Optogenetic Control of Notch1 Signaling to Investigate Trabeculation and Early Heart Development in *Zebrafish*

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Abstract

Optogenetics enables precise, non-toxic control of specific cell activities using non-toxic blue light of 470nm. In this study we will be addressing early heart development of *Danio rerio* and investigate the role of Notch1 signaling in trabeculation, a critical process in ventricular wall thickening. Defective formation of trabeculae is associated with non-compaction cardiomyopathy (NC), a **congenital heart defect** linked to a disruption in Notch signaling. To achieve spatiotemporal control of Notch1 activation, we employ the LAUNCHER system, generating transgenic zebrafish primary cell lines expressing light-activated NICD (Notch intracellular domains). Using live imaging, we will track Notch 1 activation in both endocardium and myocardium during early heart development. Ultimately, our study advances the use of optogenetics in developmental cardiology, offering new insights into the molecular mechanisms underlying congenital heart disease.

Introduction

- During heart development, thickening of the ventricular wall forms, this is called trabeculation, these structures are important for heart function.
- Notch Signaling is essential for proper trabeculae formation.
- Optogenetic tool we will be using is LAUNCHER system; it utilizes a light-activated Notch1 intracellular domain (NICD) fused to *mScarlet* for precise control of Notch1 activation.

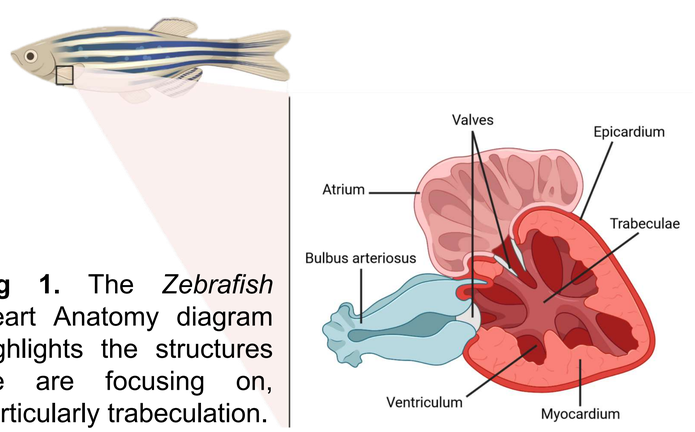

Fig 1. The *Zebrafish* Heart Anatomy diagram highlights the structures we are focusing on, particularly trabeculation.

Fontana, C. (2023) BioRender.

Material & Methods

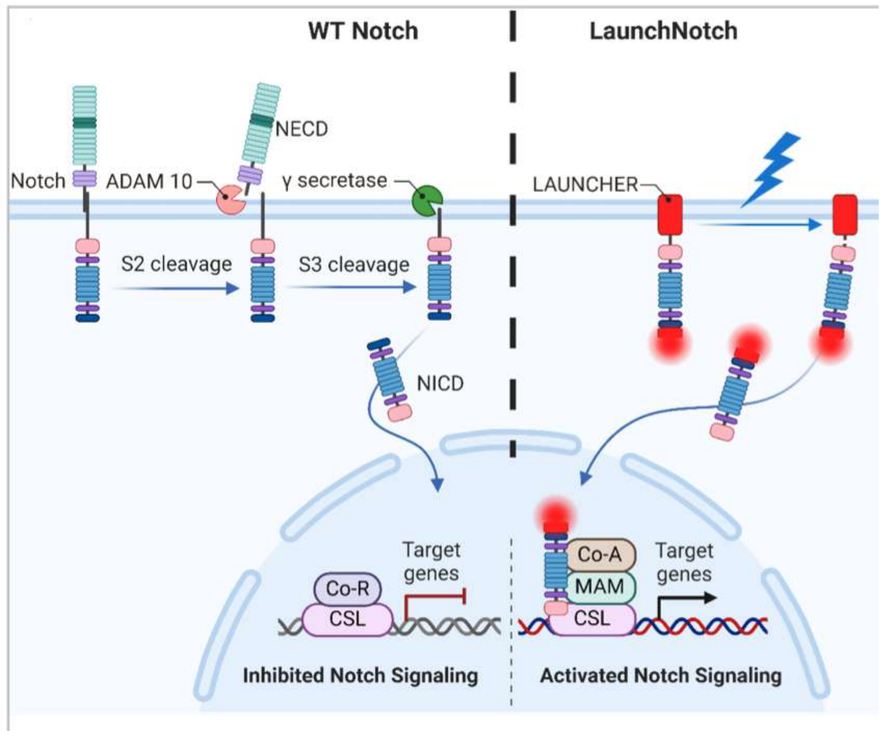

Fig 2. The LAUNCHER system diagram Illustrates the optogenetic control of Notch1 signaling in myocardium and endocardium cells.

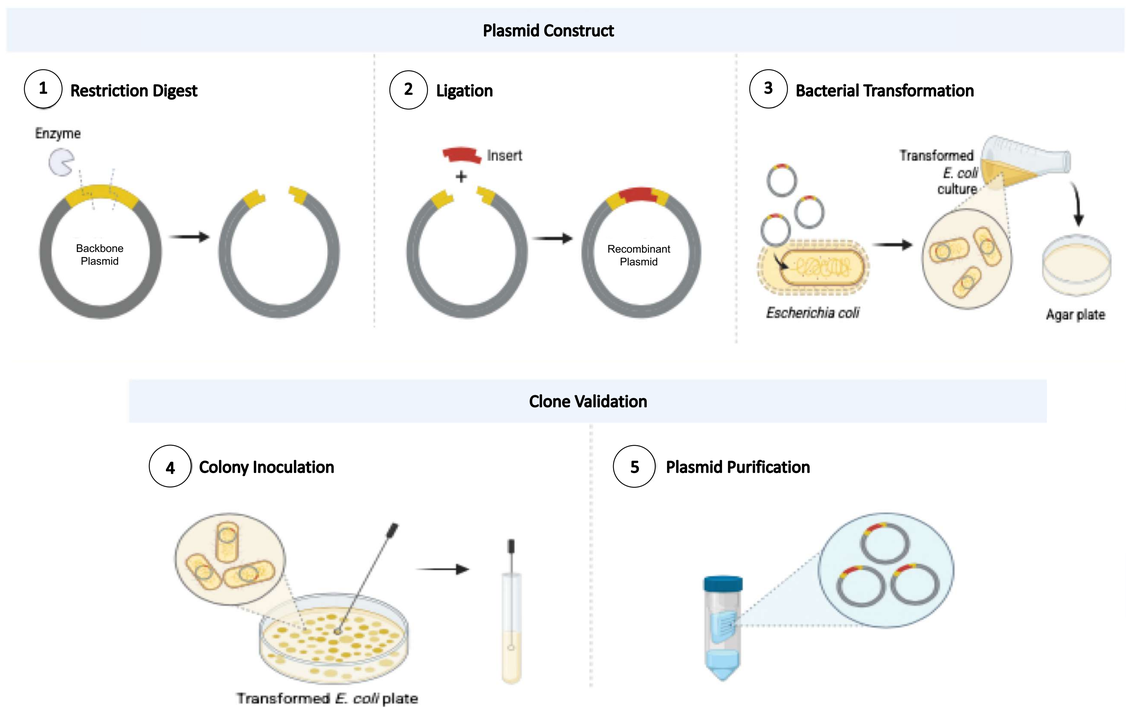

Fig 3. Molecular Cloning outlines our cloning process; Our strategy involves a two-part molecular cloning process which involves sequential insertion of *mScarlet* and NICD.

Results

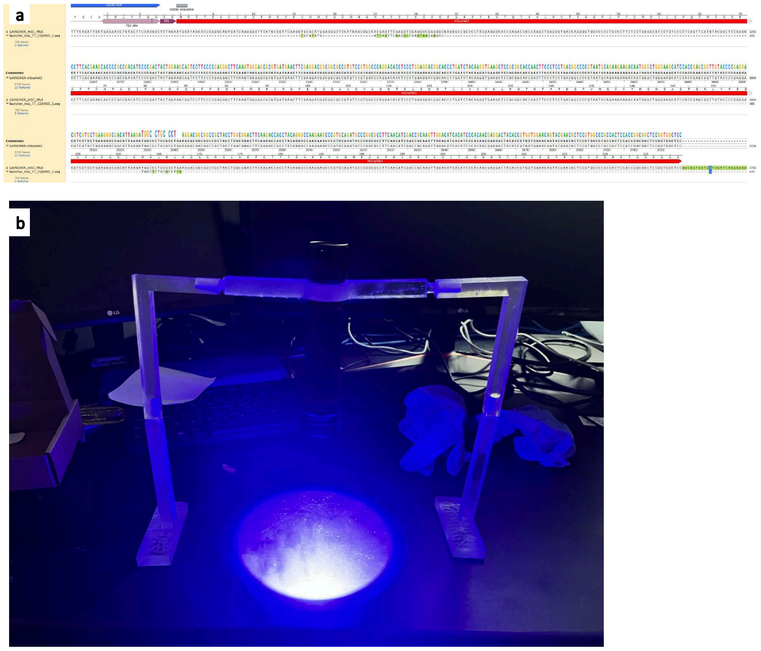

Fig 4. a. Sanger sequencing results to confirm the insertion of mScarlet in LAUNCHER plasmid. **b**. Stand for illumination of transfected cells. The light is emitting 470nm of non-toxic blue light.

Discussion/ Future Work

- *mScarlet* has been successfully integrated to LAUNCHER (control)
- Future steps will focus on inserting NICD in the construct for Optogenetic manipulation.
- Following this we will perform a chemical transfection in the zebrafish cardiomyocytes.

References

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- Bakkers J. Zebrafish as a model to study cardiac development and human cardiac disease. 2011