Cartilage-specific *col2a1a* enhancer drives hair cell-specific *atoh1a* reporter expression in zebrafish cartilage

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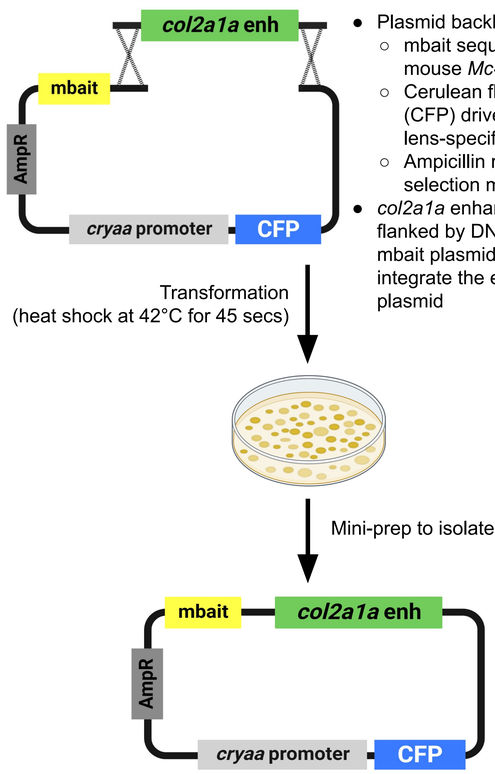
Abstract

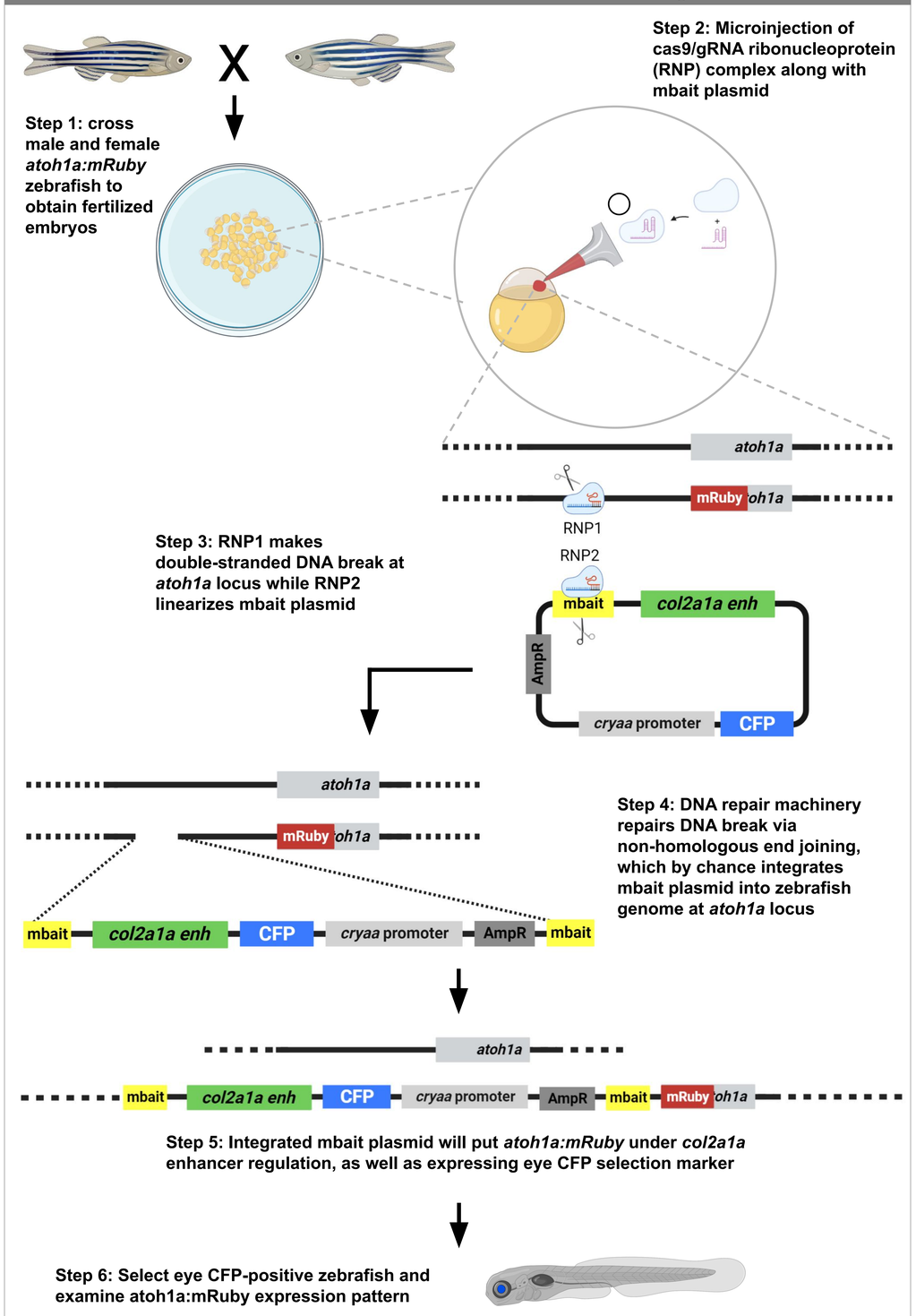
Gene expression is a precisely regulated process essential for the proper functioning and development of an organism. At the heart of this intricate control lies the interaction between two crucial DNA elements: enhancers and promoters. Enhancers are specific DNA regions that act as regulatory elements, facilitating the activation of gene transcription through the binding of transcription factors. On the other hand, promoters are situated upstream of genes, and they serve as binding sites for vital proteins like RNA polymerase to initiate transcription. The gene regulation can be explained using promoter–enhancer interactions, where the DNA loops in to bring enhancers close to the promoter and necessary proteins attach to drive transcription. This raises the question of whether enhancer promoter interaction is restricted to the gene or due to the proximity. We propose to knock an enhancer into a locus where it is not endogenously found and see whether the enhancer can interact with the native promoter to alter the expression of the native gene. Here, I insert a *col2a1a* enhancer that is specifically active in the zebrafish cartilage into a upstream region of the zebrafish *atoh1a* locus. The *atoh1a* gene expression is restricted to the zebrafish hindbrain and inner ear and lateral line hair cells during development. I can visualize the expression of *atoh1a* using a knock-in *atoh1a:mRuby* reporter zebrafish. I hypothesize that the insertion of *col2a1a* enhancer is able to expand the expression pattern of *atoh1a* expression into zebrafish cartilage. I used CRISPR-cas9 technology to integrate *col2a1a* enhancer into the *atoh1a* locus using a mbait plasmid. I designed two gRNAs for this experiment. One will target the zebrafish *atoh1a* upstream region, while the other will target the mbait sequence, which is not found in the zebrafish genome. This would allow cas9 to target zebrafish *atoh1a* locus and the mbait plasmid and make double-stranded DNA breaks. Cellular DNA repair machinery would then attempt to repair the DNA breaks, which could by chance incorporate the mbait plasmid into the zebrafish genome. The mbait plasmid contains a lens-specific fluorescent selection marker, which I screened for positive integration. I observed that in zebrafish with col2a1a enhancer knock-in, atoh1a expression expanded into the zebrafish lower jaw cartilages.

Plasmid Cloning

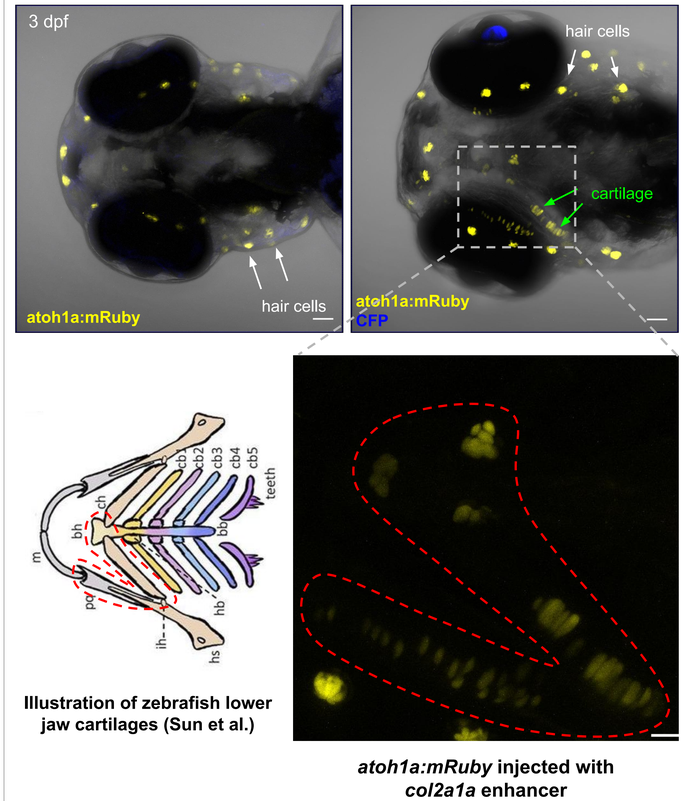


- Plasmid backbone contains:
  - mbait sequence from mouse *Mc4r* gene
  - Cerulean fluorescent protein (CFP) driven by zebrafish lens-specific promoter
  - Ampicillin resistance selection marker
- *col2a1a* enhancer sequence flanked by DNA homologous to mbait plasmid backbone to integrate the enhancer into the plasmid

Knock in of *col2a1a* enhancer into the *atoh1a* locus using CRISPR-Cas9



Results



Summary

The observation that *col2a1a* enhancer is able to drive *atoh1a* expression in the zebrafish cartilage has several implications:

- *col2a1a* enhancer can interact with *atoh1a* promoter when brought in proximity to each other, suggesting that promoter-enhancer interactions are less gene-specific than proximity-based. This is also supported by known observations that single enhancer can regulate the expression of multiple nearby genes.
- A popular theory in evolution is that, transposable elements containing enhancer sequences were excised and inserted randomly into the genome. This could lead to altered expression patterns of various genes in different cell types and tissues, which could ultimately contribute to the evolution of different species. The results of this experiment support this theory as the integration of *col2a1a* enhancer altered the expression pattern of *atoh1a*.

In the future, we would test other enhancers and genes to see whether other tissue-specific enhancers can alter the expression of atoh1a, and whether the expression pattern of other genes can be manipulated by different enhancers. In addition, we would also test whether these ectopic gene expression changes can lead to any significant phenotypic changes.

Reference

Sun Y, Kumar SR, Wong CED, Tian Z, Bai H, Crump JG, Bajpai R, Lien CL. Craniofacial and cardiac defects in *chd7* zebrafish mutants mimic CHARGE syndrome. Front Cell Dev Biol. 2022 Dec 7;10:1030587. doi: 10.3389/fcell.2022.1030587. PMID: 36568983; PMCID: PMC9768498.

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