Engineering Bacterial Immunity

Combating Antibiotic Resistance Using CRISPR-Cas Pathway

What is iGEM?

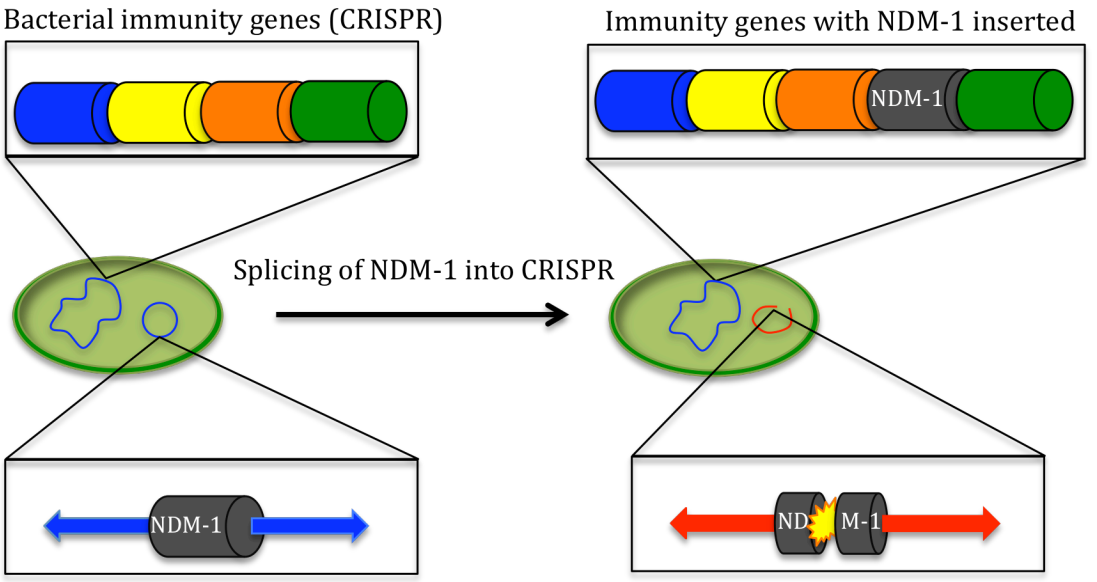
The International Genetically Engineered Machine (iGEM) competition is the premiere undergraduate synthetic biology competition, encouraging students around the world to develop novel applications for genetic engineering. The competition has grown from 5 universities in 2004 to over 160 universities in 2011 worldwide. This year iGEM will be holding a regional competition for the Americas at the Institute for Biological Engineering in Indianapolis, where a portion of contestant universities will move on to the World Championship. The World Championship will be held at the Massachusetts Institute of Technology in November 2011. Projects are judged by their innovation and application of gene networks to form products with novel functions. Examples of past projects include a *H. pylori* vaccination candidate, bacteria that solve sudoku puzzles, heavy metal detection using *E. coli*, and a waterborne parasite detection system. As ASU's inaugural team we plan to focus on NDM-1 acquired antibiotic resistance and the CRISPR-Cas pathway.

Global Challenge

The WHO and CDC consider antibiotic resistance to be one of the most pressing global health threats, compromising the effectiveness of our most important tool in fighting bacterial infection. NDM-1 allows bacteria to be resistant to a broad range of commonly used antibiotics and has spread rapidly from India and Pakistan throughout the world. Alternative solutions must be promptly and intelligently employed to counter this threat.

Bacterial Immunity

CRISPR functions as a bacterial immune system by incorporating and targeting foreign genetic elements. The modularity of this system presents the opportunity for unique applications. Our innovative solution uses CRISPR to silence the NDM-1 gene, consequently developing a new method that can be utilized for many novel gene manipulations in bacteria. This project combats an important global issue with cutting-edge science. As Arizona State University's first iGEM team, we have chosen an ambitious project that has great potential to advance the field of synthetic biology, bringing an international spotlight to ASU and the Phoenix community.



Our Project in Depth

NDM-1 in Perspective

Global antibiotic resistance is a concern of the utmost importance to the World Health Organization and healthcare everywhere. Bacteria that have acquired antibiotic resistance jeopardize world healthcare as a whole, because they increase mortality rate of normally curable infections, and there is no coherent approach to containing and countering resistant strains. New Delhi Metallo-Beta-Lactamse (NDM-1) containing bacteria are particularly ominous because the NDM-1 enzyme hydrolyzes a broad range of potent beta-lactam antibiotics (e.g. carbapenems). This enzyme is effective in rendering normal lines of treatment for bacterial infection useless. NDM-1 positive strains originated in India and Pakistan and have recently spread to the UK, Europe, and Canada. There has also been a drastic increase in the number of reported NDM-1 positive cases in the United States, according to the Centers of Disease Control and Prevention. Viable antibiotics as a resource are becoming more and more deficient. Alternative solutions to resistance must be promptly sought and intelligently employed to counter the threat of antibiotic resistant bacteria.

The CRISPR Mechanism

The CRISPR-Cas pathway can be compared to a prokaryotic immunity or RNA interference that can be directed to silence a gene of interest. This mechanism of bacterial survival affords us an interesting method to tackle the aforementioned problem. Clustered regularly interspaced short palindromic repeats (CRISPR) gene loci have been demonstrated to equip both prokaryotes and archaea with a defense mechanism against exogenous DNA and RNA sequences.[1,2] CRISPR genes appear in an array that contains contiguous spacers, repeats, and an operon of structural genes. The transcripts from the spacer/repeat region undergo hair pinning due to the palindromic sequence structure. The peptide products of the CRISPR-associated structural genes (CAS) work cooperatively with crRNA to silence a complimentary target (Diagram 1).[3] The function is a prokaryotic analog to both RNA interference and immunity. CRISPR quickly presents itself as a potentially useful tool in prokaryotic gene manipulation. Our goal as ASU's first iGEM team is to develop a CRISPR plasmid that contains elements to target and silence the NDM-1 gene sequence (Diagram 2). While targeting NDM-1, we recognize that CRISPR can potentially target any gene of interest, thus we will develop a robust platform for gene silencing. The final product of this project will be a fully functioning CRISPR array that will be submitted to the Standard Registry of Biological Parts, an open-source collection of DNA building blocks, as a BioBrick, a modular component for genetic engineering (Diagram 3).

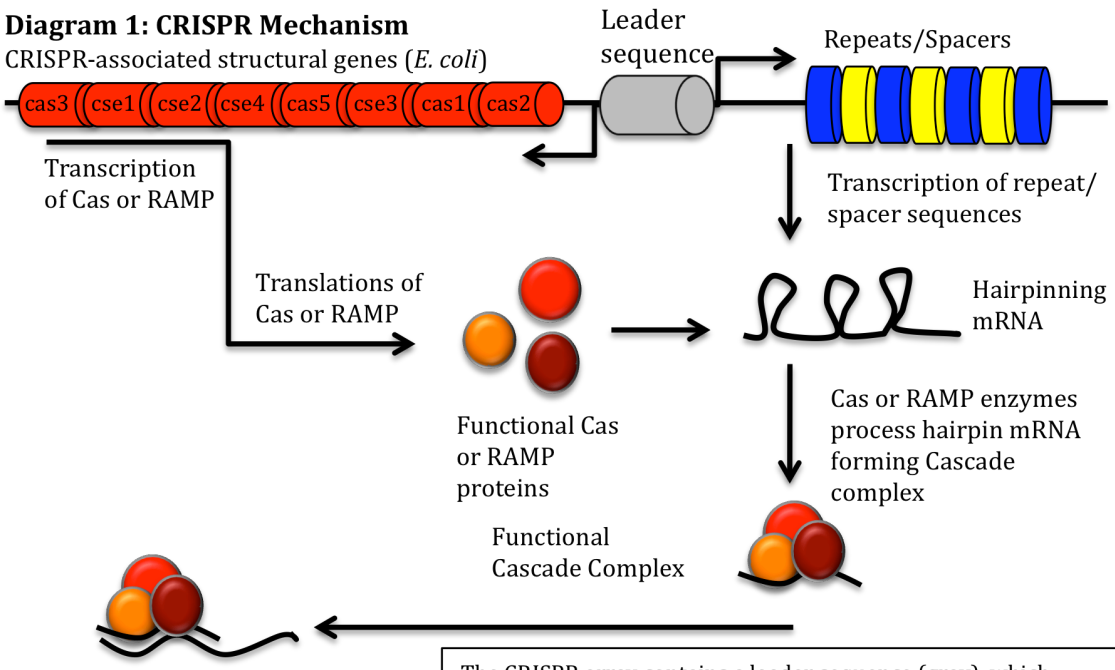
Our Team

ASU's inaugural iGEM team consists of ten highly motivated undergraduates from diverse backgrounds in genetics, biomedical engineering, molecular biology, computer science, and biochemistry. Our project is completely student-driven and an incredible educational opportunity. In the context of the iGEM competition, it provides us with the means to directly contribute to the field of synthetic biology in a significant way and present our findings on an international stage.

Support: How You Can Help

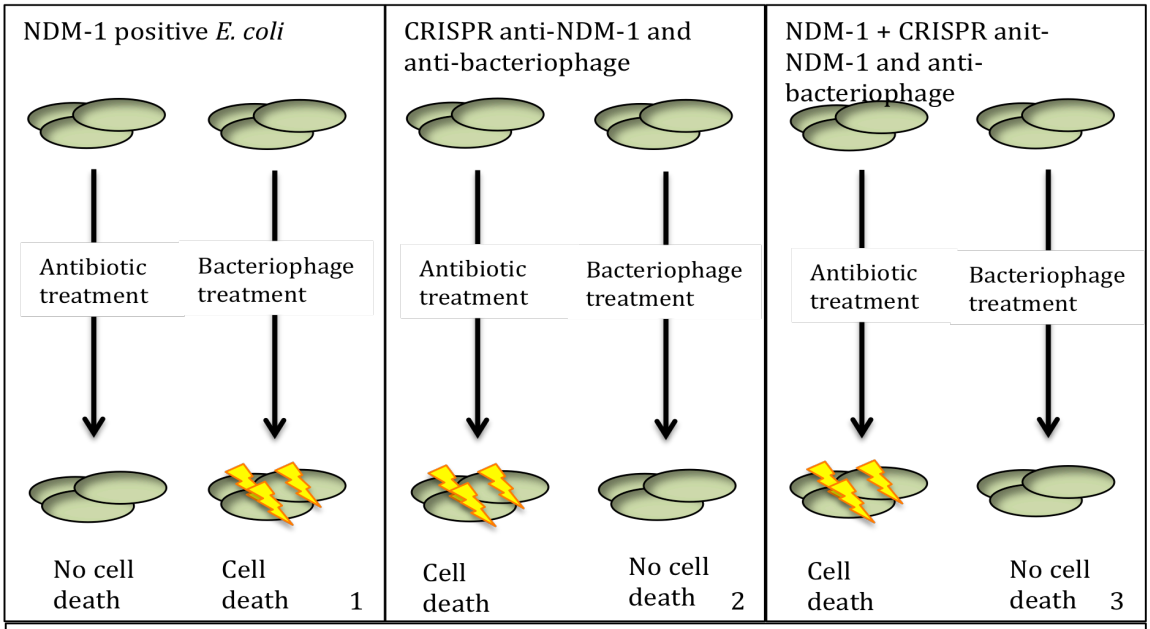
The success of this project will undoubtedly depend on the generosity of others. We are seeking support in the form of monetary sponsorship, lab materials, and sharing of expertise and opinions. Any contribution will be very much appreciated.

Diagram 1: CRISPR Mechanism



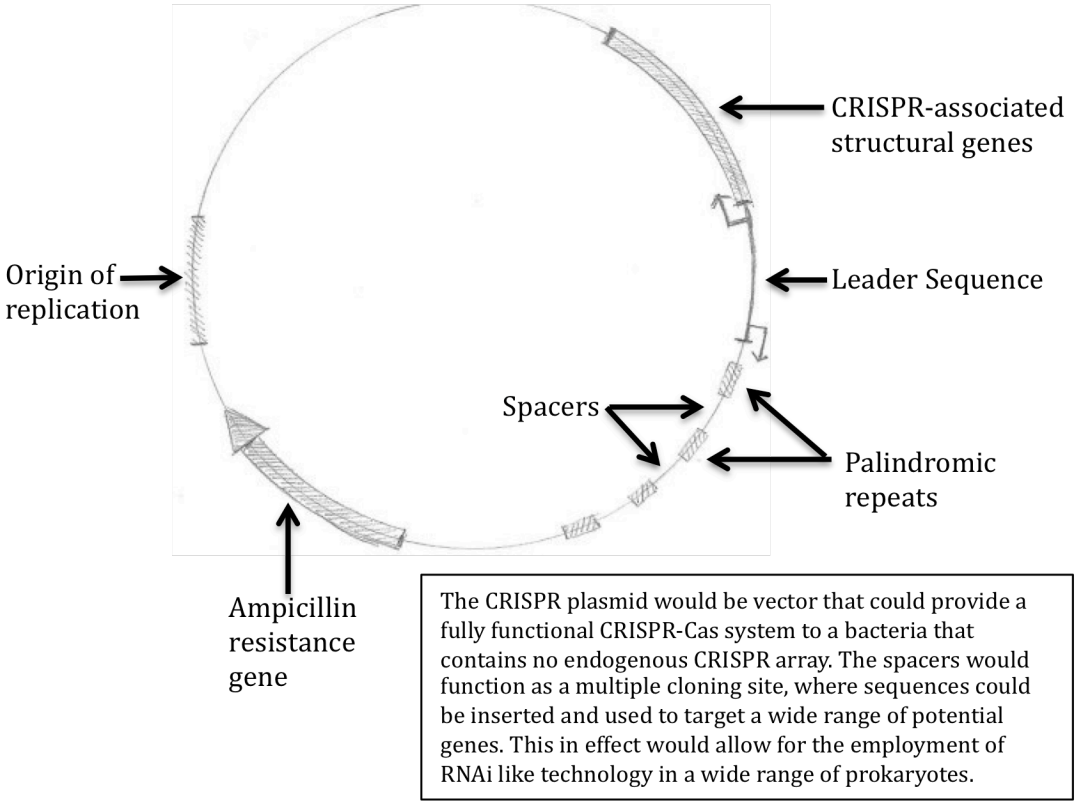
The CRISPR array contains a leader sequence (gray), which functions as a promoter for both the CRISPR-associated (Cas) or Repeat-associated mysterious proteins (RAMP) and the repeat (blue)/spacers(yellow). Once the reapeats/spacers sequence is transcribed, it forms hairpins, which are recognized by the Cas or RAMP enzymes and combined to form the Cascade complex. The Cascade complex finds a complimentary target and degrades it.

Diagram 2: Basic Experimental Concept



NDM-1 positive *E. coli* (box 1) would be resistant to antibiotic challenges but would be susceptible to bacteriophage introduction. On the other hand (box 2) *E. coli* containing a CRISPR array targeted against NDM-1 and the bacteriophage would be vulnerable to antibiotic treatment but not phage introduction. The combination of both NDM-1 and the CRISPR array (box 3), should have diminished resistance to antibiotic challenges and increased survival in phage treatment.

Diagram 3: CRISPR Plasmid Basic Concept



The CRISPR plasmid would be vector that could provide a fully functional CRISPR-Cas system to a bacteria that contains no endogenous CRISPR array. The spacers would function as a multiple cloning site, where sequences could be inserted and used to target a wide range of potential genes. This in effect would allow for the employment of RNAi like technology in a wide range of prokaryotes.

Contact Information

If you have any questions, comments, or considerations please email us at: asu.igem@gmail.com

References

1. Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, Romero D, Horvath P. CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes. *Science* 315, 1709-1712 (2007).
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3. Brouns SJ, Jore MM, Lundgren M, Westra ER, Slijkhuis RJ, Snijders AP, Dickman MJ, Makarova KS, Koonin EV, van der Oost J. Small CRISPR RNAs guide antiviral defense in prokaryotes. *Science* 321,960-964 (2008).