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CRISPR Gene Editing in the Sea Squirt, *Ciona intestinalis*

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[1]Cell and Molecular Biology, [2]Biological Sciences

Introduction

Our aim was to use CRISPR, a cutting-edge genome editing technique, to induce a mutation in marine invertebrate embryos that will cause them to lose pigment in their eyespots as larvae. We will be using embryos from the model chordate *Ciona intestinalis*, also known as the sea squirt.

The CRISPR system will be used to to inactivate tyrosinase, the gene which encodes for an enzyme responsible for producing the pigment seen in the dark eye spots of *Ciona* larvae.

Figure 1. *Ciona intestinalis* [1]

CRISPR

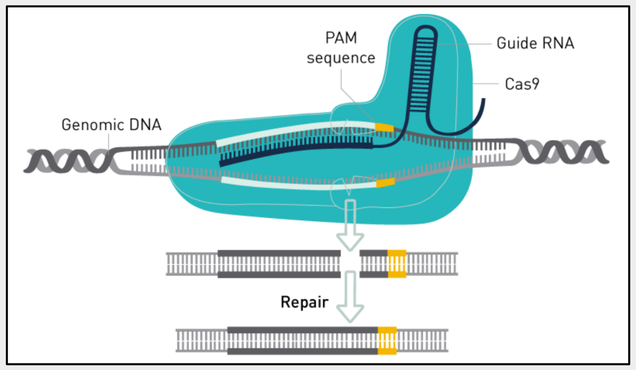

Figure 2. The CRISPR/Cas9 genome editing system [2]

CRISPR is not a machine or a physical tool, but rather it is a system that involves introducing a protein into a cell along with a DNA segment that attracts a DNA cutting protein called Cas9 to a desired location.

The Cas9 protein then induces a double stranded break at the location, silencing the gene located there.

References

1. *Skaphandrus*, http://skaphandrus.com/en/marine-animals/species/Ciona-intestinalis.
2. "Genome Editing (CRISPR/Cas9)." *Diagenode*, www.diagenode.com.
3. Knight, Kathryn. "Wriggling Sea Squirt Larvae Teach Us How Nerves Control Behaviour." *Journal of Experimental Biology.*

Methods

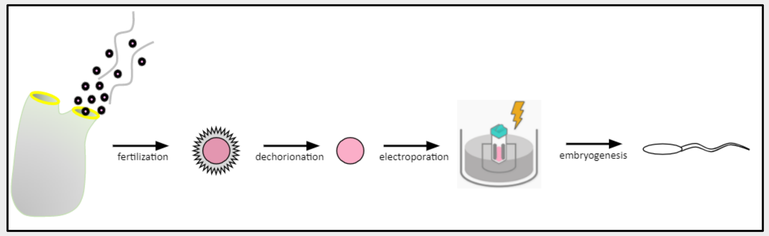

Figure 3. Experimental procedure for implementation of CRISPR

- Build the CRISPR construct by performing polymerase chain reactions (PCRs) using four primers and two plasmid templates
- Spawn *Ciona intestinalis* via dissection
- Dechorionate embryos to remove the outer membrane
- Electroporate the PCR product and a Cas9 vector into the dechorionated embryo
- The construct binds to the *Ciona* DNA. The Cas9 recognizes it and cuts the DNA there, disrupting the gene
- Allow the mutant embryo to develop to late tailbud stage to view changes in eye spot appearance

Deletion of Tyrosinase

The tyrosinase gene encodes for the enzyme responsible for producing melanin. After silencing the gene, the eyespots of the *Ciona* larvae lose their pigment and appear transparent under the microscope.

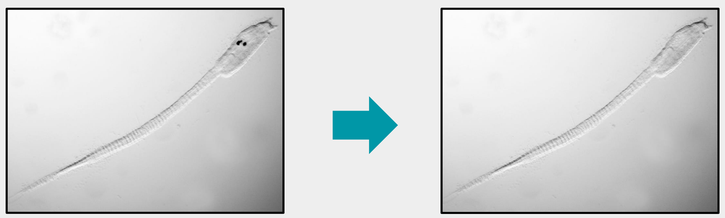

Figure 4. *Ciona intestinalis* larvae at late tailbud stage with and without visible eyespots (~1 mm) [3]

Results

After numerous variations in PCR conditions, our lab was able to produce the CRISPR construct at the correct size of approximately 1400 base pairs.

The next phase will be to electroporate this PCR product into *Ciona* embryos along with the Cas9 protein vector.

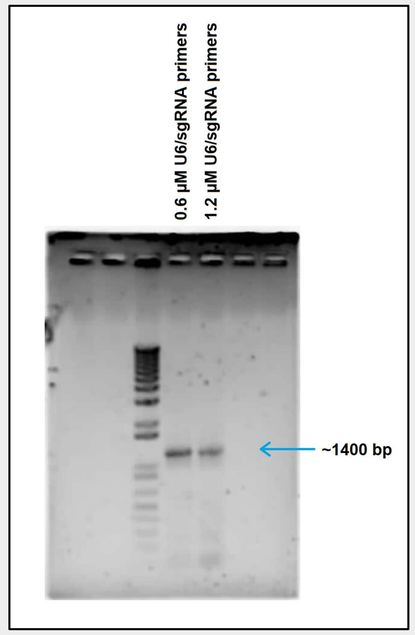

Figure 5. Gel electrophoresis showing differential CRISPR construct band strength at varying primer concentrations

Discussion

The rationale for inactivating the melanin-producing gene in *Ciona* is that it produces a visible phenotype, the loss of pigment in the eye spots of the larvae, thereby demonstrating the successful use of the CRISPR system.

In the future, we can use the same procedure to silence other *Ciona* genes, namely those involved in temperature response pathways.

These tests will further our understanding of how projected increases in ocean temperatures will impact reproduction in *Ciona* populations, and potentially in other aquatic species as well.

Acknowledgements

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