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New England Biolabs Debuts SARS-CoV-2 Primer Resource as Aid to Combat Variants

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NEW YORK – New England Biolabs has unveiled a tool to help diagnostics developers monitor mutations in the SARS-CoV-2 virus that may impact primer binding domains.

The tool, called Primer Monitor, is free to use. It requires developers to make their primer sequences public in order to evaluate them and receive notifications about new mutations of interest.

There is also an option to be notified about mutations affecting primers already in NEB's database, such as those used in the test from the US Centers for Disease Control and Prevention and the so-called Charité assay.

Brad Langhorst, a sequencing expert at NEB, built the primer monitoring tool over the past year and has gradually added capabilities to it. It was released publicly in March, and this week he and the NEB team updated it to include the notification functionality.

"The tool's job is to tell you if a primer set you are working with or are interested in is potentially affected by variants that are appearing in a specific region of the world," Langhorst said in an interview.

After users enter their primer sequences, Langhorst reviews them and aligns them on the reference genome, then the set becomes publicly visible on the online tool. A few groups have already added to the tool, he said, noting that they are primarily academic. Vendors, however, are traditionally protective of primer sequences for commercial assays.

"I called Thermo Fisher and Roche, and they won't disclose their primers," Langhorst said. Color Genomics, on the other hand, has primer sequences for its loop-mediated isothermal amplification-based test publicly available on its website, and so those are included in the NEB tool.

That said, many vendors are using other tests as templates, Langhorst noted, and their LAMP or qPCR primer sets may be in the tool already. If vendors or labs know which primer set a particular diagnostic test uses, they can use that to monitor via the NEB tool and would not need to enter any sequence information.

In addition, individuals can sign up to be notified if variants appear above a particular threshold in a specific region of a viral genome within a specified geographic area. For example, Langhorst pointed out a variant with a mutation a few bases back from the 3' end of the so-called N2 region of the SARS-CoV-2 virus targeted by the CDC assay. This variant currently makes up 47 percent of sequences in the Cagayan Valley region of the Philippines, which could possibly impact any assay based on the CDC's primers and probes.

As part of development, Langhorst said he has incorporated as many primer sets as possible from public data and US Food and Drug Administration Emergency Use Authorization documents.

"Many are using one primer, so they are prone to a variant showing up in a particular region — maybe not even one of the variants of concern like alpha or delta," he said. The NEB tool could essentially give labs an advanced warning about the potential for false negative results in their area.

Langhorst said that the tool is based on custom software and work that NEB was doing internally already, since it needed to stay on top of the mutations that could impact the primers and probes in assays it sells.

Steven Chiu, product marketing manager for DNA amplification at NEB, said that the sequence data comes through an agreement forged with GISAID but that the firm is also using a data visualization software called Tableau. "It is basically representing it in a really clean, easy-to-digest, visual package," Chiu said.

Doing the primer monitoring work manually requires searching GISAID by geographic region and downloading sequences, calling the variants, and mapping onto the primers, Langhorst said. But the GISAID website only allows downloading a dozen data sets at a time, he noted, and this process needs to be repeated every few days.

Since NEB was already doing this work, the firm decided to offer the tool for free because "the benefit to the world is much greater than trying to keep this product to ourselves," Langhorst said.

Primer IP

The FDA issued an alert in January about viral variants potentially impacting EUA assays.

This was followed by nonbinding guidance recommendations in February suggesting diagnostics developers continuously monitor global SARS-CoV-2 sequence databases and alert the agency if any mutations pop up in regions that might impact EUA test performance.

The agency disclosed in its guidance that since "molecular tests target specific regions of the viral genome, the FDA is monitoring the potential effects of genetic variation on FDA-authorized molecular tests and has been doing so on an ongoing basis throughout the pandemic."

The FDA had also publicly stated previously that it has all of the primer/probe data in confidential EUA submissions from developers, and that the agency has staff checking these proprietary sequences against viral variant sequences.

Specifically, "We have the list of all EUA primers and probes for molecular assays, and we have been interrogating the sequence databases to see if there are any mutations that could impact test performance," said Timothy Stenzel, director of the FDA's Office of In Vitro Diagnostics and Radiological Health, in January.

The FDA currently lists the EUA assays that may have issues with variants on a dedicated website.

Multitarget tests that have one target prone to dropout due to mutation in the primer region can be used as a way to track that mutation, as has been the case with the S-gene dropout in Thermo Fisher Scientifics' TaqPath assays. Thermo disclosed the sequences of its primers and probes for one of the mutations in the B.1.1.7, or alpha variant on its website but this type of disclosure does not appear to be a common industry practice for diagnostics developers.

For example, one of the first cases of a variant impacting test performance occurred last year in Belgium. A locally spreading mutation seemed to affect one of the targets — against the virus' E gene — in a multitarget test from Roche Diagnostics. A research team was unable to quickly confirm its results, however, because the company would not disclose proprietary primer/probe sequences.

"Sadly, the majority of the manufacturers do not make their primer sequences public," said Chris Mason, a scientist at Weill Cornell who also co-authored an upcoming paper about NEB's primer monitoring tool. He argues that making commercial primer sequences public could have advantages for public health.

"Viral variants are non-uniform across the world's populations, and thus appropriate diagnostic adjustments need to be responsive to the changes in specific areas," he noted in an email.

Using the NEB tool, a lab could theoretically set up notifications for a particular vendor's primer sets combined with its local region information and be notified if there may be impacts. In turn, this could allow labs "to make informed choices about risk of assay failure, need for adjustments, or to help [the] design of new tests and diagnostic tools," Mason said.

Langhorst said that the primer sequence is "not particularly valuable" intellectual property to begin with. "It is really easy to get — you're just one Sanger sequencing run away from learning what somebody else's primers are, so I don't know why people keep it secret," he said.

Mason and his colleagues at Cornell are using LAMP and PCR tests for diagnostic and screening assays. In order to monitor the primers for potential impact of circulating variants, the team had previously manually downloaded sequencing data from GISAID. Now Mason and his colleagues are using the NEB tool to continuously monitor the primers in its tests.

"We wanted this tool to make it much easier, faster, and more accessible for people with tests who are worried about variants of concern and variants of interest," he said.

In addition to the on-site functionalities, the Tableau visualization allows users to go into the public data and reformat it to their own preferences, Langhorst said. The development data is also available on GitHub, according to a white paper.

Langhorst will continue to update the tool daily with new data provided by GISAID. Going forward, because the tool was built to be rather generic, he said, it could potentially be adapted to monitor primers for assays to detect other pathogens like respiratory syncytial virus or influenza.

He will be presenting the tool at a Centers for Disease Control and Prevention SARS-CoV-2 Sequencing for Public Health Emergency Response, Epidemiology, and Surveillance, or SPHERES, event next week, which he hopes will attract more users. He noted that he is also available to collaborate with kit developers.

"There are hundreds of EUAs, and a lot of people are trying to do this monitoring themselves," he said. "It would be great if we could just work together as a team and save resources, so we don't have to have a computational person in every single company trying to keep up with this."

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