New platform optimizes genome-editing enzyme activity

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Protein engineering is an important means of generating enzymes, antibodies and genome-editing proteins with new or enhanced properties, but the use of such bioengineering is restricted because the effect of multiple mutations on protein function is difficult to predict. Large-scale functional assessment of protein sequence variants would therefore be useful for protein engineering, which prompted University of Hong Kong (HKU) scientists to develop a simple but powerful platform for rapid simultaneous profiling of genetic mutations and mapping relationships between them.

Known as **CombiSEAL**, the platform should improve the ability to engineer diverse proteins systematically, as well as other biomolecules and systems, including synthetic DNAs and genetic regulatory circuits, which have multiple biomedical and biotechnology applications, the researchers reported July 15, 2019, in *Nature Methods*.

"CombiSEAL is the first platform to do systematic assembly and profiling of a bar-coded protein library carrying multiple site-directed mutations," said study leader Alan S. L. Wong, an assistant professor in the Laboratory of Combinatorial Genetics and Synthetic Biology, School of Biomedical Sciences, and Department of Electrical and Electronic Engineering at HKU.

"The bar-coded library can leverage next-generation sequencing to give a quantitative readout, allowing us to track the identity and relative frequency of each protein variant within a library, before and after the selection process, in order to analyze the functional impact of combinations of mutations," Wong told *BioWorld Science*.

Combinatorial optimization of a protein sequence relies on creating and screening a large number of variants, but current approaches are limited in their ability to systematically and efficiently build and test variants with multiple modifications in a high-throughput manner.

In their new study, Wong and his HKU research team presented the CombiSEAL platform, which enables scalable assembly and parallel characterization of barcoded protein variants with combinatorial modifications.

Unlike previously available methods, "CombiSEAL enables simple one-pot, seamless and scalable assembly of variants from a repertoire of fragments tagged with barcodes specifying predetermined mutations at defined positions," he said.

Mass action

The HKU team showed that their CombiSEAL platform systematically characterized a library of 948 combination mutants of the widely used *Streptococcus pyogenes* Cas9 (SpCas9) nuclease enzyme, optimizing its genome-editing activity in human cells.

SpCas9 (SpCas9) is an RNA-guided endonuclease that catalyzes the site-specific cleavage of double stranded DNA, which is important in clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 gene editing.

The ease with which the editing activities of the pool of SpCas9 variants could be assessed at multiple on- and off-target sites with the CombiSEAL platform accelerates the identification of optimized variants and facilitates study of mutational epistasis, whereby the functional impact of one mutation depends on the presence of one or more other mutations.

These SpCas9 systematic mutant library classification findings are important, as "protein engineering is impeded by the unpredictability of functional consequences of combining multiple mutations," noted Wong.

"Direct experimentation [with] sequence variants is important for the identification of rare combinations conferring desired properties of proteins that are useful for biomedical and biotechnological applications."

The researchers successfully identified the Opti-SpCas9 enzyme as possessing enhanced gene editing specificity without sacrificing potency and broad targeting range.

"Opti-SpCas9 has a high on-target editing efficiency and enhanced editing specificity," explained Wong. "Unlike other high-fidelity SpCas9 enzymes, Opti-SpCas9 was the only variant in our tests that was compatible with guide RNAs containing an additional 5' guanine for transcription under the U6 DNA region promoter, without comprising its on-target activity.

"This is an important feature, because U6 is a widely used promoter in performing CRISPR screens," he said.

The new CombiSEAL platform should therefore be broadly applicable for engineering proteins through combinatorial modifications en masse.

"We believe this simple yet powerful strategy will accelerate the next-generation engineering of enzymes, antibodies, and genome-editing proteins with new or enhanced properties," said Wong.

"Our new technology platform could be easily implemented in many laboratories for the massive parallel engineering of DNAs and proteins relevant to a multitude of biomedical, therapeutic and biotechnology applications.

"We have filed a patent application based on this work and we will be will continuing our efforts to engineer new proteins with therapeutic value." (Choi, G.C.G. et al. Nat Methods 2019, Advanced publication).

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