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Transplanting a Bacterial Immune System: Determining the Function of a Novel CRISPR System

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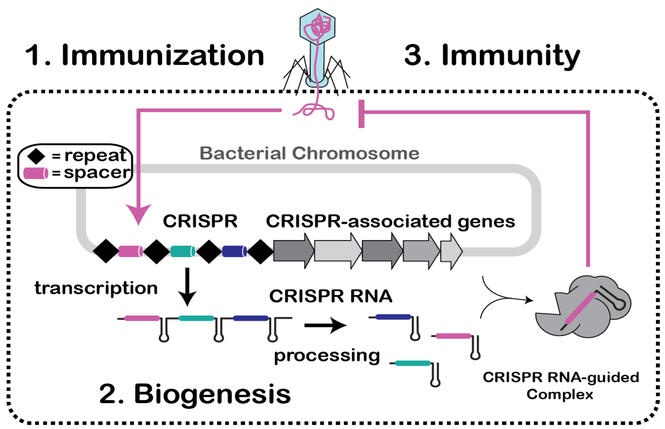
I. Introduction

CRISPR (Clusters of Regularly Interspaced Short Palindromic Repeats) loci and *cas* (CRISPR-associated) genes provide adaptive immunity (see panel below) in bacteria and have recently been repurposed for genome editing.

Systems are structurally and functionally diverse.

- 2 classes, 6 types, 33 subtypes
- Very few have been studied experimentally
- None of the Type IV systems have been characterized



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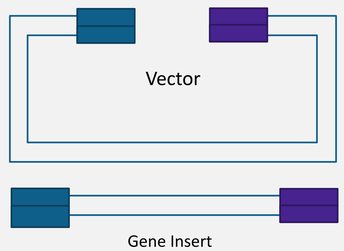
II. Methods

Nothing is known about Type IV CRISPR system function. To discover the structure and function of the system, we created a circular piece of DNA (vector) that contains all of the Type IV CRISPR system genes.

Transfer vectors were made with a single gene and various affinity tags.

Restriction enzymes were used to cut the vectors. The genes were then inserted one at a time into the destination vector.

The vector was co-transformed with a second vector containing a CRISPR into *E. coli* cells.



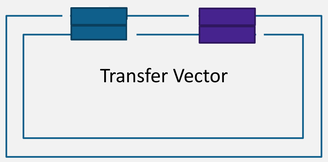
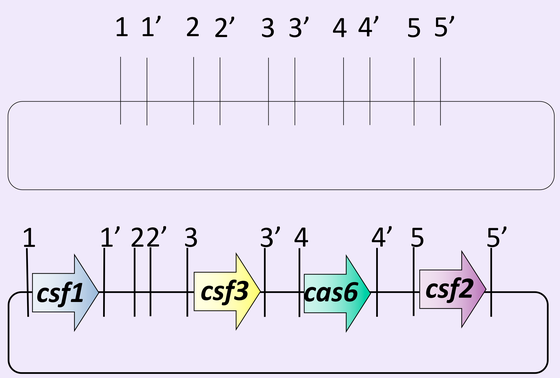


Figure 1 – A destination vector containing restriction sites for five cassettes was used to insert all four CRISPR genes

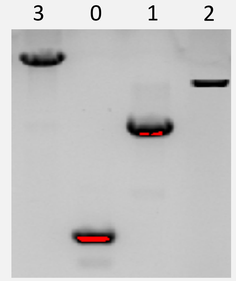


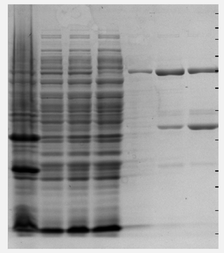
III. Results

Colony PCR (Polymerase Chain Reaction) is used as a diagnostic tool to ensure a gene was successfully inserted into the destination vector.

The top image shows the results for samples with subsequent genes added to the vector. As more genes are added, the size of the PCR product increases.

Protein expression tests are underway to determine the optimal growth conditions for co-expression of all Type IV genes.





This image shows expression of an MBP-tagged Type IV CRIPSR protein.

IV. Conclusions

- We successfully transplanted the genes of a Type IV immune system into *E. coli* cells.
- Our system is expressing proteins.
- Trials are underway to identify conditions that express an entire Type IV system complex.

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