Rapid method of assembling new gene-editing tool could revolutionize genetic research

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Development of a new way to make a powerful tool for altering gene sequences should greatly increase the ability of researchers to knock out or otherwise alter the expression of any gene they are studying. The new method allows investigators to quickly create a large number of TALENs (transcription activator-like effector nucleases), enzymes that target specific DNA sequences and have several advantages over zinc-finger nucleases (ZFNs), which have become a critical tool for investigating gene function and potential gene therapy applications.

"I believe that TALENs and the ability to make them in high throughput, which this new technology allows, could literally change the way much of biology is practiced by enabling rapid and simple targeted knockout of any gene of interest by any researcher," says J. Keith Joung, MD, PhD, associate chief for Research in the Massachusetts General Hospital (MGH) Department of Pathology and co-senior author of the report that will appear in Nature Biotechnology and has received advance online release.

TALENs take advantage of TAL effectors, proteins naturally secreted by a plant bacteria that are able to recognize specific base pairs of DNA. A string of the appropriate TAL effectors can be designed to recognize and bind to any desired DNA sequence. TALENs are created by attaching a nuclease, an enzyme that snips through both DNA strands at the desired location, allowing the introduction of new genetic material.

TALENs are able to target longer gene sequences than is possible with ZFNs and are significantly easier to construct. But until now there has been no inexpensive, publicly available method of rapidly generating a large number of TALENs.

The method developed by Joung and his colleagues – called the FLASH (fast ligation-based automatable solid-phase high-throughput) system – assembles DNA fragments encoding a TALEN on a magnetic bead held in place by an external magnet, allowing automated construction by a liquid-handling robot of DNA that encodes as many as 96 TALENs in a single day at a cost of around $75 per TALEN. Joung's team also developed a manual version of FLASH that would allow labs without access to robotic equipment to construct up to 24 TALEN sequences a day. In their test of the system in human cells, the investigators found that FLASH-assembled TALENs were able to successfully induce breaks in 84 of 96 targeted genes known to be involved in cancer or in epigenetic regulation.

"Finding that 85 to 90 percent of FLASH-assembled TALENs have very high genome-editing activity in human cells means that we can essentially target any DNA sequence of interest, a capability that greatly exceeds what has been possible with other nucleases," says Jeffry D. Sander, PhD, co-senior author of the FLASH report and a fellow in Joung's laboratory. "The ability to make a TALEN for any DNA sequence with a high probability of success changes the way we think about gene-altering technology because now the question isn't whether you can target your gene of interest but rather which genes do you want to target and alter."

The research team also found that the longer a TALEN was, the less likely it was to have toxic effects on a cell, which they suspect may indicate that shorter TALENs have a greater probability of binding to and altering unintended gene sites. Joung notes that this supports the

importance of designing longer TALENs for future research and potential therapeutic applications.

In 2008, Joung and colleagues at other institutions established the Zinc Finger Consortium (http://zincfingers.org), which has made a method of engineering ZFNs broadly available to academic laboratories. His team is now making the information and materials required to create TALENs with FLASH available within the academic community, and information about accessing those tools is available at http://TALengineering.org. Gene editing nucleases, including both ZFNs and TALENs, were recently named "Method of the Year" for 2011 by the journal *Nature Methods*.

Joung says, "While I believe that TALENs ease of design and better targeting range will probably make them a preferred option over ZFNs made by publicly available methods, ZFNs' smaller size and the less repetitive nature of their amino acid sequences may give them advantages for certain applications. For the time being, it will be important to continue developing both technologies." Joung is an associate professor of Pathology and Sander an instructor in Pathology at Harvard Medical School,

Provided by Massachusetts General Hospital