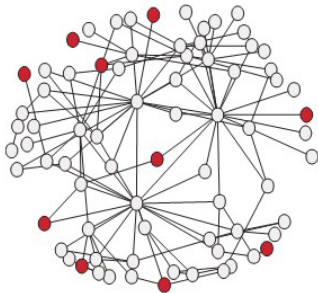


Many-gRNA CRISPR system for simultaneous binding to many targets

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Higher-order KD/KU screens

Technology Summary

This novel method enables the design of compact, stable DNA sequence cassettes, called Extra Long sgRNA Arrays (ELSAs), that produce up to 20 CRISPR guide RNAs. By combining sequence design, biochemical characterization, and machine learning, the inventors created a large toolbox of nonrepetitive CRISPR genetic parts that maintain their functionalities without introducing repetitive DNA sequences. Optimization algorithms assemble these nonrepetitive CRISPR genetic parts into highly compact ELSAs that express the desired number of guide RNAs. Their largest ELSA contains 100 nonrepetitive genetic parts, expresses 20 guide RNAs, but is only 4186 DNA base pairs long. The compactness and nonrepetitiveness of these ELSAs make them particularly easy to build and introduce into cells. ELSAs can be used for CRISPR genome editing and gene regulation.

Application & Market Utility

The inventors demonstrated how ELSAs can be utilized: 1. They introduced ELSAs into cells to knock-down the expression of several metabolic enzymes, redirecting flows of carbon, energy, and electrons and increasing production of a valuable chemical by over 150-fold. 2. They introduced ELSAs into cells to create a multi-auxotrophic strain, illustrating their long-term stability. 3. They introduced ELSAs into cells to deactivate antibiotic stress responses that cause cell persistence after antibiotic treatment. ELSAs have widespread applications.

Next Steps

Speaking with Dr. Salis regarding the design of Extra Long sgRNA Arrays (ELSA) for potential end-users' cellular engineering applications as well as the use of his design algorithms for engineering ELSAs.

TECHNOLOGY READINESS LEVEL

4-7

Seeking

Investment | Licensing | Research

Keywords

- CRISPR/Cas9
- gene editing
- cellular engineering
- sgRNA
- design algorithms

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