Determining the Function and Structure of Sms1, A Type V CRISPR Effector Endonuclease

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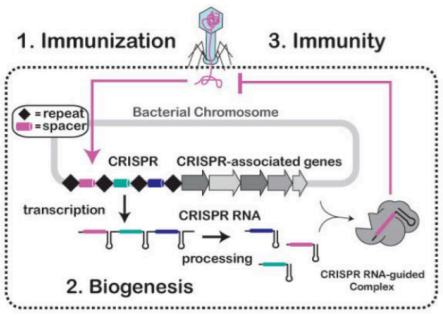
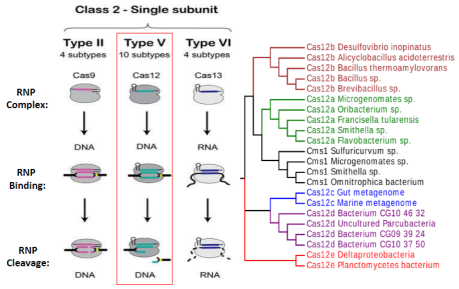
Introduction to CRISPR

CRISPR-Cas systems are prokaryotic adaptive immune systems. Bacteria use CRISPR systems as a defense against foreign nucleic acid invasion such as a phage infection.



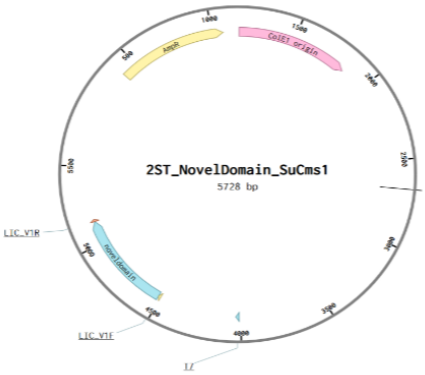
Type V CRISPR-Cas Systems Are Diverse

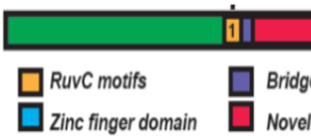


Hypothesis: Cms1 endonucleases contain a novel domain that helps assist in the cleaving of DNA.

Cms1 Endonucleases Contain a Novel Domain

- Type V Cms1 endonucleases contain RuvC motifs, a bridge helix, a zinc finger domain and a novel domain.
- LIC cloned Novel Domain into the Strep - tag containing 2ST vector.





Expression Method for Novel Domain

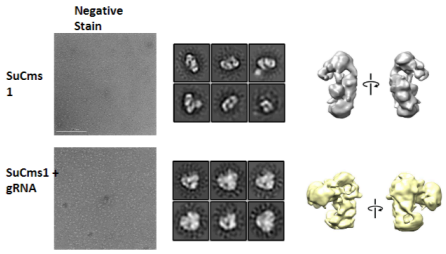
Protocol Outline

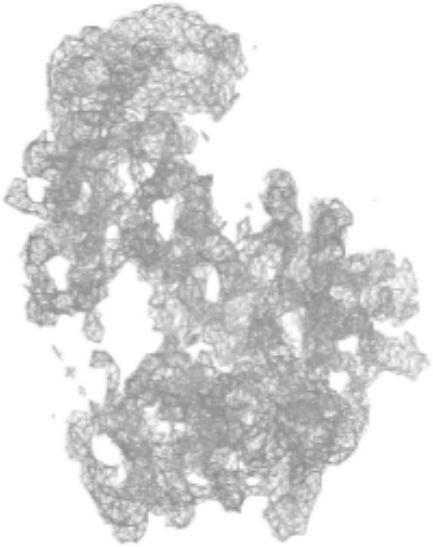
1. Clone novel domain DNA into expression plasmid.
2. Transform into competent cells.
3. Vary the growth protocol (i.e. temperature and time) to find conditions for optimal expression.

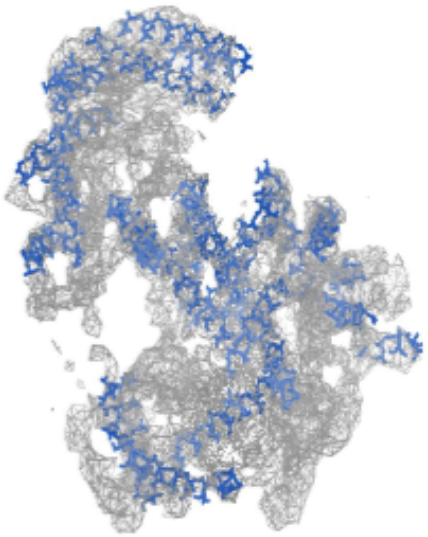
Expression Results:

- The total molecular weight we expected our novel domain to be was around 35 kDa.

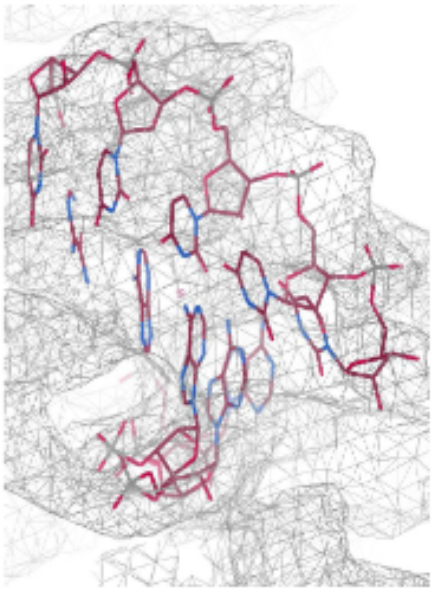
SuCms1 Undergoes a Conformational Change





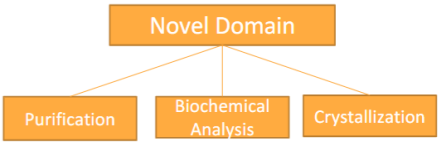


4 Angstrom Map
Secondary Structure



Modeling
Idealized A form RNA

Conclusions and Future Decisions



- Long term we hope that once we crystallize the novel domain we will use X-Ray Diffraction to hep add to the theorized structure that we currently have to help us better understand function and hopefully uncover new properties to help further gene editing technologies.

References

1. Begemann, Matthew B et al. 2017. *Cold Spring Harbor Laboratory*, doi:10.1101/192799. Accessed 27 Sept 2018.
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3. Zhang et al. EvolView, an online tool for visualizing, annotating and managing phylogenetic trees. Nucleic Acids Res (2012) pp. 4. (http://nar.oxfordjournals.org/content/40/W1/W569) He et al. Evolview v2: an online visualization and management tool for customized and annotated phylogenetic trees, Nucleic Acids Res, (2016). (http://oxfordjournals.org/content/44/W1/W236)