Transplanting a bacterial immune system: Design, construction, and expression of a multi-subunit Type IV CRISPR system

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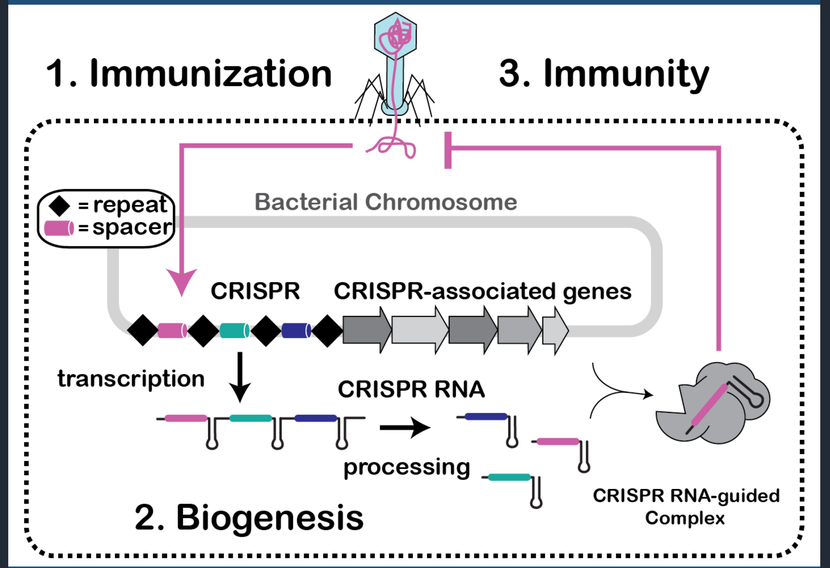
Introduction

CRISPR (Clusters of Regularly Interspaced Short Palindromic Repeats) loci and cas (CRISPR associated) genes provide adaptive immunity against invasive elements such as viruses and plasmids in bacteria and archaea, and these immune systems have recently been repurposed for genome editing technologies [1,2]. Each system provides immunity in three distinct stages; acquisition, biogenesis, and interference (see panel below) [5-6]. However, the systems are structurally and functionally diverse, consisting of two classes, six types, and thirty three distinct subtypes [4]. Of these thirty three distinct subtypes only a few have been studied biochemically and structurally. Specifically, no Type IV CRISPR systems have been studied to date and their biological and mechanistic functions remain unknown.

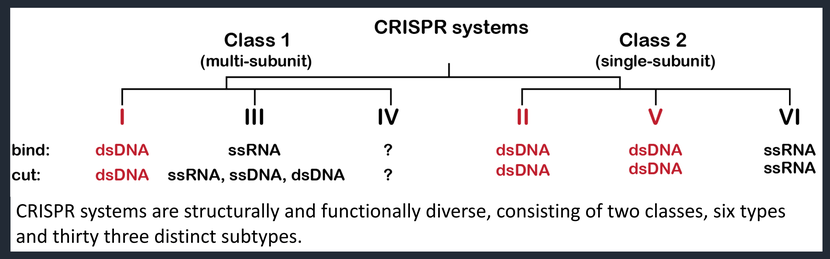
The overarching goal of the Jackson Lab is to determine the structure and function of uncharacterized CRISPR systems, but many uncharacterized systems are found in organisms that are difficult to culture in the lab. To study the CRISPR systems of these obscure organisms we want to transplant the immune system into a easy to grow bacteria (*E. coli*) that lacks a CRISPR system. **We hypothesize that the creation of a polycistronic vector containing all Type IV genes is necessary to allow us to express a Type IV system in *E. coli*.**

The purpose of this project is to create a polycistronic vector containing all Type IV genes. Ligation-independent cloning was used to create transfer vectors with each of the Type IV CRISPR genes along with either no-tag, a histidine-tag, a strep-tag, or a maltose binding protein tag. Various polycistronic vectors were made with various tag combinations.

CRISPR System and Function



Shown is the mechanism of the adaptive immune system of a CRISPR system; acquisition (immunization), biogenesis, and interference (immunity). Through these steps a cell is able to defend itself against foreign DNA by incorporating the nucleic acid into the CRISPR system.



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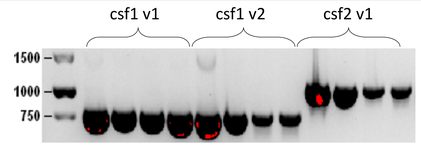
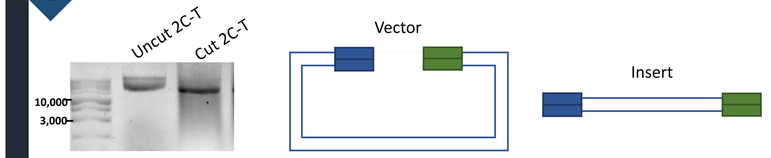
Methods

1. • Ligation Independent Cloning: PCR amplification to add LIC tags



Primers were designed to amplify the Type IV CRISPR system genes. Ligation-independent cloning adds long DNA tags to the primer sequence to create single-stranded overhangs on the PCR product.

2. • Ligation Independent Cloning: Restriction Digest – linearize plasmid



A restriction digest of transfer vector 2C-T. The band of the cut sample was gel extracted to ensure that no circular plasmid remained.

3. • Ligation-Independent Cloning: Create single stranded overhangs

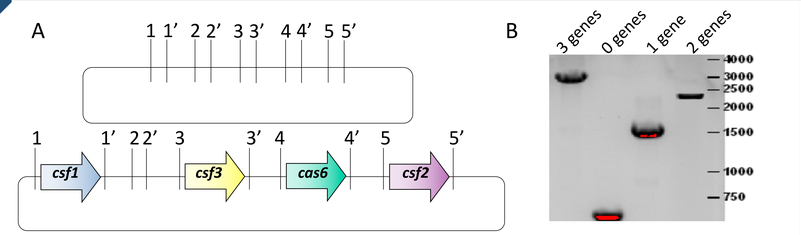
Create single stranded overhangs using T4 DNA Polymerase with exonuclease activity. These overhangs make it possible for the insert and vector to anneal together without using ligase.

4. • Ligation-Independent Cloning: Transform into competent *E. coli* cells

Organism	Gene	Tags
AF	*csf1*	No tag, N-terminal His, N-terminal MBP, N-terminal Strep
AF	*csf2*	No tag, N-terminal His, N-terminal MBP
AF	*csf3*	No tag, N-terminal His, N-terminal MBP
AF	*cas6*	No tag, N-terminal His, N-terminal MBP

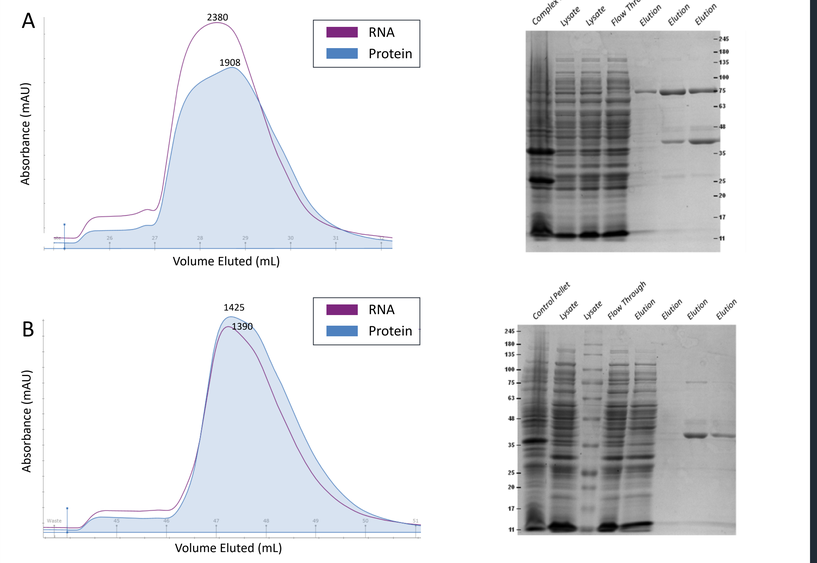
The *Acidithiobaccillus ferrooxidans* (AF) Type IV CRISPR genes that have successfully been cloned into transfer vectors using ligation-independent cloning.

5. • Traditional Cloning: Polycistronic Vector

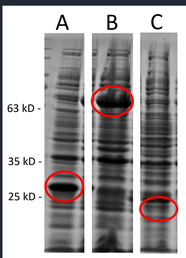


Type IV CRISPR system genes will be inserted into a polycistronic vector using traditional cloning methods. The destination vector and transfer vector with the gene insert are both digested with the specific enzymes for the cassette, then gel extracted, ligated, and transformed into competent cells. The completed polycistronic vector will contain all four Type IV CRISPR system genes.

Results



The complex (*csf1, csf2, csf3, cas6,* and CRISPR) and a control of csf2 with an MBP tag were grown and induced at 22 degrees Celsius. Both samples were ran over a MBP column for protein expression in order to determine if a complex was formed.



Construct	IPTG (mM)	Temperature		
		16°C	22°C	37°C
AF_3T4_His_4XCRISPR	1	1	2	3
AF_3T4_MBP_4XCRISPR	1	1	2	3
AF_6T4_His_4XCRISPR	1	1	3	2
AF_6T4_MBP_4XCRISPR	1	1	3	2

A - B. Protein gel images of an AF complex comprised of a polycistronic vector (*csf1, csf2, cas6*), a tagged *csf3* (His and MBP, respectively), and a CRISPR. The circled bands are the tagged *csf3* protein. C. Protein gel image of a *Mahella australiensis* (Ma) His-tagged *cas6* as a positive control. The circled band shows the lack of a protein band that is expressing in the complexes. The table shows the ranking of expression trials at three temperatures, 1 indicating the strongest expression and 3 indicating the weakest.

Conclusions

- We successfully transplanted an immune system into *E. coli* cells
- Our system is expressing proteins
- We will continue to try various constructs of the complex in order to determine if a complex can form *in vivo.*

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