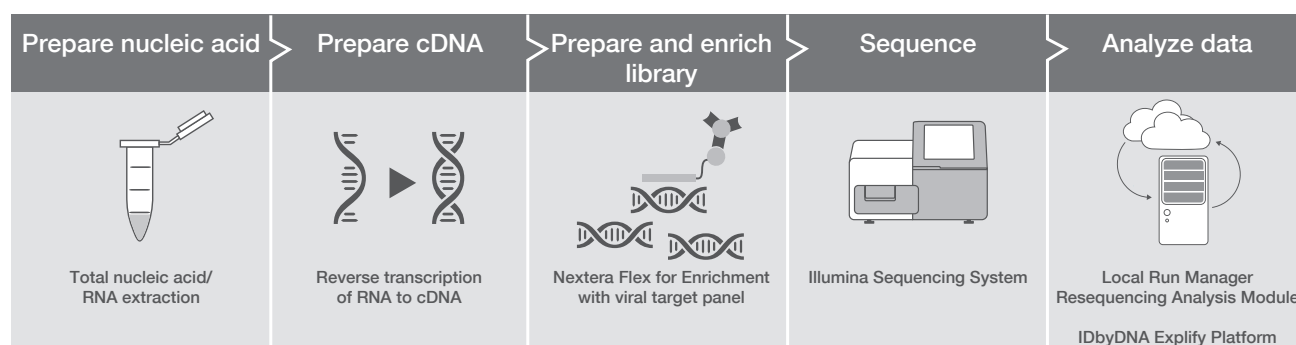


# Detecting coronavirus with the Respiratory Virus Oligo Panel

Tips and resources for detecting coronavirus and other respiratory viruses using NGS and a targeted enrichment workflow.

## Introduction

Next-generation sequencing (NGS) with library enrichment has proven to be a highly sensitive method for detecting and characterizing common respiratory viruses, including coronavirus strains (Figure 1).<sup>1</sup> This technical note describes general workflow recommendations and product options evaluated by Illumina for detecting coronavirus using the targeted enrichment workflow with Nextera™ Flex for Enrichment, the Respiratory Virus Oligo Panel, and Illumina NGS systems.



**Figure 1: Enrichment workflow for coronavirus detection**—The streamlined NGS workflow for coronavirus detection integrates sample preparation, library preparation, target enrichment, sequencing, and data analysis.

## Prepare nucleic acid

### Extract RNA

Illumina does not provide nucleic acid extraction kits. Several commercially available kits were evaluated internally for use with this workflow. Table 1 lists products tested internally; however, customers can choose any extraction method that they are comfortable using as long as there is sufficient yield for use in the chosen double-stranded cDNA conversion method.

**Table 1: Extraction kits tested internally at Illumina**

Product name	Vendor	Catalog no.
For targeting RNA viruses		
QIAmp Viral RNA Mini Kit (50 samples)	QIAGEN	52904
QIAmp Viral RNA Mini Kit (250 samples)	QIAGEN	52906
For targeting DNA and RNA viruses		
AllPrep PowerViral DNA/RNA Kit (50 samples)	QIAGEN	28000-50

Listed products reflect what is used at Illumina. Customers can use any extraction method of their choosing.

## Convert RNA to cDNA

Extracted RNA is then converted to double-stranded cDNA. Table 2 lists cDNA conversion kits evaluated by Illumina and other commercially available kits. Note that RNA degradation is not required prior to library preparation when using Nextera Flex for Enrichment library prep. If the protocol includes a thermal degradation step, incubate for 1 second instead of the recommended amount of time. For best results, use freshly extracted nucleic acid samples to perform reverse transcription.

**Table 2: Commercially available double-stranded cDNA preparation kits**

Product name	Vendor	Catalog no.
<b>Kits tested by Illumina</b>		
Maxima H Minus Double-Stranded cDNA Synthesis Kit (10 reactions)	ThermoFisher Scientific	K2561
<b>Additional kit options not tested by Illumina</b>		
NEBNext Ultra II First Strand Synthesis Module (24 samples)	New England BioLabs	E7771S
NEBNext Ultra II First Strand Synthesis Module (96 samples)	New England BioLabs	E7771L
NEBNext Ultra II Non-Directional RNA Second Strand Synthesis Module (20 reactions)	New England BioLabs	E6111S
NEBNext Ultra II Non-Directional RNA Second Strand Synthesis Module (100 reactions)	New England BioLabs	E6111L
SuperScript Double-Stranded cDNA Synthesis Kit (10 reactions)	ThermoFisher Scientific	11917010
SuperScript Double-Stranded cDNA Synthesis Kit (50 reactions)	ThermoFisher Scientific	11917020

Listed products reflect Illumina recommendations. Customers can use any double-stranded cDNA conversion method of their choosing.

## Prepare library

cDNA is used to prepare libraries for sequencing. The following guidelines are recommended when using Nextera Flex for Enrichment library prep with the Respiratory Virus Oligo Panel\* and the standard protocol in the [Nextera Flex for Enrichment reference guide](#) (Table 3):

- Use 50-100 ng input cDNA, with a minimum of 10 ng cDNA required
- Use 30 µl input volume; if cDNA yield is low, elute in a smaller volume
- Follow the formalin-fixed paraffin-embedded (FFPE) instructions for Solid Phase Reversible Immobilization (SPRI) beads (81 µl) for pre-enrichment library clean-up if the cDNA yield is low
- Use the temperature and cycle numbers listed in Table 4

Once libraries are prepared, 12 libraries are pooled for enrichment. When selecting libraries for pooling, it is highly recommended to choose libraries with the same mass. While 500 ng per library is ideal, it is possible that yield will be lower. If that is the case, load the same mass for each library, even if that mass is only 200 ng. Failure to do so can result in significant read imbalance and subsequent lower quality analysis.

**Table 3: Reagents for library preparation kit**

Product name	Vendor	Catalog no.
Nextera DNA Flex Pre-Enrichment Library Prep and Enrichment Reagents-96 samples (8-, 12-plex enrichment reactions)	Illumina	20025524
Respiratory Virus Oligo Panel	Illumina	20042472
<b>Indexing options</b>		
IDT for Illumina Nextera DNA Unique Dual Indexes Set A (96 Indexes, 96 Samples)	Illumina	20027213
IDT for Illumina Nextera DNA Unique Dual Indexes Set B (96 Indexes, 96 Samples)	Illumina	20027214
IDT for Illumina Nextera DNA Unique Dual Indexes Set C (96 Indexes, 96 Samples)	Illumina	20027215
IDT for Illumina Nextera DNA Unique Dual Indexes Set D (96 Indexes, 96 Samples)	Illumina	20027216
IDT for Illumina Nextera DNA Unique Dual Indexes Sets A-D (384 Indexes, 384 Samples)	Illumina	20027217

**Table 4: Recommended Nextera Flex for Enrichment workflow parameters**

Step	Parameter
Amplify tagmented DNA	12 cycles
Hybridize probes	ramp down to 58°C
Capture hybridized probes	58°C
Amplify enriched library	12 cycles at 58°C

\* Targets common respiratory viruses, recent flu strains, and SARS-CoV-2; includes human target sequences as positive controls.

## Sequence

The Nextera Flex for Enrichment reference guide specifies a 2 × 101 bp read length; however, due to the smaller insert size of the RNA, a 2 × 76 bp read length is recommended for use in this workflow. Table 5 lists Illumina sequencing systems recommended for use in this workflow. Follow the Denature and Dilute Guide specific to the chosen system to prepare the libraries at the proper loading concentration. In general, Illumina recommends generating 500K–1M reads per sample due to potential index imbalances resulting from different enrichment efficiencies for each sample. For customers newer to NGS, 1M reads per sample is recommended.

**Table 5: Recommended Illumina sequencing systems**

System	Catalog no.
iSeq™ 100 System	20021532
MiniSeq™ System	SY-420-1001
MiSeq™ System	SY-410-1003
NextSeq™ 550 System	SY-415-1002
NextSeq 2000 System	20038897

All listed Illumina sequencing systems will provide equivalent yield.

## Analyze data

For data analysis, Illumina recommends three options:

1. **Local data analysis using Local Run Manager (LRM) Resequencing Analysis Module.** This module provides alignment to the reference genome, depth of coverage for the reference genome, a summary of the identified small variants including single nucleotide variants (SNVs), insertions and deletions (indels), a summary of the fragment length observed, and duplicate information for library diversity.
2. **Cloud-based analysis using the IDbyDNA Explify Respiratory Viral Oligos Panel (RVOP) Analysis App on BaseSpace Sequence Hub.** This analysis tool provides data quality control (QC), results interpretation, curated databases, and custom report generation.
3. **Cloud-based analysis using the DRAGEN™ Enrichment App on BaseSpace Sequence Hub.** This pipeline provides rapid analysis of data generated by the enrichment workflow for any genome. Reports include sequencing QC metrics, insert size distribution, and fold enrichment for the detected viruses in the panel. Results files, including alignments (BAM) and variants (VCF), are easily downloaded, individually or in bulk, via the BaseSpace Downloader.

## Learn more

To learn more about how Illumina solutions are helping characterize coronavirus, visit [www.illumina.com/areas-of-interest/microbiology/infectious-disease-surveillance.html](http://www.illumina.com/areas-of-interest/microbiology/infectious-disease-surveillance.html)

To learn more about the enrichment workflow for detecting coronavirus using Illumina NGS systems, visit [www.illumina.com/content/dam/illumina-marketing/documents/products/appnotes/NGS-enrichment-coronavirus-app-note-1270-2020-002.pdf](http://www.illumina.com/content/dam/illumina-marketing/documents/products/appnotes/NGS-enrichment-coronavirus-app-note-1270-2020-002.pdf)

To learn more about accessing the IDbyDNA Explify RVOP Analysis App, contact Illumina Technical Support or IDbyDNA Client Services at [clientservices@idbydna.com](mailto:clientservices@idbydna.com)

## References

1. Illumina (2020) Enrichment workflow for detecting coronavirus using Illumina NGS systems. ([www.illumina.com/content/dam/illumina-marketing/documents/products/appnotes/NGS-enrichment-coronavirus-app-note-1270-2020-002.pdf](http://www.illumina.com/content/dam/illumina-marketing/documents/products/appnotes/NGS-enrichment-coronavirus-app-note-1270-2020-002.pdf)). Accessed April 1, 2020.