Serological test signals viral triggers of diseases like diabetes and celiac disease

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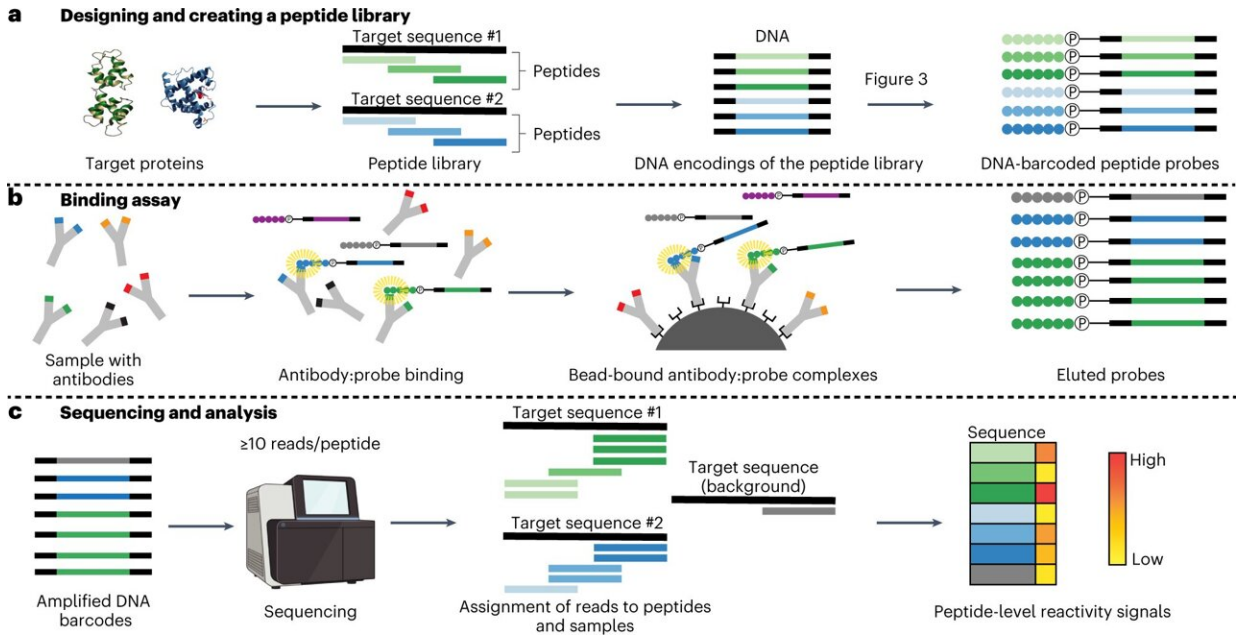

Overview of protocol. a, Design of a peptide library begins with a set of target protein sequences. An informatic approach (such as the combined sliding-window/set-cover algorithm described in the 'Design of peptide-encoding oligonucleotides' section of 'Experimental design') is then used to design a library of peptides of a user-defined length that cover the supplied target sequences (green and blue bars). Next, amino acid sequences are informatically converted into DNA encodings, and constant regions (black segments) are added to each end. The corresponding DNA oligonucleotide library is prepared by massively parallel DNA synthesis and converted in bulk to a corresponding library of DNA-barcoded peptides ('PepSeq probes') by using in vitro transcription and translation and taking advantage of intramolecular coupling mediated by a tethered puromycin (P)-containing molecule. b, A library of PepSeq probes,

prepared as in a, are then incubated with biological samples of interest, allowing antibodies to bind their cognate epitopes (binding indicated by the yellow halos). The antibodies are then captured onto protein-bound beads via their constant regions, unbound PepSeq probes are washed away and bound PepSeq probes are eluted. c, The DNA tags of eluted PepSeq probes are amplified by PCR, and the relative abundance of each probe is quantified by using high-throughput sequencing. Changes in relative abundance provide a semi-quantitative measure of cognate antibody abundance/affinity for each peptide in the design. Created with BioRender.com. Credit: *Nature Protocols* (2022). DOI: 10.1038/s41596-022-00766-8

A new serological test can not only help humanity prepare for and respond to the next pandemic, but it also can be pivotal in the search for viral triggers of diseases like diabetes and celiac disease.

Jason Ladner, an assistant professor in the Department of Biological Sciences and the Pathogen & Microbiome Institute (PMI), was a leading innovator on PepSeq, a technology that allows scientists to test antibody binding against hundreds of thousands of protein targets at one time, instead of testing one at a time. When we're trying to track a contagious virus, like coronavirus, and figure out how to respond to it, getting answers quickly can be the answer between life and death.

This protocol is laid out in detail in an article published earlier in November in *Nature Protocols*.

The paper, "PepSeq: a fully in vitro platform for highly multiplexed serology using customizable DNA-barcoded peptide libraries," describes the novel approach for conducting highly multiplexed serology assays and details the team's approach for designing and synthesizing custom PepSeq libraries, as well as how to use these libraries to conduct assays and interpret the data. The hope, Ladner said, is to provide a roadmap

for scientists everywhere to use the protocol in their own research, moving us closer to answers on some major infectious diseases.

It's an important step forward as concerns about bioterrorism, zoonotic diseases and the next pandemic are never far away. Understanding these pathogens will help scientists develop vaccines and track their movement and evolution.

"Serology assays are important for diagnosing many human and animal infections," he said. "However, traditional assays often lack sensitivity and/or specificity. Our approach can help to identify which proteins most commonly stimulate an antibody response during infection and which epitopes are specific for the pathogen of interest, rather than being cross-reactive across related pathogens."

What is PepSeq?

At its most basic, the protocol allows scientists to test how protein targets , or antigens, bind to antibodies hundreds of thousands at a time. Antigens, which are pieces of a pathogen—like a virus or a bacteria—are the targets of antibodies in the host's blood. New antibodies are produced in response to each new infection, which are highly specific for particular antigens and can persist for months or even years. Therefore, antibodies help to protect from future infections with similar pathogens, but they also provide a record of past exposures.

One of the big questions on the medical side, then, is which antibodies protect against infection. This is where humanity found itself in 2020—not knowing which were the right antibodies that the body needed to develop to respond to COVID-19. That information was needed to create an effective vaccine and, in the future, could help develop vaccination approaches that would broadly protect against coronaviruses like SARS-CoV-2, including those that we have yet to

identify.

An even bigger question involves identifying which antibodies are needed to attack a pathogen's antigens. This is where humanity found itself in 2020—not knowing which were the right antibodies that the body needed to develop to respond to COVID-19. That information was needed to create an effective vaccine.

PepSeq, which was initially developed by a team that included John Altin, now a researcher at TGen, Ladner's collaborator and a co-author on the study, contributes to this process in two key ways. First, it allows scientists to simultaneously assess antibody binding across a large number of different antigens. Because antibodies are evidence of a host's past infections, knowing what antibodies a person has can offer clues about what pathogens they have been exposed to in the past. This assay allows researchers to broadly explore exposure history by testing for evidence of past infections by all viruses known to infect humans.

"This can help us to better understand the epidemiology of infectious diseases, and it is also empowering us in our search for potential viral triggers for non-infectious diseases like diabetes and celiac disease," Ladner said.

Second, the antigens used in PepSeq are short peptides, which means they are typically no more than 64 amino acids in length. That means that each time an antibody binds to a PepSeq antigen, the signal is specific to a particular protein region, allowing Ladner and his team to dissect the antibody response in minute detail. It helps suss out which portions of a pathogen are most commonly targeted by antibodies and which antibodies are specific to particular pathogens, like the novel coronavirus that causes COVID-19, and which cross-recognize—and potentially cross-protect against—closely related pathogens, like the coronaviruses that can cause the common cold.

"In particular, we've been very interested in a couple of epitopes that are highly conserved between SARS-CoV-2 and 'endemic' human coronaviruses that have been infecting humans for decades and generally just cause a common cold," he said. "We've found that the dynamics of the antibody response to these epitopes are distinct, and responses directed against these regions could provide broad protection against coronaviruses."

The third key factor overarching every assay: PepSeq allows these tests to be run quickly and with a much smaller sample volume than other assays—crucial because often these samples are precious and in short supply.

What happens now?

This study provides the information needed for any researcher to use PepSeq. It also provides their rationale for the decisions they made in the process so others can build on the technology. As innovative as this protocol is, the researchers aim for others to innovate even further. Ladner's team is leading the development of bioinformatics protocols and custom software packages that will create the PepSeq libraries and allow for the interpretation of data collected through the protocol.

From a big-picture standpoint, having these kinds of conversations helps the field as a whole progress, Ladner said. It helps develop trust in the process and the results.

"Even if no one outside of PMI and TGen ends up using PepSeq, we want the broader scientific community to trust that the results are reliable, and the best way to do that is through transparency about the process," he said. "In this type of protocol manuscript, we can go into much greater detail about the methods than we can in a manuscript focused on the results of a particular research project that utilizes

PepSeq."

Ladner also is refining a protocol for new sample types, including human saliva, urine and blood-fed mosquitoes. He's also using PepSeq to better understand the distribution and ecology of viruses that affect other animals but not humans (yet). This will allow his team to explore antibody reactivity in other animals, like bats, which will demonstrate what types of viruses are commonly infecting these reservoir species.

Although most of this research is aimed at questions in the fact-finding part of the process, it should lay a foundation that leads to a more effective response during future outbreaks, as well as vaccines and therapeutic treatments for viral illnesses.

"One of the main goals for vaccination is to stimulate the production of protective antibody responses, and PepSeq can help us to understand the antibody response to vaccination," Ladner said. "The results from PepSeq assays could certainly help to inform strategies and approaches that are more directly linked to outbreak preparation and response."

More information: Sierra N. Henson et al, PepSeq: a fully in vitro platform for highly multiplexed serology using customizable DNA-barcoded peptide libraries, *Nature Protocols* (2022). DOI: 10.1038/s41596-022-00766-8

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