HOW NEXT-GENERATION SEQUENCING CAN HELP IDENTIFY AND TRACK SARS-CoV-2

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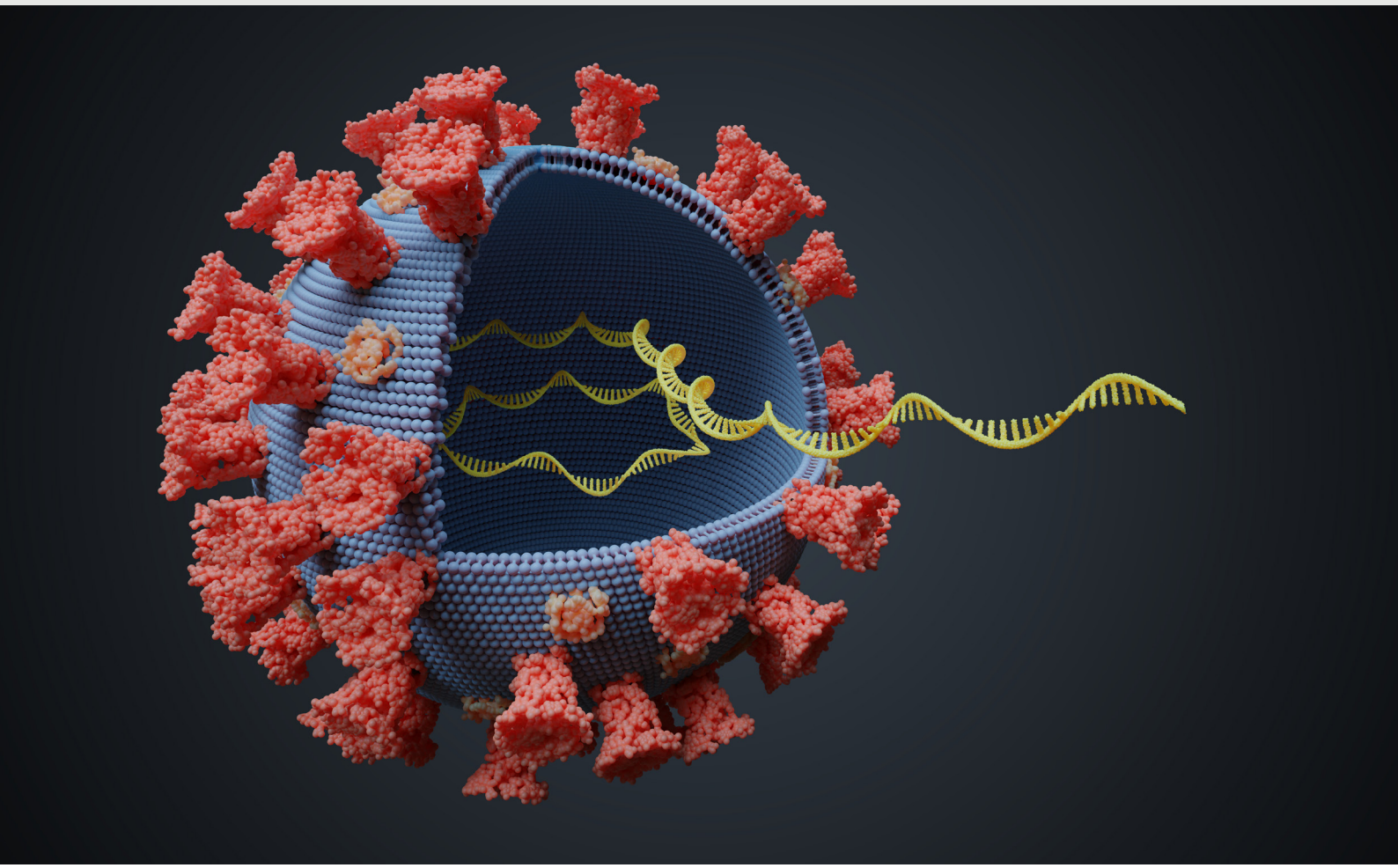

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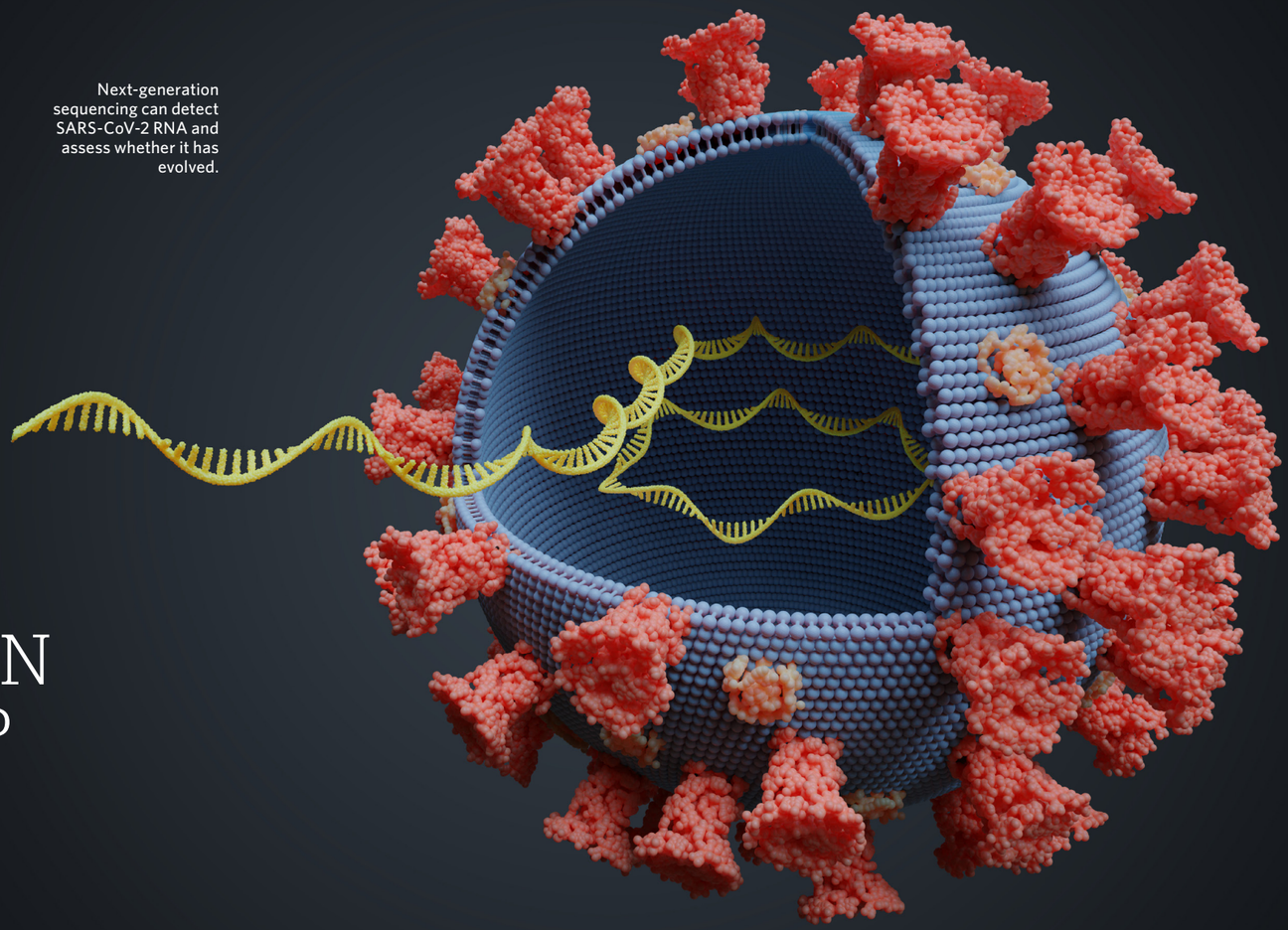

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Next-generation sequencing can detect SARS-CoV-2 RNA and assess whether it has evolved.

HOW NEXT-GENERATION SEQUENCING CAN HELP IDENTIFY AND TRACK SARS-COV-2

ILLUMINA HAS DEVELOPED TWO WORKFLOWS to enable detection of coronaviruses from clinical samples, aiding diagnosis, surveillance and treatment development efforts.

SARS-CoV-2, the virus responsible for the Covid-19 pandemic, is a novel pathogen first identified in China in late December 2019. Since the first SARS-CoV-2 genome was published on 24 January, researchers have been rapidly gathering information about the virus to help inform public health initiatives.

By the end of April, more than 12,000 SARS-CoV-2 genome sequences had been uploaded to GISAID, an open access database that tracks viral evolution and spread around the world. This number lags far behind the three million-plus cases of infection detected by polymerase chain reaction (PCR) tests, highlighting the difficulty in containing the spread of the virus and keeping track of its genetic sequence over time.

Part of the problem is that SARS-CoV-2 causes a broad spectrum of clinical symptoms, from very mild to critical. In order to be effective, infection control and prevention measures need to account for all types of transmission, including by people who are asymptomatic or presymptomatic. Similarly, other infections might affect the course of Covid-19; awareness of these could also help to identify patients who are at higher risk of severe illness.

Current front-line PCR testing only determines the presence of the virus. "It doesn't tell you anything about the genetic sequence of the virus, the presence of co-infection or any information about the patient's immune response," says Gary Schroth, distinguished scientist and VP at Illumina. "A next-generation sequencing (NGS) system to back up the PCR is really important to ensure that the test is still performing well and to obtain this valuable information."

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To help researchers work towards these goals, Illumina has devised two methods for sequencing SARS-CoV-2 from clinical samples: one workflow based on shotgun metagenomics, and one on target enrichment.

Shotgun metagenomics workflow

Shotgun metagenomics is a high-throughput sequencing method that identifies and genotypes all microbial communities in a sample without prior knowledge of what microbes are present. The comprehensive examination of all organisms in a sample is crucial if novel pandemic pathogens, like SARS-CoV-2, are to be identified.

Illumina's shotgun metagenomics workflow integrates sample preparation, library preparation, sequencing and analysis — either using local data or with cloud-based tools. For the latter, Illumina has partnered with metagenomics company

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WHAT NGS APPROACH SHOULD I USE TO DETECT CORONAVIRUS?

Method	Min. read recommendation	Applications
Shotgun Metagenomics Link	10,000,000	• Identify the breadth of novel and known pathogens in a sample • Study coronavirus genetic variation • Study the host response to infection
Target enrichment Link	500,000	• Identify the presence of coronavirus and other key respiratory viruses in a sample using the Respiratory Virus Oligo Panel • Study coronavirus genetic variation

IDbyDNA; customers can access the Explify Platform, a proprietary database of curated DNA and RNA reference sequences to identify more than 35,000 viruses and 13,000 bacteria.

The shotgun technique can determine whether patients are co-infected with other viruses, such as influenza, or bacteria, which could be useful for informing future treatment decisions and predicting patient outcomes. Knowledge of the virus's genetic make-up also allows researchers to understand how SARS-CoV-2 is evolving in order to monitor transmission and ensure that diagnostic tests and treatments that target viral genome products remain effective.

"THE ENRICHMENT WORKFLOW IS IDEAL FOR BENCHTOP SYSTEMS."

The shotgun approach also provides information on patients' response to infection. "When you sequence a nasal swab in this way, you don't just get the RNA from the virus, you also get the RNA from the human transcriptome in your nose," Schroth explains. The RNA of inflamed epithelial and activated immune cells can reveal what the patient's immune system is doing in response to the virus. This could help researchers understand how SARS-CoV-2 causes disease and why it affects some people more than others.

Target enrichment workflow

Target enrichment allows researchers to reliably sequence specific genes in a sample, thus, decreasing the amount of data requiring analysis.

Illumina's enrichment workflow detects coronavirus strains with equivalent accuracy to shotgun sequencing. This workflow combines Illumina's Nextera Flex for Enrichment, which generates targeted NGS libraries, and a newly available respiratory virus oligo panel, which captures and allows the resequencing of more than 40 pathogens. The viral enrichment panel detects genomic mutations across different samples, helping to define the epidemiology of transmission.

The two workflows differ in the sequencing depth (number of reads per sample) required (see Table). "The enrichment workflow is ideal for benchtop systems. Because it provides very specific information, you really only need 0.5-1 million reads to get good results from a sample," says Schroth. By contrast, the shotgun method requires 20-50 million reads per sample. "Shotgun requires a lot more data, but you get a lot more information out of it," he adds.

These two complementary workflows highlight how NGS can be used to discover novel pathogens like SARS-CoV-2, and can be used for surveillance — determining how the virus is evolving and the consequences of those changes. The workflows can be performed alongside traditional testing methods and used to optimize infection control strategies. ■

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