CRISPR-Assisted Genetic Engineering
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**Background: What is CRISPR?**
Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) are a genomic feature of many bacterial and archaeal species. CRISPR functions as an adaptive immune system, protecting the host from invasion by plasmids and bacteriophages.

Its locus consists of a set of CRISPR-associated (Cas) genes, a leader sequence, and an array. This array consists of repeating elements along with "spacers", which direct the CRISPR machinery to target and destroy a complementary sequence of DNA or RNA in the cell.

**A New Platform for Gene Silencing**
ASU's 2011 iGEM team developed a project which sought to develop a modular platform for gene manipulation. During the execution of our project we:

- Explored multiple approaches for testing a system for gene silencing
- Expanded our implementation of the BioBrick concept to include using the endogenous genomes of organisms
- Designed a software tool that offers accessibility to others labs who hope to utilize our platform for gene manipulation

**Design**
We configured our experimentation with CRISPR systems using the following modular design elements:

- **Spacer Selection:** Spacers (~30bp) can be created to selectively target any sequence of DNA or RNA proto-spacers.
- **Proto-spacers** have short nucleotide tags called proto-spacer adjacent motifs (PAMs). These short sequences are recognized by the Cas machinery.

**Array Construction:**
- **Leader Sequence:** Promoter sequence that proceeds the RSR array
- **Cas Genes:** The Cas genes can be utilized in two ways:
  - Amplify gene and insert into testing plasmid
  - Insert array into strain with functional Cas genes

**Endogenous E. Coli Cas Testing**
Samples containing the Leader+RSR construct showed significantly increased transformation efficiency, contrary to the expected suppression due to the presence of the array.

**L. Innocua: DNA-Targeting**
Advantages of this CRISPR system:

- **Compactness:** A single Cas gene (Cas9) is much smaller and more easily amplifiable than those of E. coli or B. halodurans
- **Better Regulation:** A trans-encoded CRISPR RNA (tracrRNA) is required for function and allows for an extra level of regulation
- **More Options:** The proto-spacer adjacent motif (PAM) is smaller than in other CRISPR hosts, increasing the variety of spacers

**Future Applications**
A cell-free CRISPR system could be used for:
- Verifying successful CRISPR construction and function
- Characterizing spacer integration into the RSR array and model spacer targeting
- Providing a mechanism to study the evolution of CRISPR in a simplified environment

A functioning RNA-targeting CRISPR system could be used to modulate gene expression in a given production pathway

**Acknowledgements**
- Intern Juan Padilla
- Deans Mark Jacobs and Margaret Nelson
- Dr. William Ditto, Dr. Marco Santello
- Deans Paul Johnson and Jim Collofello
- ASU President Dr. Michael Crow