Immunofluorescent Staining of Zebrafish Retinal Ganglion Cells

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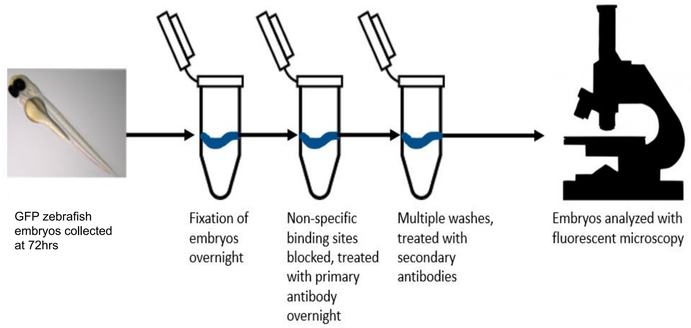
Celebration of Scholars 2023: Exposition of Student & Faculty Research, Scholarship & Creativity

Introduction

- Retinal ganglion cells (RGCs) send signals from the eye to the brain, with their axons forming the optic nerve
- Zebrafish are capable of regenerating their optic nerves after damage.
- There are different subtypes of RGCs, and we want to understand if they develop and regenerate differently after injury.
- We are developing protocols and tools to use antibody staining to image specific RGC subtypes as they develop and regenerate in embryonic zebrafish.

Experiment

- Mated Isl2b:GFP zebrafish
- Fixed embryos 3 days post-fertilization
- Stained embryos using the primary and secondary antibodies
- Washed multiple times between treatments in PBS/BSA/Triton
- Imaged the stained embryos using a confocal microscope



Results and Discussion

- Identified several antibodies that label RGC subtypes. However, staining is not reliable or consistent.

Future Directions

- Optimize permeabilization to increase the efficacy of the staining process and to reduce noise.

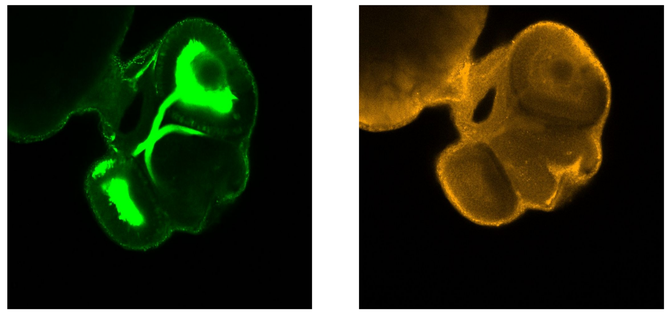


Figure 1. Isl2b:GFP embryo stained with Chicken anti-GFP primary and Rhodamine Red Donkey Anti-Chicken secondary. Red staining is not visible; however optic chiasm can be seen in green.

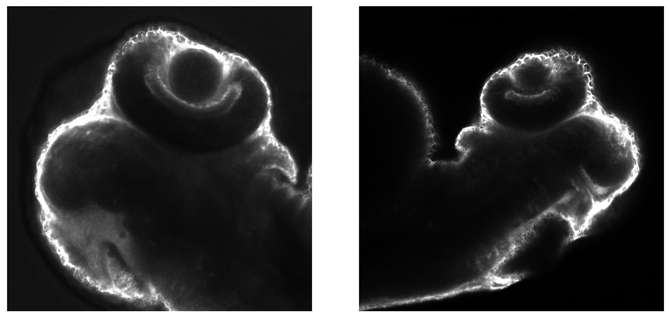


Figure 2. Zebrafish embryo stained with ZN-5 antibody, 568 secondary; the optic nerve is visible, although significant noise is present.

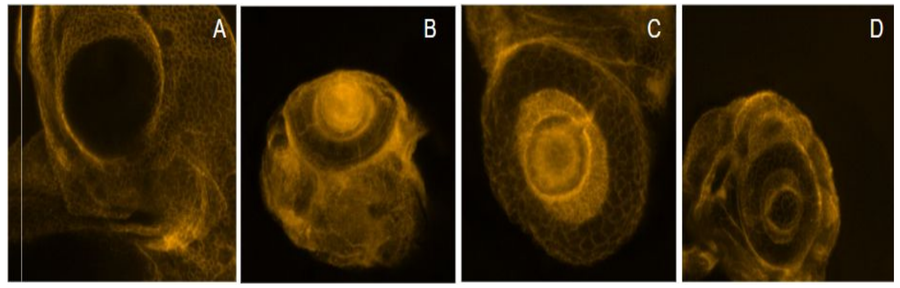


Figure 3. Zn-5 antibody stained embryos. All embryos fixed at 3dpf. A- control, fixed overnight at 4°C in 4% PFA, no permeabilization or clearing treatment; B- fixed in 100% acetone for 10 minutes at -20°C; C- fixed overnight in 4% PFA, permeabilized with acetone prior to staining; D- fixed overnight in 4% PFA, cleared using the Clear^{T2} method.

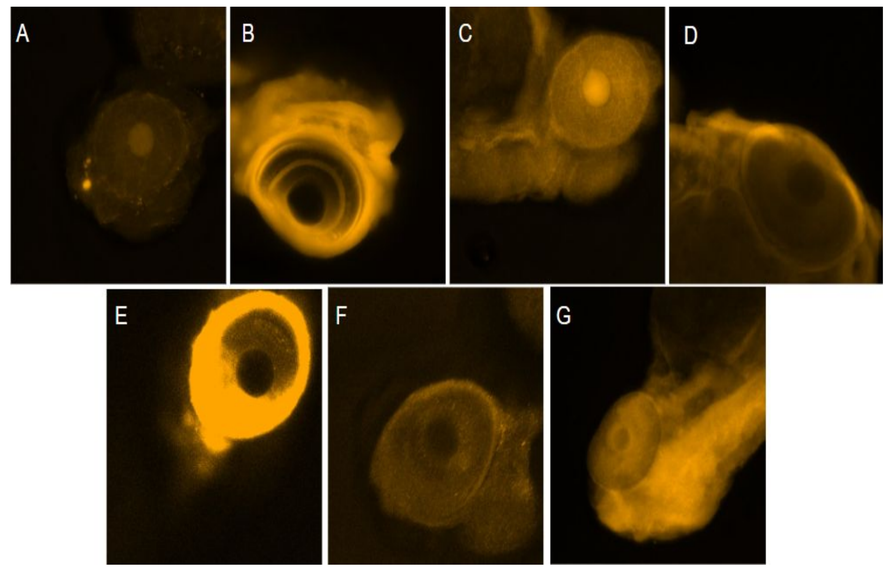


Figure 4. Embryos stained with antibodies RGC-subtype specific antibodies. All embryos were fixed overnight in 4% PFA at 4°C. A- ChAT antibody at 1:100 dilution; B- CHRNB2 antibody at 1:100 dilution; C- CACNB3 antibody at 1:100 dilution; D- OPN4 antibody at 1:200 dilution; E- SLC17A6 antibody at 1:100 dilution; F- OTX1B antibody at 1:250; G- SLIT1A antibody at 1:400 dilution.

Acknowledgements & References

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