Online game challenges players to design on/off switch for CRISPR

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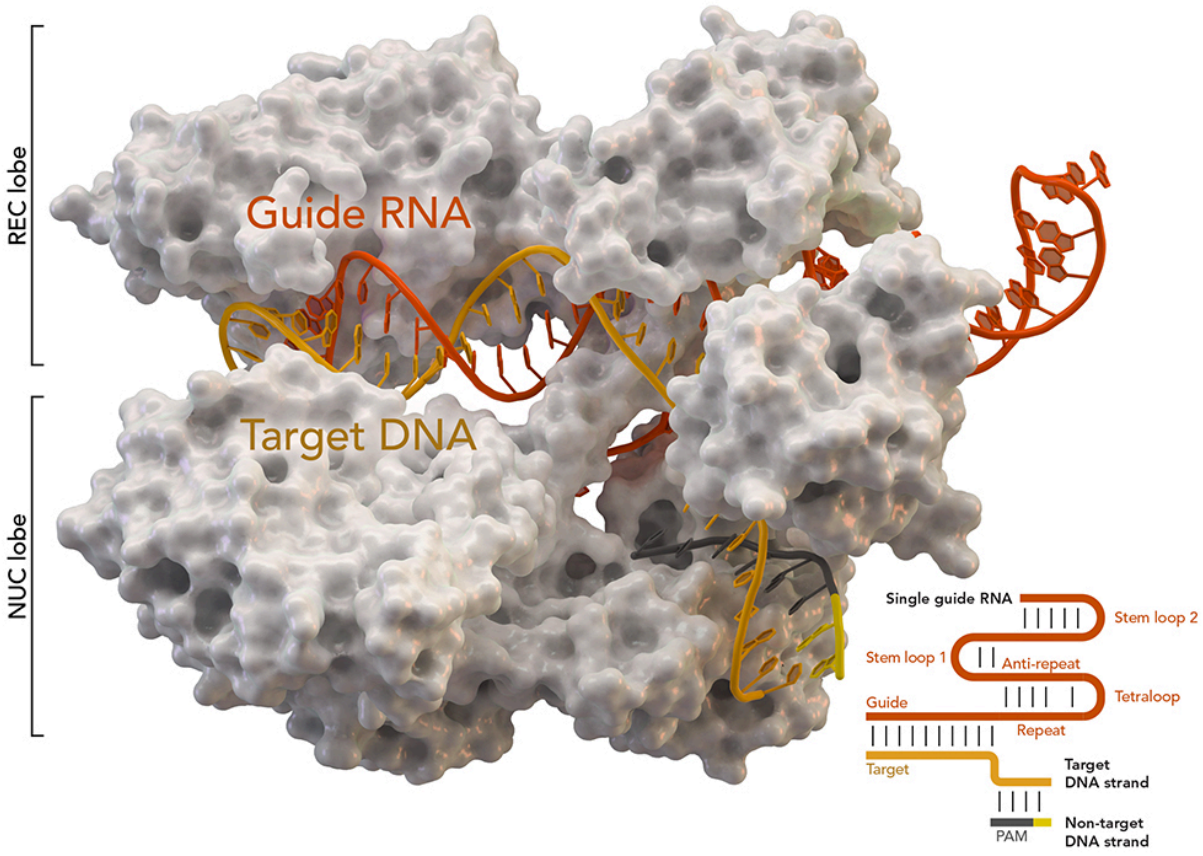

CRISPR-associated protein Cas9 (white) from Staphylococcus aureus based on Protein Database ID 5AXW. Credit: Thomas Splettstoesser (Wikipedia, CC BY-SA 4.0)

A Stanford team has launched a new challenge on the Eterna computer game. Players will design a CRISPR-controlling molecule, and with it open the possibility of new research and therapies.

A team of researchers at the Stanford University School of Medicine has launched a new challenge for the online computer game Eterna in which players are being asked to design an RNA molecule capable of acting as an on/off switch for the gene-editing tool CRISPR/Cas9.

Molecular biologists will then build and test the actual molecules, based on the most promising designs provided by the players.

A gene editor as powerful as CRISPR could have unexpected effects inside living cells, so it makes sense to turn it off when it's not needed. In addition, an on/off switch might be able to put CRISPR-influenced genes on a sort of timer, activating and deactivating them on a schedule that could mimic the way we schedule taking doses of drugs.

Anyone can play the Eterna RNA-design game. "All you need is a good internet connection, the interest and the time," said Howard Chang, MD, PhD, professor of dermatology and director of the Center for Personal Dynamic Regulomes at Stanford. The center, funded by the National Institute of General Medical Sciences' Center of Excellence in Genomic Science program, is providing $15 million to support the project.

"Great ideas can come from anywhere, so this is also an experiment in the democratization of science," Chang said. "A lot of people have hidden talents that they don't even know about. This could be their calling. Maybe there's somebody out there who is a security guard and a fantastic RNA biochemist, and they don't even know it."

The challenge to design a CRISPR switch is the latest puzzle offered through Eterna, a game portal that allows players to design virtual RNA

structures. Eterna most recently hosted a game event challenging players to build an RNA molecule that could simplify the widespread use of a tuberculosis test.

What is CRISPR/Cas9?

CRISPR/Cas9 is a gene-editing tool derived from bacteria. Researchers in the 1980s discovered that E. coli, a species of bacterium that lives in the gut, has a system for reading and damaging viral DNA. When a virus infects a bacterium, it can copy and incorporate segments of the viral DNA into its own genome. (These viral DNA regions are called "clustered regularly interspaced short palindromic regions," or CRISPR.) Just as our own immune systems can use antibodies to remember previous infections, the viral DNA in CRISPR regions helps E. coli bacteria recognize a subsequent viral infection. When a bacterium's RNA molecules recognize the viral DNA, they guide the CRISPR/Cas9 complex to that DNA, and the bacterium's Cas enzymes destroy the viral DNA by cutting it.

In 2012, researchers found a way to deploy the bacterial CRISPR/Cas9 immune system—including CRISPR, a protein called Cas9 and a "guide" RNA—to recognize, cut and replace any DNA sequence. Since then, the CRISPR/Cas9 system has enabled rapid gene editing projects in everything from viruses to corn to human stem cells.

How might scientists use a CRISPR on/off switch?

"CRISPR is a technology for cutting or binding DNA," said Chang. "But you don't want it to be on all the time." It would be useful to have a switch that could turn gene activity on and off on some sort of schedule. A doctor often tells a patient to take a drug twice a day for two weeks. But if the "drug" were generated by the patient's own genes, he said,

small molecules might be used to switch on that gene activity for an hour or two twice a day for two weeks and then stop.

Right now, Chang said, if someone used CRISPR/Cas9 to engineer cells to activate a gene, it would be hard to know how much the gene is being turned on. Plus, he said, the gene would be on all the time. "We just hope everything works out and it's the right amount. But that's a little scary, right?" said Chang.

CRISPR's not just about breaking the gene to turn it off, said William Greenleaf, PhD, associate professor of genetics. "A switch on the CRISPR/Cas9 system can control how the genes are being expressed—that is, when and where."

Where the RNA molecule comes in

In the new Eterna challenge, called OpenCRISPR, players will design a guide RNA molecule that leads CRISPR to the right sequence of DNA for editing or binding. "The RNA is the part that confers gene specificity. It's the thing that says, 'Go after gene A, not gene B,'" said Chang.

The difficulty for Eterna players is to come up with an RNA molecule that does several things, said Greenleaf. The guide RNA has to be recognized by the CRISPR-associated enzyme. The CRISPR-enzyme system has to be able to recruit biochemical activity to the targeted gene. And lastly, the activity of the CRISPR-enzyme system has to be controlled by a small-molecule drug, so there needs to be a "binding pocket" for that small molecule. The RNA molecule has to function so that the CRISPR system is active when the small-molecule drug is present and inactive when it's not. So far, experts have not been able to create such a drug-activated CRISPR, which is why Chang and Greenleaf are calling on the community of Eterna gamers for help.

The new puzzle will be quite different from the recent challenge in which Eterna players had to design a molecule that could do a mathematical calculation for a tuberculosis diagnostic test. "The CRISPR puzzle actually should be pretty easy to solve in silico, even for new players who get to the switch design levels," said Rhiju Das, PhD, associate professor of biochemistry and principal investigator for Eterna.

From in silico to in vitro: an iterative process

How those Eterna-designed switches will behave in living cells is a big question. Das said the team will be asking players for different possible solutions to the same problem. "We're not sure yet if there will be unforeseen problems with the Cas9 protein experimentally. That's partially why we want as many diverse solutions as possible for the Greenleaf and Chang labs to test, even in this pilot round," Das said.

It will be an iterative process, said Greenleaf. His Stanford lab will test the first round of solutions and then return these data to the players with refinements that will guide their design work.

"We're hoping for 10,000 to 100,000 players to contribute 10 solutions each. If we get that many, we'll indeed work to get that many synthesized and tested," Das said.

Out of the ivory tower

One of the goals of Stanford's Center for Personal Dynamic Regulomes is to get people interested in science, said Chang. "The Eterna game is a powerful way to engage lots and lots of people," he said. "They're not just passive users of information but actually involved in the process."

Like other computer games, Eterna allows players to accumulate points,

build expertise and advance to higher levels. The best players have a chance of having their designs implemented in the lab.

One thing that makes the project exciting, said Chang, is that it is an experiment in the sociology of science. "There is a misconception of science as something that happens in an ivory tower by someone in a white coat with a long beard. And they are saying things and drawing things that nobody understands. But it's not like that! It's really like a puzzle that anybody can get engaged with," he said.

Anyone interested in playing Eterna can sign up here.

In addition to the funding from NIGMS and Stanford's Center for Personal Dynamic Regulomes, the new Eterna challenge is being launched with collaborative support from the Innovative Genomics Institute at the University of California-Berkeley. Stanford's departments of Biochemistry and of Genetics also supported the work.

Provided by Stanford University Medical Center