Developing a CRISPR/Cas9 System for Volvox Carteri

Robyn Jasper, Jose Ortega, Stephen Miller, PhD

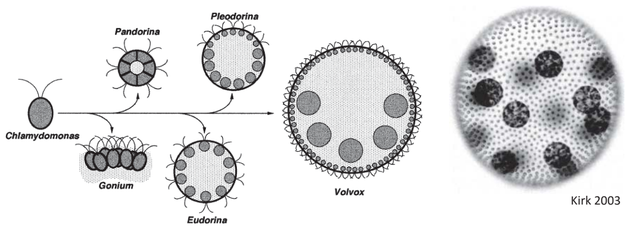
Department of Biological Sciences, University of Maryland, Baltimore County

UMBC
AN HONORS UNIVERSITY IN MARYLAND

Abstract

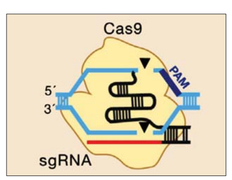
Genome editing is a tool implemented to test gene function through targeted mutations. The Cas9/CRISPR system is simpler and more precise than previously developed genome editing systems. The high precision is due to the CRISPR associated (Cas) endonuclease's ability to bind DNA via associated guide RNAs. Cas endonucleases can delete or add bases to the genome, which permits not only knockouts to determine mutant phenotypes, but also tagging genes with reporters. However, a Cas9/CRISPR system has not been adapted for use with green algae. In this project, I adapted an existing Cas9 vector for use in the alga *Volvox* using molecular cloning techniques to insert species-specific regulatory sequences and guide RNA sequence targeting a test gene with known mutant phenotype. Biolistic transformation of the vector resulted in viable transformants, which were tested for guide RNA expression and Cas9 protein expression via RT-PCR and Western blots, respectively. RT-PCR confirmed that guide RNA was made. Cas9 expression is being tested currently. Once transformants that express both components are obtained, we will characterize them for mutations. Ultimately, this system will be used to edit genes related to multicellularity in *Volvox* and to improve the alga *Chlorella* as a biofuels and neutraceuticals production organism.

Volvocine algae and *Volvox carteri*



- Common unicellular ancestor ~200 MYA
- Varying from unicellular to multicellular, and varying divisions of labor
- Multicellular
- Full cell differentiation
- Easily cultured in lab
- Mutants easily isolated

CRISPR/Cas9



CRISPR - Clustered Regularly Interspaced Short Palindromic Repeats

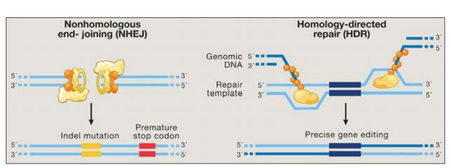
Bacterial genomic sequence involved in immune defense

Consists of repeat sequences and foreign DNA spacers

Mostly used to:

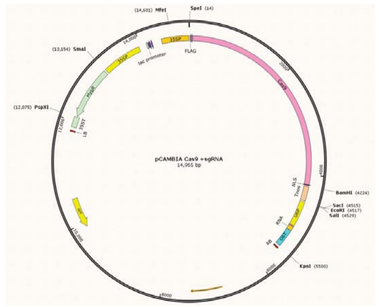
-Create knockdowns

-Insert reporter genes



Hsu 2014

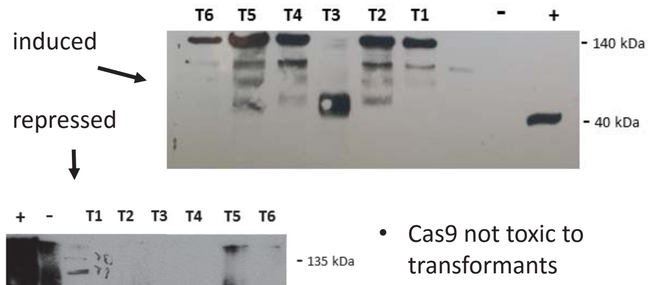
Methods



Functionality testing:

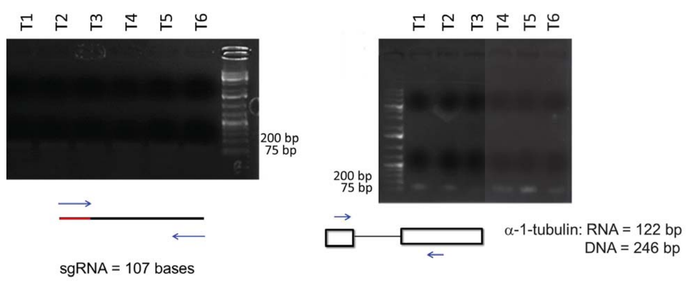
- Vector integration into genome via gDNA PCR
- Cas9 expression via Western Blot
- sgRNA expression via RT PCR
- Identify mutant phenotype via microscopy

Cas9 Expressed in Transformants



- Cas9 not toxic to transformants
- Cas9 expression induced and repressed
- Is it functional?

sgRNA Expressed in Transformants



U6 regulatory sequences are able to drive sgRNA expression

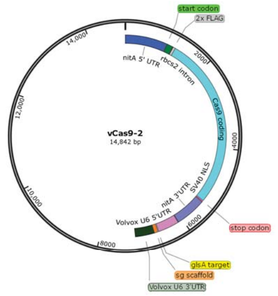
Both sgRNA and Cas9 are expressed, but the transformants do not exhibit mutant phenotype

Conclusion

- Transformants contain *cas9* sequence
- Cas9 is being expressed when induced
- Cas9 accumulation is not toxic to the cells
- sgRNA is being expressed
- The expression cassette is viable for driving expression
- No notable mutant phenotype

Is the Cas9 active?
Is the sgRNA folding properly and active?

Future Directions



- New ATG start codon
- RBCS2 intron for increased expression of Cas9
- New sgRNA targets

- Transform new plasmids and isolate more transformants
- Test Cas9 functionality by *in vitro* assays and immunolocalization
- Test sgRNA accumulation and identify the transcription start site

A functioning system can be adapted to:

- investigate multicellularity in Volvox
- investigate orthologs in Chlamydomonas
- investigate metabolic pathways for biofuel production

Acknowledgements

Meyerhoff Scholars Program

HHMI Scholars Program

MARC U*STAR

URA Award