

Illumina COVIDSeq™ Test (Research Use Only)

An integrated next-generation sequencing (NGS) solution for detection of SARS-CoV-2 RNA.

Introduction

SARS-CoV-2 belongs to a large family of coronaviruses that can 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 causes COVID-19 disease, which is associated in the majority of infections with mild respiratory symptoms. However, for patients with underlying medical conditions or advanced age, COVID-19 may lead to severe illness.¹ SARS-CoV-2 transmission between humans primarily is via respiratory route, including droplets of saliva or discharge from infected patients. COVID-19 is a public health emergency of international concern, highlighting the need for rapid, accurate viral detection.¹ To address this need, Illumina offers the Illumina COVIDSeq Test, an integrated NGS research solution for detection and characterization of SARS-CoV-2.

Illumina COVIDSeq Test

The Illumina COVIDSeq Test is an NGS product to be used on the NovaSeq™ 6000 Sequencing System and intended for the detection and characterization of SARS-CoV-2 RNA from nasopharyngeal (NP), oropharyngeal (OP), nasal swab, and mid-turbinate specimens for research applications, not for use in diagnostic procedures or patient management.

Illumina COVIDSeq workflow

The Illumina COVIDSeq Test provides a streamlined, integrated workflow from RNA extraction and cDNA synthesis, through library preparation, sequencing, and data analysis.

RNA extraction—RNA is extracted from decontaminated nasopharyngeal swabs using the QIAamp Viral RNA Mini Kit and following the recommended protocol.

cDNA synthesis—DNA complementary to the RNA (cDNA) is generated by reverse transcriptase with random hexamers.

Target amplification—SARS-CoV-2 viral genome present in the sample is amplified using two separate PCR reactions that are then pooled together. In addition to SARS-CoV-2 targets, each COVIDSeq Primer Pool also contains 11 primer pairs designed to amplify human cDNA targets. These amplicons serve as an internal control (IC), ensuring that even SARS-CoV-2 negative samples generate a library for sequencing. The internal control also serves as a quality control during data analysis.

Ordering information

Product	Catalog no.	No. of kits for 3072 samples
Illumina COVIDSeq Test (3072 samples)	20043675	1
IDT for Illumina Nextera DNA Unique Dual Indexes Sets A–D (384 Indexes, 384 samples)	20027217	8
NovaSeq Xp 4-lane Kit	20021665	2
NovaSeq 6000 S4 Reagent Kit (200 cycles)	20027466	2
QIAamp Viral RNA Mini Kit	20043667	13

Illumina • 1.800.809.4566 toll-free (US) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com

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Library preparation—The pooled, amplified fragments undergo On-Bead Tagmentation to further fragment and tag amplicons with adapter sequences. Post-tagmentation yield is normalized due to saturation of the bead-linked transposomes 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 and concentration is 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 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.

Data analysis—The Illumina DRAGEN™ COVIDSeq Test Pipeline analyzes sequencing results to detect the presence of SARS-CoV-2 virus in each sample. 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. The Illumina DRAGEN COVIDSeq Test Pipeline is available at no additional charge as part of the DRAGEN v3 Server license. Customers can contact their local Field Applications Scientist to request a link to download the pipeline after purchasing the Illumina COVIDSeq Test.

References

1. Coronavirus. World Health Organization. www.who.int/health-topics/coronavirus/coronavirus. Accessed May 25, 2020.