CRISPR/Cas9 LockOn

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# Specific Aims

Think of this as an executive summary

Giving only minimal, high-level background information

And impact assessment

The key to the section

Set of numbered **Specific Aims** statements

Each representing an element of the research to be conducted

Specific Aims are your opportunity to make a positive first impression

# Background

Clustered regularly interspaced short palindromic repeats or CRISPRs are a particular family of tandem repeats found in a wide range of prokaryotic genomes (**Grissa, Vergnaud, & Pourcel, 2007**). They consist of a succession of highly conserved regions varying in size from 23 to 47bp separated by similarly sized unique sequences termed spacers. Associated with CRISPRs are endonucleases called Cas9 (CRISPR Associated Proteins) and short “guide” RNA (gRNA) which when coupled together in the CRISPR system can become an essential tool for molecular scientists interested in targeting genes.

Starting with the gRNA, scientists can design a synthetic gRNA of about length 20bp that is complementary to a gene of interest. This targeting sequence or “spacer” sequence can be changed to be complementary to any part of the genome, i.e. target any gene of choice. However, two conditions must be met in order for the CRISPR/CAS9 system to knock a gene out. The first condition is that the spacer must be unique when compared to the rest of the genome, and the second condition is that the target is immediately upstream of a Protospacer Adjacent Motif (PAM) site. The PAM site is mandatory for target binding and the exact sequence of the PAM depends on the species of Cas9, i.e. the source bacteria from which Cas9 was derived (**Figure 1**).

Once the two conditions have been met, CRISPR/Cas9 binds to the DNA at the target site and performs a double stranded break and the resulting damaged DNA is then subject to either Non Homologous End Joining or Homologous Directed Repair depending on the experimental design.

The ability to effectively target a gene in the genome proves to be an invaluable tool for molecular biologist. One real world application would be in personalized medicine, if there is sufficient knowledge of genes associated with a disease such as Alzheimer's then scientists could cure Alzheimer's by designing a CRISPR/Cas9 system that can target and silence all genes associated with Alzheimer’s disease. Another real world application would be in population genetics, where CRISPR/Cas9 could be used in “driving” a gene in a given population into extinction or resurrection. Of course these real world applications pose ethical issues, but these applications also demonstrate the powerful utility of these “molecular scissors”.

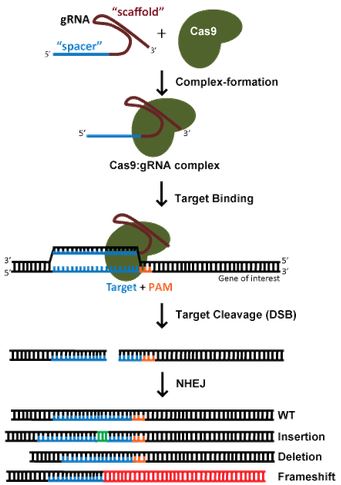


Figure : CRISPR/Cas9 Endonuclease System. Cas9 and a synthetic gRNA with the spacer sequence is coupled to form a Cas9-gRNA complex. The Cas9-gRNA complex binds to the gene of interest (blue) that is immediately followed by a PAM (orange). If there is sufficient homology between the target site and the gRNA and there exists a PAM, Cas9 cuts the DNA and exits, leaving the damaged DNA to repair itself either by Non-Homologous-End-Joining or Homology-Directed-Repair (Not Shown) to introduce mutations that will disrupt and silence the targeted gene.

# Preliminary Studies

Describes work that you:

– Have **already done**

Demonstrates that you will be able to achieve the aims that you describe

Background section focuses on other people's work, while **this section focuses** on **your** own previous work

There is a fine line here:

– Between showing that you have achieved good results in "pilot" study

– And hurting the significance of your proposal **because you have accomplished too much of it already**

Here you have the chance to show some preliminary data

# Research Plan

Demonstrates that you have determined how to **achieve the specific aims**

Describing preliminary work you have already accomplished helps establish your capabilities

Noting which Specific Aims are dependent on other aims is appropriate

You may choose to describe your backup plan if critical pieces do not turn out as expected

**Write in prose**, no bullet points!

# Proposal

We propose a tool for designing a CRISPR/Cas9 system that follows the rules for gene targeting and silencing. The programming project, named CRISPR/Cas9 LockOn, will consist of three pieces: a database, a user friendly script, and tests. The database portion of the project will consist of curating information about various Cas9 PAMs available for endonucleases and storing them in some database system along with the outputted data from our program. The second component of the project will be our program CRISPR/Cas9 Lock-On! that will be implemented in Perl and Bash. The preliminary aim of the program will be to effectively find all valid CRISPR/Cas9 targetable sites in Saccharomyces *cerevisiae* (baker’s yeast) strain S288c, but we do plan to scale up to more genomes in the future such as humans. Finally, the last part of our project will be testing and automating the program to ensure high quality user-interface (UI) experience.

The workload for each of the pieces will be divided evenly between Meryl Stav and myself Michael Tung. (table 1) All documentation of the project will be stored on github and will remain Open Source.

# Works Cited

Grissa, I., Vergnaud, G., & Pourcel, C. (2007). CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats. *Nucleic Acids Research, 35*(Web Server), 6.