nodes used, which can be multinode or single node.

Property	Definition
Node Count	Total number of nodes.
Status	Status of the analysis session: • Queued for Analysis • Running • Complete • Aborted
Delivery	Delivery status of the analysis results.

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.

- Results
  - COVID-Seq_RunReport.pdf — Contains only results for patient samples. See *PDF Report* on page 6.
  - COVID-Seq_RunReport.tsv — Contains test results for both patient and control samples. See *TSV Run Report* on page 7
  - Errors.tsv
- Logs_Intermediates_Lane_all
  - ConsensusFasta
  - FastqGeneration
    - Reports
      - Demultiplex_Stats.csv
      - Top_Unknown_Barcodes.csv
  - FindSampleValidity
  - JsonTsvReport
  - MapAlign
  - RunQc
  - SampleSheetValidation
  - VariantCalling
  - VirusDetection
- Sample_Analysis
  - <SampleIdN>
    - <SampleIdN>.fasta

- <SampleIdN>.bam
- <SampleIdN>.bam.bai
- <SampleIdN>.bam.md5sum
- <SampleIdN>.hard-filtered.vcf.gz
- <SampleIdN>.hard-filtered.vcf.gz.md5sum
- <SampleIdN>.hard-filtered.vcf.gz.gz.tbi

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.For the NextSeq 550Dx, the value is `Lane 1,2,3,4`.
Index Set 1, Index Set 2, Index Set 3, Index Set 4	The QC result for each index set within the associated lane. Values can include `PASS`, `FAIL`, or `N/A`.

- 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 `Pass`, `Fail`, or `N/A`.
Result	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.
Consensus Sequence	Indicates if the consensus SARS-CoV-2 sequence was generated for the sample.

Field	Description
Lane / Index Set	The lane and index set associated with the sample. For the 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.

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 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:

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 `Pass`, `Fail`, or `N/A`.
Result	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.
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. For the NextSeq 550Dx, the value is `1,2,3,4`.
Index Set	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`.
Index ID	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.

- 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 `Positive` or `NTC`.
Human Control	Indicates if the internal (human) control is detected in a control sample. Values can include `Detected` or `Not Detected`.
SARS-CoV-2	Indicates if SARS-CoV-2 is detected in the control sample. Values can include `Detected` or `Not Detected`.

Field	Description
Lane	The flow cell lane associated with the same. For the NextSeq550Dx, the value is `1,2,3,4`.
Index Set	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`.
Index ID	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.

Variant Calling and Consensus Sequence Generation for Informational Use

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/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 Sample_Analysis/Logs_Intermediates/ConsensusFasta/<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.

```
bs project download --id <project ID> -o <output directory>
```

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Website: www.illumina.com
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Illumina Customer Support Telephone Numbers

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

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

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