Avoiding CRISPR: Plasmid design for genetic engineering

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Abstract

Clustered regularly interspersed short palindromic repeats (CRISPR) is a natural defense system for bacteria and archaea against foreign DNA and RNA. Specifically, short snippets of foreign DNA or RNA are incorporated into protospacer adjacent motif (PAM) repeats sequences in the genome of the bacterial species, and serve as molecular memory of past infections by viruses. These repeats are transcribed by RNA polymerases and perform constant surveillance of the bacterial cell cytoplasm for foreign DNA. Once detected, PAM sequences would bind to the foreign DNA leading to the recruitment of Cas endonuclease protein that cut the foreign DNA. Plasmids are double stranded DNA vectors that serve to carry foreign genes into the cell for genetic engineering. Hence, plasmids are also foreign DNA with respect to the CRISPR system of the cell. To avoid destruction by the Cas protein, plasmid should not contain sequences that would bind to any of the PAM sequences encoded in the genome of the bacterial species. Thus, the PAM sequences of each bacterial species where genetic engineering is to be performed should be sequenced, and the knowledge gained utilized in the design of plasmid vectors that do not carry any of the sequences encoded by PAM repeats. Such an approach would help reduce the chances of destruction of plasmid vector once it was introduced to the cell, and would help improve the efficiency of plasmid transduction and genetic engineering. Collectively, CRISPR is a natural cellular defense system that could destroy introduced plasmid vector through recognition by PAM repeat sequences encoded in the cell's genome. Sequencing of the PAM sequence of the bacterial species followed by careful design of the plasmid DNA sequence would significantly reduce the chances of destruction of the vector by CRISPR once it was introduced into the cell.

Keywords: genetic engineering, CRISPR-Cas, foreign DNA, PAM repeats, vector, endonuclease, bacteria, archaea, plasmid design,

Subject areas: biochemistry, molecular biology, microbiology, biotechnology, bioengineering,

Conflicts of interest

The author declares no conflicts of interest.

Author's contribution

The author thought about how clustered regularly interspersed short palindromic repeats (CRISPR) could cut and degrade foreign DNA, and realized that plasmids for genetic engineering must be

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designed to avoid incorporating sequences that could be recognized by the CRISPR system as foreign. He wrote the abstract preprint.

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