Docket #: S18-411

DISCOVERY OF A HIGHLY THERMOSTABLE CAS9 VARIANT

Researchers at Stanford and the Chan Zuckerberg Biohub have discovered a Cas9 protein variant from *Ignavibacterium* that is thermostable at elevated temperatures.

The application of genome editing technologies, such as clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins, has revolutionized molecular biology and biomedicine. CRISPR-Cas9 systems are naturally occurring in many archea and bacteria and function to target and cleave invading nucleic acids. Many Cas9 proteins have been identified, each with its own temperature requirements for robust nuclease activity. However, most widely used Cas9 systems are challenged by elevated temperatures. For example, the highest previously reported active temperature for a Cas9 is 70?C.

Stage of Research
The inventors have identified a thermostable Cas9 enzyme that expands the temperature range of the CRISPR-Cas9 system. Through mini-metagenomic sequencing of hot spring samples from Yellowstone National Park, the inventors discovered and characterized a novel hypothermophillic Cas9 protein, IgnaviCas9, from an unculturable Ignavibacterium. IgnaviCas9 has a natural propensity to cleave DNA across a wide temperature range, circumventing the need for protein engineering. The inventors applied IgnaviCas9 in bacterial RNA-seq library preparation to deplete undesired amplicons and demonstrate its potential applicability in molecular biology, genomic and industrial workflows.

Stage of Development
Research – in vitro

Applications

- Implementation into RNA-seq workflow to selectively degrade unwanted amplicons and primer-dimers
- Genetically engineering thermophilic bacteria for industrial processes, like fermentation

Advantages

- Expanded temperature range of the CRISPR-Cas9 system
- Highly thermostable with nuclease activity at temperatures up to 100?C
- High stability for potential in vivo applications

Publications

- Schmidt ST, Yu BY, Blainey PC, May AP, Quake SR. Nucleic acid cleaveage with a hyperthemophillic Cas9 from an uncultured Ignavibacterium. Proc. Natl. Acad. Sci. USA. 116(46):23100-23105 (2019).

Patents

- Published Application: WO2020081808

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