Transgenic Hydra
Editing the Opsin Gene with CRISPR

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Background

Hydra are small, freshwater animals that fall into the phylum Cnidarian. Hydra, although typically asexual, can be environmentally induced to produce gametes and embryos.

CRISPR, or Clustered Regularly Interspaced Short Palindromic Repeats, is a relatively new gene editing tool that utilizes a guide RNA and an endonuclease enzyme (cas9). The guide RNA positions cas9 and at the gene of interest, where cas9 will act as molecular scissors by creating a double strand break in the opsin gene, leading to the insertion of a GFP coding sequence in the opsin gene by homologous recombination.

When expressed, GFP will allow for a green fluorescent phenotype to appear wherever the opsin gene is expressed in the hydra.

The aim of this research is to establish protocols for growing fertilized embryos suitable for injection with CRISPR reagents so that we can create genetically modified hydra.

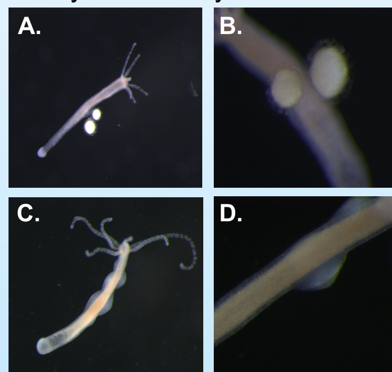

Figure 1. A. female polyp, **B.** mature eggs, **C.** male polyp, **D.** testes.

Methodology

Embryo Production: Male and female hydra are put together and allowed to breed. 10 fertilized eggs are placed with males for 24 hours and then placed into individual wells to be incubated in the dark for two weeks. Hatchlings were fed as normal polyps.

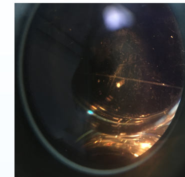

Figure 2. A microscopic picture of an embryo in a well.

DNA Extraction for Construction of Homology Arms: A mature, female polyp was placed with CTAB buffer and 100 ng/ml Proteinase K overnight (Winnepenninckx et al. 1993). DNA was extracted with an equal volume of chlorophorm: isoamyl alcohol and precipitated using 3M sodium acetate in 100% ethanol. The resulting DNA will be used with PCR to amplify the region of interest in the hydra genome.

Figure 3. DNA extraction reagents in PCR tube.

Results

Embryo Production: 8 hatchlings currently feeding showing an 80% survival rate

CRISPR: DNA template for homology arm construction has been completed.

Future Work

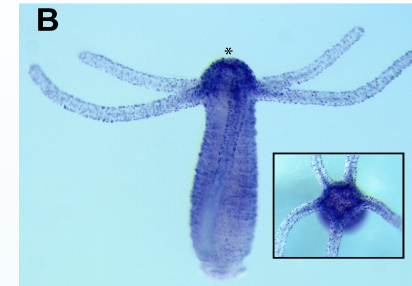

Figure 4. Polyp with a blue stain on opsin expression regions. If successful, our experiments will replace the blue stain with GFP fluorescence in live animals. Asterisk = mouth

We will use our protocols for the production of viable embryos to construct transgeneic hydra using CRISPR.

We will use microinjection to insert CRISPR reagents and modified homology arms into embryos. The embryos will undergo embryogenesis (the process of embryos maturing to hatchlings), resulting in a percentage of hatchlings that possess transgenic tissue (Juliano et al. 2014).

The hatchings will be observed under fluorescence microscopy, from which polyps with ample transgenic tissue will be chosen to collect regenerate tissue.

PCR and gel electrophoresis will be done to confirm transgenic tissue.

References

Juliano, C. E., Lin, H., Steele, R. E. Generation of Transgenic Hydra by Embryo Microinjection. J. Vis. Exp. (91), e51888, doi:10.3791/51888 (2014).

Winnepenninckx, B., Backeljau, T., & De Wachter, R. (1993). Extraction of high molecular weight DNA from molluscs. Trends in Genetics: TIG, 9(12), 407

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