Design and Construction of Single-Spacer Type-IV CRISPR Sequences

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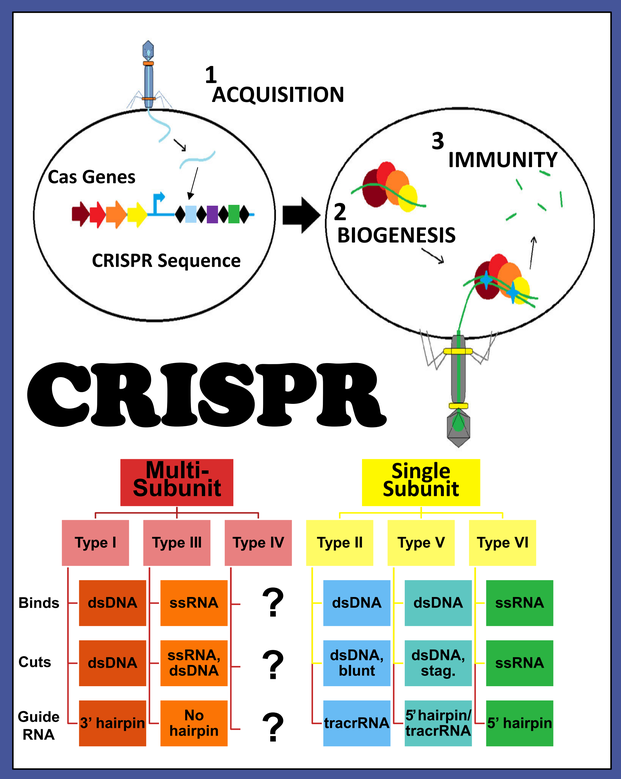
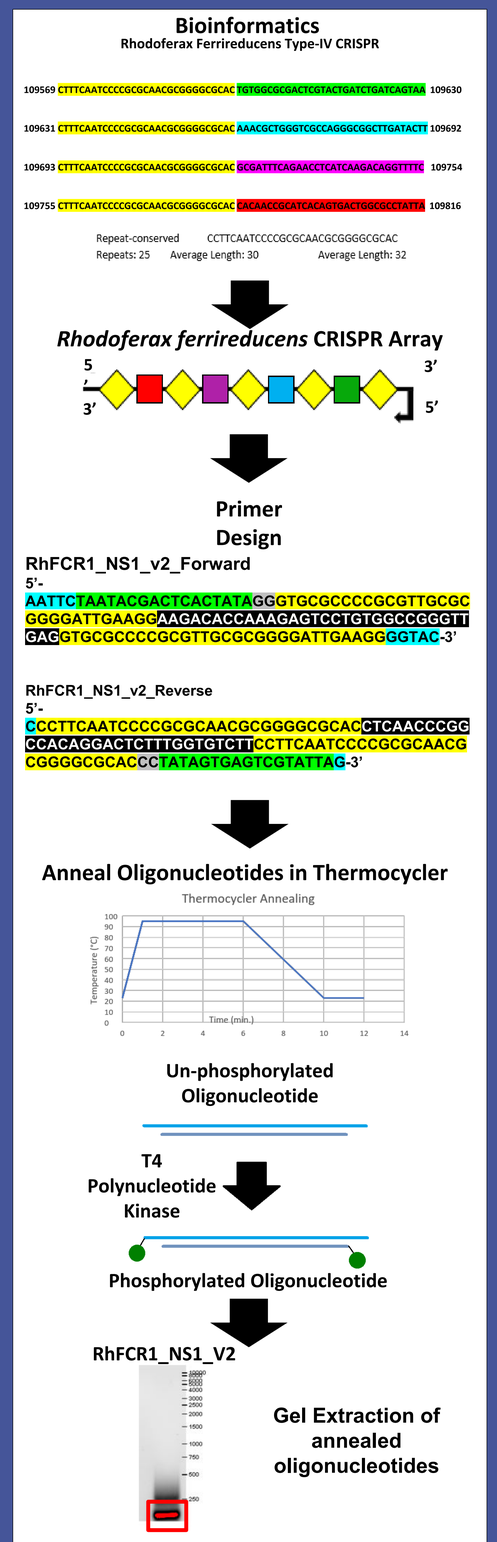
Introduction

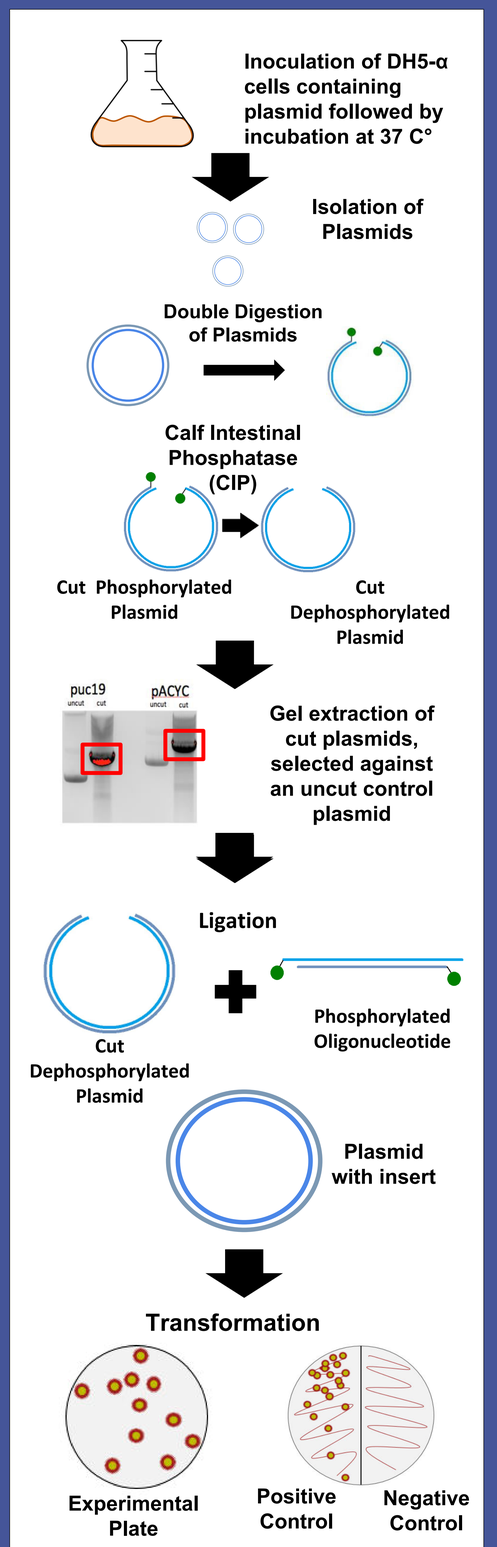
CRISPR systems are RNA-guided microbial adaptive immune systems that have been repurposed for applications in medicine, pharmacology, and agriculture. The hallmark of CRISPR immune systems is the CRISPR sequence contained in the host chromosome, which consists of short direct-repeats of 20-40 bases, followed by unique spacer sequences of about the same length. In 2005 several bioinformatics groups discovered that CRISPR spacer sequences in bacteria and archaea were identical to known microbial pathogens such as viruses and plasmids, suggesting that CRISPRs serve as a molecular memory of encounters with foreign nucleic acid. Since 2005 basic research on these systems has revealed the CRISPR is transcribed and processed into a library of small RNAs. Each CRISPR derived RNA (crRNA) combines with CRISPR associated (Cas) proteins to form ribonucleoprotein complexes that survey the intercellular environment and use the crRNA as a guide to bind complementary DNA or RNA sequences. Once the complex is bound, it elicits an immune response that activates cis- or trans-acting nucleases that destroy the target before it can infect the host. All CRISPR systems follow these general steps to provide immunity, and because CRISPR systems can be programmed with RNA guides to target nucleic acid, they have been repurposed as genome editing tools. However, CRISPR systems are incredibly diverse, and we hypothesize that additional CRISPR-based technologies are waiting to be discovered through basic research. To determine the structure and function of a type IV CRISPR system, we aim to recombine the genetic components of the system into E. coli and express the system. We aim to determine what components are required to provide immunity from nucleic acid hosts, and will attempt to purify CRISPR complexes from these cells to investigate their function *in vitro*. Our role in this overarching goal is to design and synthesize the CRISPR component of the immune system.



Preparation of Insert

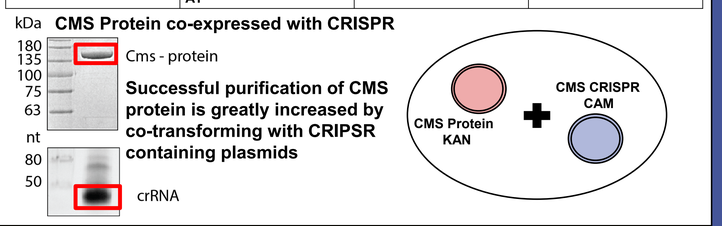


Traditional Cloning



Results and Application of CRISPR

Organism	Repeat	Spacer	Plasmid
Acidithiobacillus Ferrooxidans	CTTCTCAGCCGCGCGTGTG GCGGCATACCGC	GCAGTCAGGGCATCTGCAA CTTGCGCGAT	pACYC, PUC19
	GTATGCCGCCAGGTGC GCGGCTGGGAAGA	TATGACGCAGATCCGC GCGGGATCA	pACYC, PUC19, pCDF, pCOLA
	GTATGCCGCCAGGTGC GCGGCTGGGAAGA	CTTTACACTTTATGCTTC CGGCTCGTATGT	pACYC, PCDF
Rhodoferax Ferrireducens	GTGCGCCCCGCGTTGC GCGGGGATTGAAGG	TGTGGCGCGACTCGTAC TGATCTGATCAGTAA	PUC19
	GTGCGCCCCGCGTTGC GCGGGGATTGAAGG	TCGCTCGAAGGTATTGA CACGACGCTGACCACG A	PUC19
	CCTTCAATCCCCGCGCA ACGCGGGGCGCAC	GTCGAGTGCAAAACCTT TCGCGGTATGGCATG	pACYC
Mahella Australiensis	GTGCAGATACCTTCTAT GAGGGGTTAAAAG	TTCACTATAAATGAAAA TTTCAGGCAGCGCTCGG ATT	pACYC
CMS	GTTTAAAACCACTTTAA AATTTCTACTATTGTAG AT	TGGAGCAACACCTGAA GGAAGGCTTGAT	pACYC



Conclusions & Future Directions

With the completion of several native CRISPR sequences, derived from various organisms containing a type-IV Cas system, we can begin biochemical analysis. Currently, we are working on determining the viability of our completed CRISPR sequences, via in-vitro T7-transcription. Additionally, we are working on constructing polycistronic vectors (microbial based plasmids that contain several type-IV Cas genes) for bioassay testing. Bioassays will allow the Jackson lab to determine the requirements for the various aspects of CRISPR function: Acquisition, biogenesis, and immunity. The Jackson lab is now in the process of purifying the individual protein components of the type-IV Cas system. We aim to crystallize each protein, and determine the structure of the proteins via x-ray crystallography.

References

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Ryan Jackson Lab Start Up