Testing Effectiveness of SnapTag Self Labeling Protein System in Zebrafish Embryos

Medha Singamsetty, Emily Shaw, Le Trinh

Dept of Biological Sciences, Translational Imaging Center, Bridge Institute, University of Southern California, Los Angeles, CA, USA

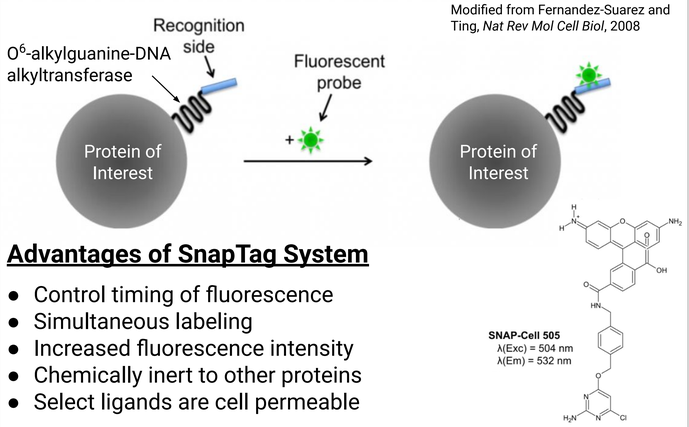
Bridge **U**nder**G**rad **S**cience (**BUGS**) *Summer Research Program*

USC University of Southern California

Abstract

The SnapTag self-labeling protein system has emerged as a promising tool in molecular biology, enabling efficient and specific labeling of target proteins within live cells. Previous work has established SnapTag as an effective labeling system *in vitro,* but has yet to be tested in a whole organism model such as zebrafish *(Danio rerio)*. This project investigated the effectiveness of SnapTag in zebrafish embryos by creating a SnapTag expression construct, synthesizing the construct into mRNA, and then injecting the mRNA into zebrafish embryos. The embryos were then introduced to the SnapTag ligand and imaged using confocal microscopy. Our findings demonstrate a lack of tissue penetration of the SnapTag ligand, SnapTag 505 in zebrafish embryos, which indicates that further experimentation is necessary to evaluate the efficacy of SnapTag labeling in zebrafish.

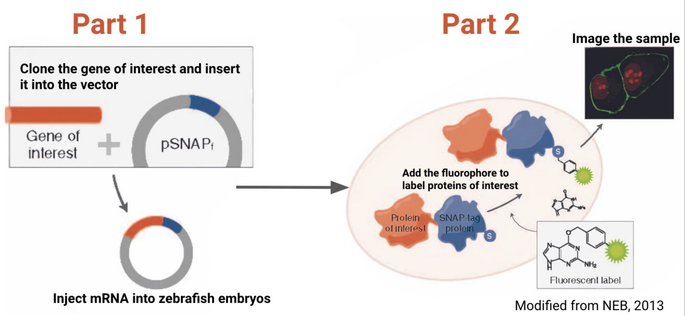
SnapTag System



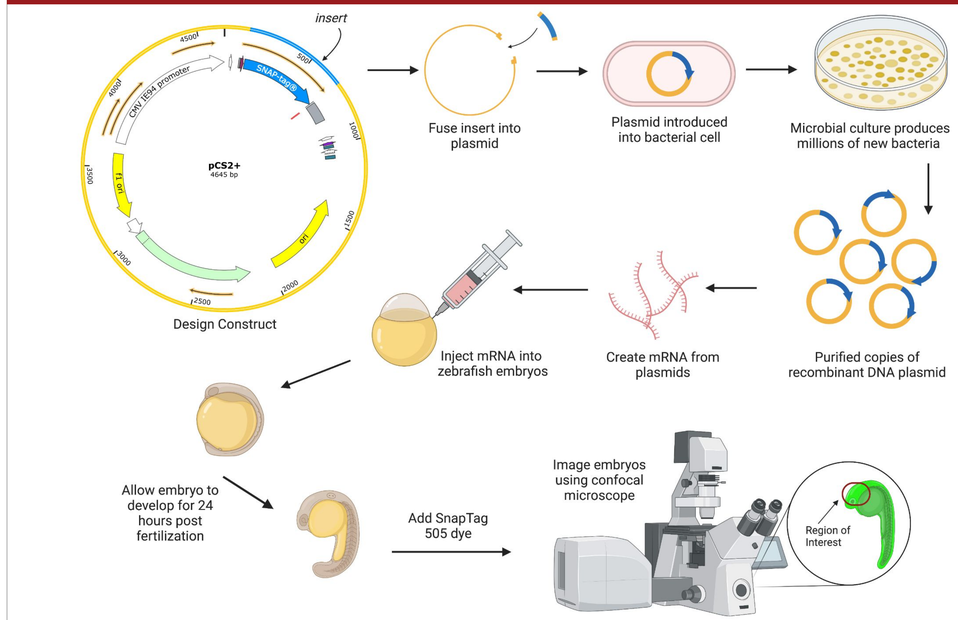
Advantages of SnapTag System

- Control timing of fluorescence
- Simultaneous labeling
- Increased fluorescence intensity
- Chemically inert to other proteins
- Select ligands are cell permeable

Project Overview

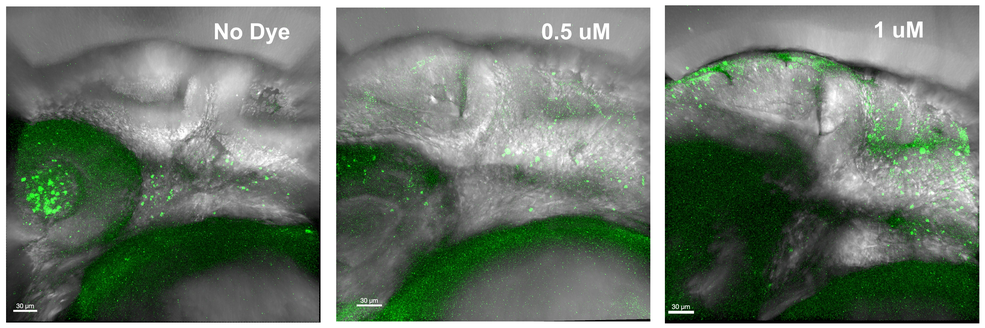


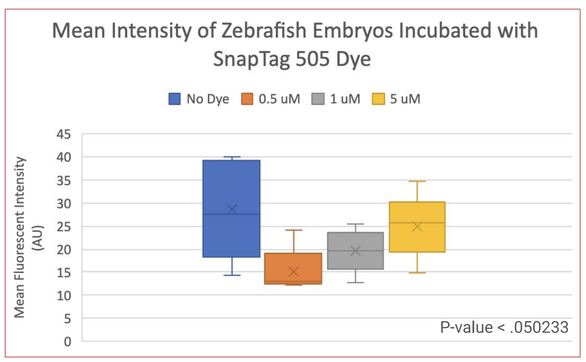
Materials and Methods



Results of SnapTag 505 Dye Calibration in Zebrafish Embryos

Mid-hindbrain regions of Zebrafish embryos incubated in various concentrations of Snap 505 dye

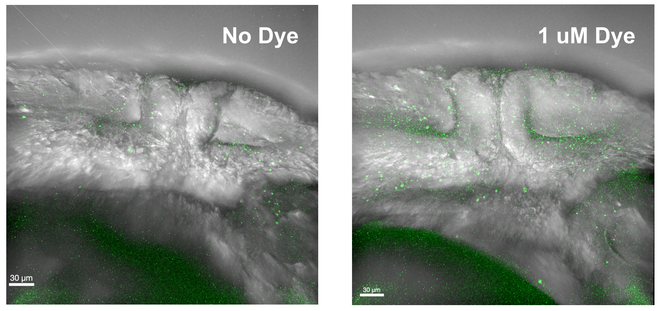


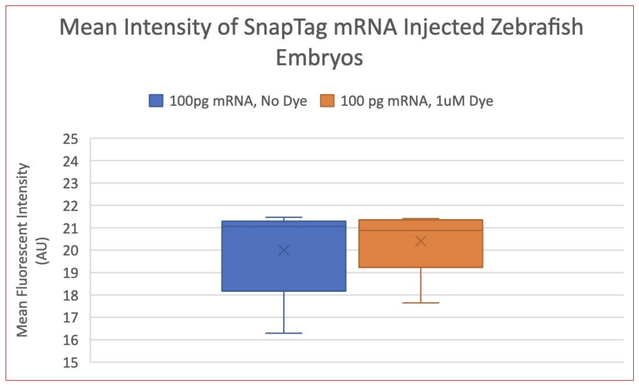


Our findings indicate no significant difference of fluorescent signal given various concentrations of SnapTag 505. There is also minimal background binding of the dye in the embryos.

Results of SnapTag Labeling in Zebrafish Embryos

Mid-hindbrain regions of Zebrafish embryos injected with 100pg of SnapTag mRNA and no dye or incubated with 1uM of Snap 505 dye





Our results indicate that fluorescent expression of SnapTag with dye is not significantly different from SnapTag without dye, suggesting tissue penetration issues.

Conclusion and Future Directions

- Snap 505 dye concentration has little effect on background fluorescence in zebrafish embryos.
- SnapTag bound with SnapTag 505 dye does not appear to be generating a significant fluorescent signal in zebrafish embryos.
  - The fluorescent intensity did not change significantly once the dye (ligand) was added.
  - This is likely due to dye penetration issues.
- Next steps will be testing other SnapTag cell permeable dyes in zebrafish embryos, like TMR-Star.

Acknowledgements

I'd like to thank the Fraser Lab for hosting me, and especially my PI Le Trinh and mentor Emily Shaw for guiding me through my project. I'd also like to thank Arkadi Shwartz for all the help using the microscopes, and Falk Schneider for the NLS SnapTag DNA. Finally, I'd like to thank my lab partner Christopher Robertson and the Bridge Institute.

CONTACT

bridge.usc.edu/bugs