Method for RNA-guided Endonuclease-based DNA Assembly
Technology #18595

Applications

The current invention can be used for complex DNA assembly in vitro using multiple DNA fragments. The DNA assembly is not restricted by the sequence of the DNA fragments, and can be performed efficiently in one reaction mixture.

Problem Addressed

Current methods for DNA assembly, such as homology-directed assembly or restriction-ligation assembly, are limited by various sequence-based constraints. Homology-directed assembly becomes inefficient as the number of DNA fragments increases. Even minor unintended homology regions of DNA can result in decreased efficiency. Restriction-ligation assembly is limited by unique restriction enzyme site availability, back-ligation rate, and the total number of assembly junctions determined by the length of sticky end overhangs. The current invention utilizes RNA-guided single strand endonuclease to generate longer sticky end overhangs at user-defined sequences, thus, resolving most of the limitations of the current technologies.

Technology

The current invention relies on RNA-guided endonuclease such as Cpf1 to create a sticky ended DNA break at desired sites. This is performed simply by incubating Cpf1 protein, guide RNAs, and a dsDNA template together. This results in 4-5 nucleotide overhangs, which can be used to assemble and ligate DNA fragments. This reaction is efficient in ligase buffer, allowing for “one-pot” DNA assembly with ligation happening in the same reaction mixture. A variety of engineered Cpf1 proteins are available with different protospacer adjacent motif (PAM) requirements able to be targeted by the endonuclease. Since guide RNAs can be designed against almost any sequence, this invention removes many of the sequence-based constraints of current technologies.

Advantages

- RNA-guided endonuclease-based DNA assembly doesn’t have the same sequence-based constraints of current technologies
- Cpf1 sits on the targeted DNA with low dissociation rate, thus, limiting back ligation
- Cpf1 generates longer sticky ends of 4-5 nucleotides, compared to most restriction enzymes, thus, allowing for increased number of unique assembly sites

Categories For This Invention:

Life Sciences
Biotechnology
Genomics
Research Tools

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