Improvements Move Broad's CRISPR-Based Diagnostic Technology Closer to Field, Clinic

Mar 08, 2018 | Christie Rizk

Premium

NEW YORK (GenomeWeb) – Researchers in Feng Zhang's lab at the Broad Institute have added more CRISPR enzymes to their nucleic acid detection platform, SHERLOCK, amping up its sensitivity and making it capable of even smaller quantitative measurements.

SHERLOCK actually stands for Specific High sensitivity Enzymatic Reporter unLOCKing, but the allusion to Arthur Conan Doyle's fictional detective is no accident. And if the tool is a technological Holmes, then Zhang lab researchers Jonathan Gootenberg, Omar Abudayyeh, and their colleagues could be called its John Watsons — the work they do makes SHERLOCK a better detective.

Recently in *Science*, Gootenberg and Abudayyeh were joint first authors on a paper describing the new improvements on the technology. It now has four-channel single reaction multiplexing using orthogonal CRISPR enzymes, quantitative measurement of input down to 2 attomolar concentration, a 3.5-fold increase in signal sensitivity through a combination of Cas13 with auxiliary CRISPR-associated enzyme Csm6, and a lateral flow read-out.

"SHERLOCKv2 can detect Dengue or Zika virus ssRNA as well as mutations in patient liquid biopsy samples via lateral flow, highlighting its potential as a multiplexable, portable, rapid, and quantitative detection platform of nucleic acids," the authors wrote in their paper.

The same researchers revealed the first version of SHERLOCK in a *Science* paper in April 2017. They described their use of Cas13a, which can target, bind, and cleave a nucleic acid target with great specificity. Once activated, this protein will indiscriminately cut up any RNA oligonucleotide, even those that don't match the original target sequence, leading the researchers to believe that it could be used for diagnostic purposes.

In October 2016, researchers led by University of California, Berkeley professor Jennifer Doudna also

quenched fluorescent signal, which could then be used as a readout for target detection. And to boost the sensitivity, the Zhang team then used isothermal amplification in order to generate target RNAs for Cas13a to detect.

For version two of SHERLOCK, the Zhang lab looked to improve on many of these capabilities, and the improvements have moved SHERLOCK one step closer to being useful as a diagnostic tool in the field and in the clinic.

For one thing, the team looked to make SHERLOCK easier to read. Instead of having a fluorescent signal indication, they changed it to a bilateral flow strip made of paper, a visual readout that doesn't require any additional instrumentation. The paper test is similar to a pregnancy test, the Broad noted in a statement, and the results can be seen with the naked eye.

But making it easier for a field worker or clinician to read SHERLOCK isn't all that useful if the platform's detection capabilities are subpar. So to improve those capabilities, the team also combined various Cas13 enzyme types with Cas12a and Csm6 to allow for the simultaneous detection of multiple nucleic acids at once. "We have worked really hard to get multiplexing. We can develop different [signals] by using different enzymes," Gootenberg said. "When the different enzymes are activated and have collateral activation — they start cutting nucleic acids in the solution — some of them will prefer different letters or different pairs of letters. Some of them will like DNA over RNA. And by combining all of those enzymes in one tube, if you have a panel of different viruses you might want to detect simultaneously, you can do that all in one sample."

Further, he added, the increased sensitivity makes SHERLOCK more capable of detecting rare viruses or viruses such as HIV that are present at low concentrations.

Importantly, SHERLOCK can tell not only whether a pathogen is present, but also how much of it is present. "For some diagnostics, it's sufficient to say that there's a presence or absence of a pathogen," Gootenberg said. "But when you're looking at certain things like nucleic acid levels that are indicative of cancer in liquid biopsies, you need to be able to say, 'It's increased or decreased by two-fold,' not just that it's there or not there. So we've worked on demonstrating that you can make the reaction quantitative."

But all these improvements might not have been possible without some creative thinking. Take Csm6, for example. It's a fairly obscure enzyme from a Type III CRISPR system, which is a different CRISPR system than Cas13, and generally not the first enzyme that a researcher might reach for when considering which enzymes to multiplex with Cas13.

"This is our first time working with Csm6. It's part of the CRISPR systems that generally involve large, multi-protein complexes to carry out defenses against phage," Abudayyeh said. Recent research has shown that Csm6 is activated by an effector complex, which recognizes a phage and then activates Csm6, he added. In turn, Csm6 indiscriminately cleaves the RNA in an organism, whether it's the phage RNA or the host RNA.

"Some people think it's an abortive cell immunity defense system, where if you can't get over the phage infection, maybe the bacterium just ends up dying because Csm6 cleaves all the RNA in the cell," Abudayyeh noted. "So we thought Csm6 was a little bit like Cas13 because Cas13 also indiscriminately cleaves RNA, and plays a potentially similar cell-abortive immune role." But getting it to work in SHERLOCK would mean figuring out how to activate it.

Through various biochemical experiments described in their new paper, the researchers showed that Csm6 is activated by cyclic adenylate molecules or linear adenine homopolymers terminated with a 2′,3′-cyclic phosphate. And in the biochemical experiments for Cas13 that they conducted in 2016, they showed that

be cool if you could take an RNA molecule that has six adenines on it, and then a stretch of four uridines, which one of our Cas13 enzymes can cleave, and the idea is that it would cleave the uridines off and leave the six adenines and the 2′,3′-cyclic phosphate, which is the perfect activator for Csm6. And you essentially have Cas13 recognize the target specifically, cleave this molecule and activate Csm6, and now you have two enzymes indiscriminately cleaving your reporter to generate a signal," Abudayyeh said. "And when we started testing this out, it worked really well. And we were able to incorporate it as a SHERLOCK enhancer to generate more signal."

At the same time, he added, this experiment demonstrated an exciting biological phenomenon — two completely different CRISPR systems are apparently able to interact and communicate with each other. It left the team wondering whether this is something that could happen in live cells when both types of systems are present.

But not simply content with mixing RNA-cleaving enzymes from different CRISPR systems, the team also added Cas12a — a DNA cleaving enzyme — to enhance the orthogonality. "When you have a DNA cleaving enzyme with collateral activation, you can use that as an independent way from all the RNA enzymes to gain another [signal]," Gootenberg said. "And that's another thing we show when we have a four-color readout — three of those colors are for RNA enzymes and one is for DNA. It's a good way to have another independent thing to read out."

The team even worked in different Cas13 orthologs. One of them, LwaCas13a, was used in the original SHERLOCK paper. In this study, the researchers showed that it's capable of cleaving uridines. This paper also showed that the different Cas13a and Cas13b subtypes have different cleavage preferences, and so could be used in various combinations to detect different diseases all at the same time. For example, the researchers combined reporters for LwaCas13a, PsmCas13b, CcaCas13b, and AsCas12a, and were able to detect two DNA targets (the acyltransferase gene from the disease-causing *Pseudomonas aeruginosa* bacterium and the thermonuclease gene from the *Staphylococcus aureus* bacterium), as well as Zika virus and Dengue virus RNA dilutions and allele-specific genotypes in human saliva samples.

And to demonstrate SHERLOCK's utility in the clinic as well as the field, the team took liquid biopsy samples from cancer patients at Brigham and Women's Hospital and ran them through the diagnostic tool, showing that it could detect their mutations at different levels of occurrence. "Patient tumors are more and more sequenced these days at the time of detection, so we could design personal SHERLOCK assays where you can track their mutations over time, or in response to treatment," Abudayyeh said. "And the fact that you have an easy paper-based readout, any clinic, regardless of how well developed it is, could easily track that information on a frequent basis."

And their improvements to SHERLOCK never stop. The team is currently working on a study that explores SHERLOCK for testing primary patient samples, in order to see how it could be deployed in the field in case of an outbreak of a disease such as Zika. Gootenberg noted that the researchers are hoping to publish a paper on that effort soon. "One of the visions for the technology is to have it as a field-deployable technology and we're moving in that direction," he added.

SHERLOCK's malleability could certainly aid them in this effort. "The really great thing about the SHERLOCK assay is how easy it is to redesign and deploy," Abudayyeh said. "We've shown that we can detect dozens of different targets and we can design these assays in as little as a week. That speaks to how robust it is, and how well it works for each given set of CRISPR RNAs. It's as easy as designing a new CRISPR RNA and then showing that it works."

And while the researchers are continuing to look at enzyme discovery or even enzyme engineering to see how they can improve the assay, they are also looking at how its current form can be used to full effect to help as many people as possible, Gootenberg noted.

Importantly, Gootenberg and Abudayyeh said, this study showed that basic biological research can have ripple effects. "This is just a really great example of how a basic science discovery, like a new nuclease — Cas13, Cas12, even Csm6 — can be developed into a platform with real potential for making an impact on human health and society," Abudayyeh said.

"We were excited to do this project to profile the different enzymes, to see how they work, to put them together, and see how they all work in concert," Gootenberg added. "It's really fun to be able to use the basic biology to help push the applications."

Filed Under Diagnostics Cancer Infectious Disease Gene Silencing/Gene Editing cancer of unknown primary dengue Zika virus Broad Institute CRISPR diagnostics

We recommend

ATCC Licenses CRISPR/Cas9 Tech From Broad Institute
GenomeWeb

Sage Licenses CRISPR-Cas9 Technology from Broad Institute
GenomeWeb

Broad Institute Licenses CRISPR/Cas9 to AMRI
GenomeWeb

Horizon Expands CRISPR-Cas9 Portfolio with Broad Institute Licensing Deal
GenomeWeb

Syngenta Licenses Broad Institute CRISPR IP
GenomeWeb

Patent for Editing
GenomeWeb

Powered by