MOL231: Development of tools for manipulating nervous system architecture in a sea anemone

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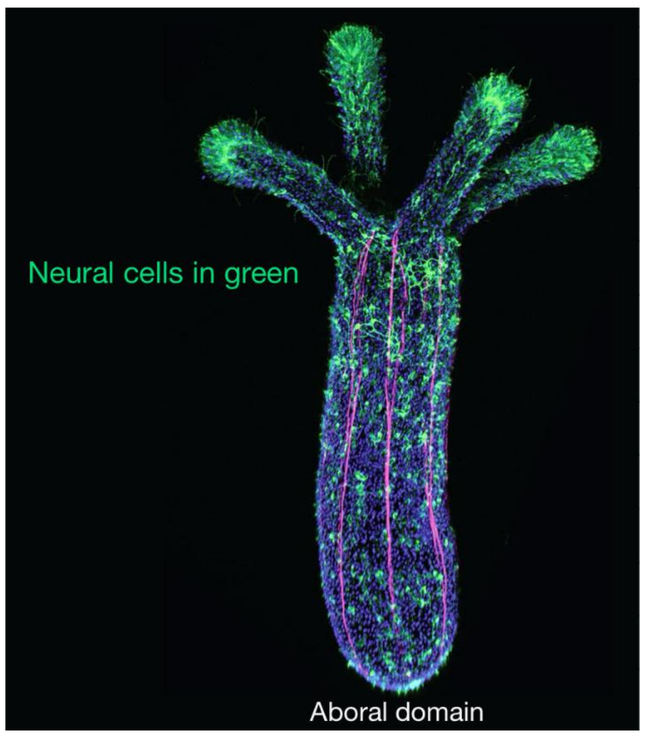

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Background:

Nematostella vectensis has a diffuse nerve net instead of a centralized brain and is able to regenerate nervous tissue. **Six3/6** and **FoxD1** are transcription factors that define and pattern the aboral region where neural and sensory cells develop, while **ashA** promotes the formation and differentiation of neurons within this region (1). Together, these genes influence nervous system organization.

This makes it possible to investigate whether increasing neural density in the aboral region could contribute to a more centralized nervous system.

Aim:

The aim of this study was to assess whether Six3/6 and FoxD1 are co-active in the same regions by crossing the corresponding transgenic lines, and to optimize components required for Gibson Assembly to generate a construct aimed to increase neurogenesis.

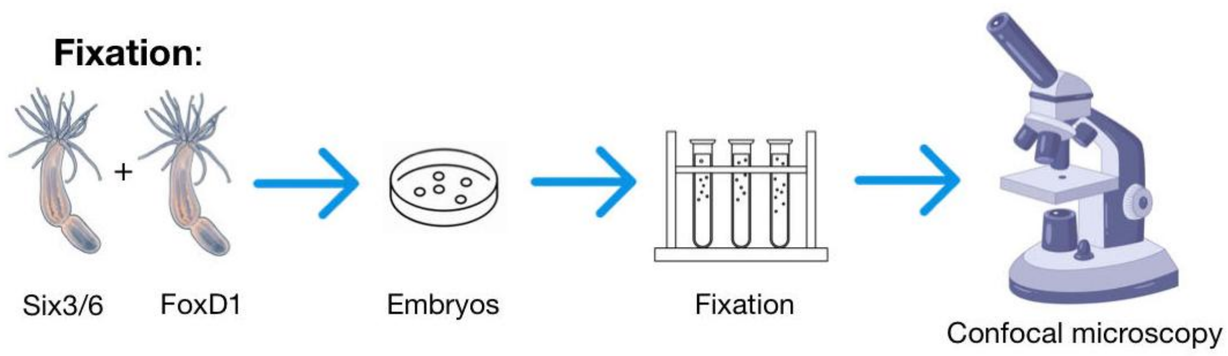
Methods:

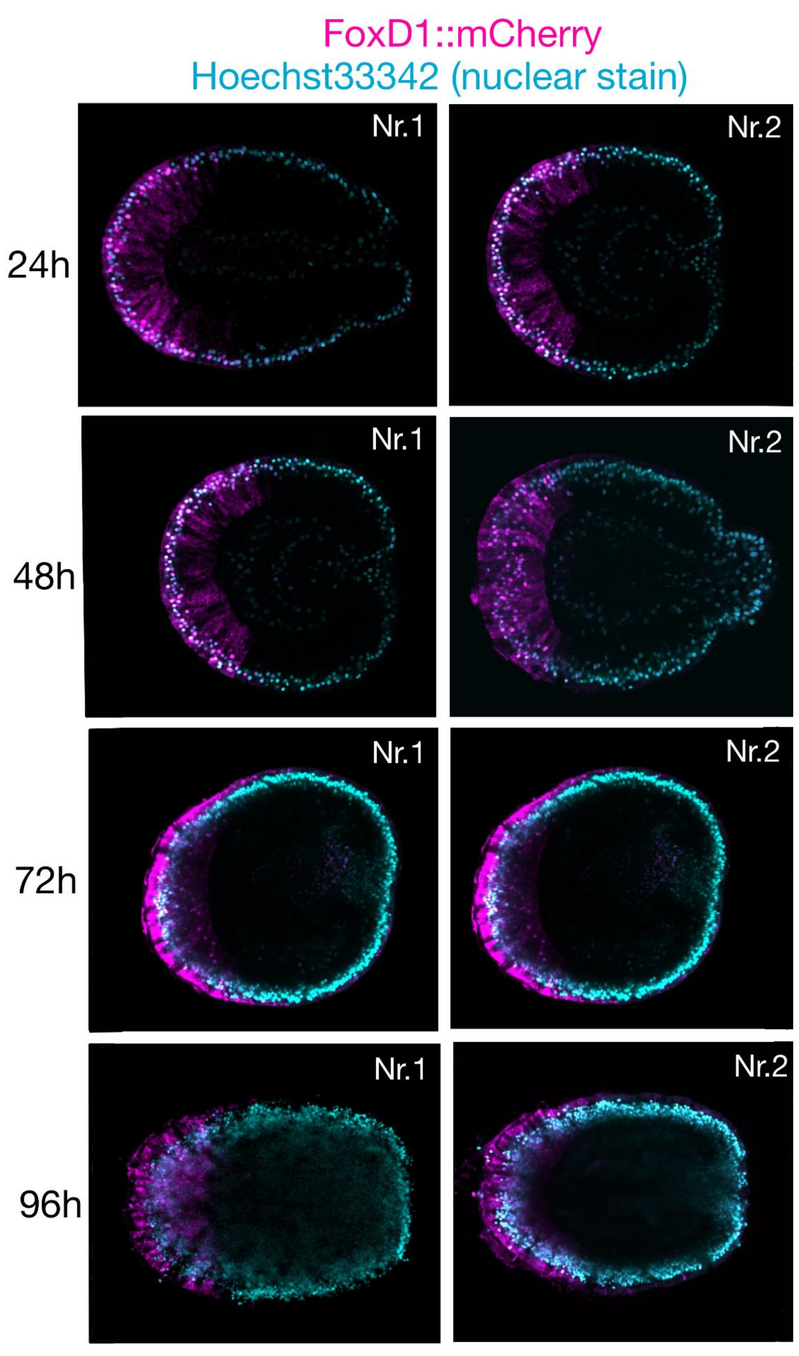
Expression analysis:

- Fluorescence microscopy to confirm Six3/6::GFP and FoxD1::Cherry expression.
- Embryo **fixation** and antibody staining to localize expressing cells.
- Confocal microscopy

Gibsson Assembly preparation for future **transgenesis**:

- PCR amplification of ashA, GFP and plasmid backbone.
- Gel electrophoresis verification

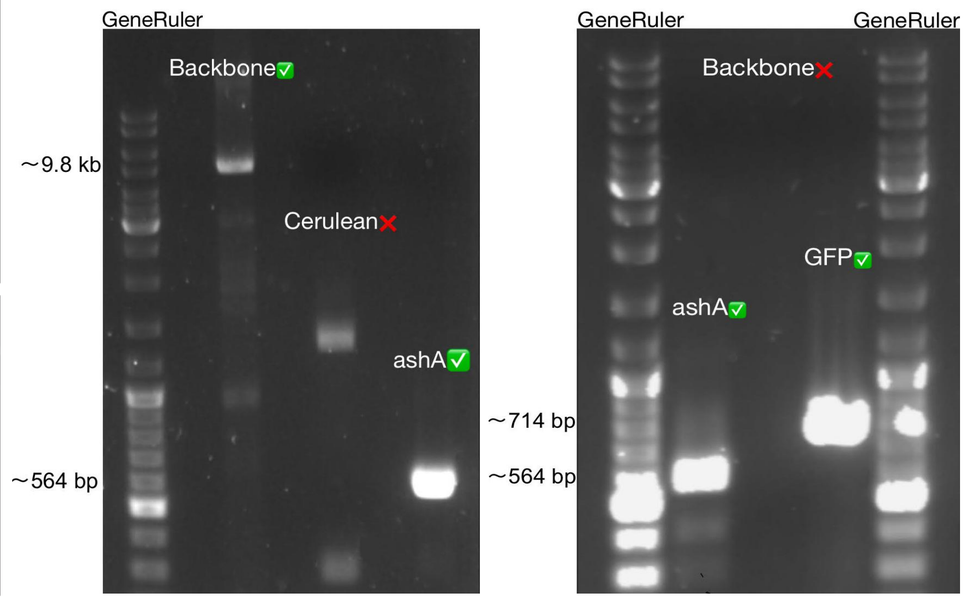




Results:

← Antibody staining for the FoxD1 transcription factor was successful, while staining for Six3/6 requires further optimization.

PCR amplification conditions were optimized after initial difficulties detecting the backbone fragment on gel electrophoresis.
Following primer redesign, successful amplification was achieved. However, the cerulean replacement construct requires further validation. ↓



Conclusions:

- Analysis of the antibody staining suggest that the Six3/6 promoter is suitable for driving ashA expression in the aboral region, at least up to 96 hours of development.

Future work:

- Complete the Gibson Assembly.
- Optimize staining of Six3/6::GFP embryos.
- Assess whether transfection with an ashA-containing plasmid increases neural density.
- Investigate whether increased neural density can maintain functional synaptic connectivity and lead to a more centralized nervous system.

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References:

1. https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1001488
2. https://www.nikonsmallworld.com/galleries/2019-photomicrography-competition/nematostella-vectensis-sea-anemone-stained-for-neural-cells-green-muscles-pink-and-nuclei-blue