Enhanced CRISPR-based trackable protein engineering using modeling

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Project Goals: CRISPR EnAbled Trackable genome Engineering (CREATE) is a strategy that combines CRISPR/CAS9 editing with multiplexed oligo synthesis, enabling mapping of mutations to traits of interest. In this project, we attempted to apply CREATE to protein engineering. This research project addresses two important issues in developing optimal CRISPR-based trackable protein engineering: efficient cutting of the target site by modulating CAS9 promoters and homologous recombination by controlling homology arm. Modeling aided optimization would provide a better understanding of CREATE system and overcome bottlenecks in implementing CREATE for protein engineering. The long-term goal of the project is to develop optimal protein engineering system in various microbial cell factories including bacteria and yeast.

Humans have been used microbes to produce fermented foods and beverages for a long time. Recently, microbes have been harnessed to produce value-added products as cell factories. Many research groups are investigating strategies to use microbial cell factories for sustainable and economical protein production such as enzymes and antibodies. Because the microbes have extensive regulation between metabolic enzymes, protein engineering by error-prone PCR and rational design sometimes leads to disruptive cellular metabolism and regulatory mechanism. In addition, our knowledge is limited to uncover the underlying system controlling metabolic homeostasis (1). Advances in synthetic biology technologies such as CRISPR/Cas9 system enabled researchers to overcome bottlenecks in microbial genome editing (2). CREATE is an advanced approach based on CRISPR/CAS9 editing and barcode tracking, and it enables multiplex editing and mapping at the genome scale (3).

Currently, we are attempting to apply this CREATE tool to protein engineering. To overcome the bottlenecks in improving editing efficiency of the CREATE system, additional optimization of CRISPR/CAS9 editing and cassette design is necessary. First, modulating CAS9 promoters can control CRISPR toxicity in microorganisms. Modeling aided optimization might improve CAS9 cutting efficiency, resulting in enhanced throughput of the CREATE technology. Second, cassette design including the homology arm (HA) length and the distance between PAM-codon/target site effects the editing efficiency. The validation of cassette design based on the factors is also essential for high-throughput and precise protein engineering. The results can be used as the conditions for cassette design automation.

Our long-term goal is to develop the optimized system for protein engineering including antibodies and enzymes. For example, CREATE-based single chain antibody mutants might provide high-affinity antibodies with trackable high-throughput mapping of desired phenotypes. We could evaluate the

contribution of each mutation to the improved affinity. Also, CREATE-based enzyme evolution might provide a better understanding of protein fitness landscapes than random mutagenesis in optimizing protein function. We envision that the CREATE technology will enhance understanding of complex biological networks in protein engineering and further improve biofuel production in microbes.

References

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