A reporter zebrafish line for live fluorescent visualisation of bone extracellular matrix

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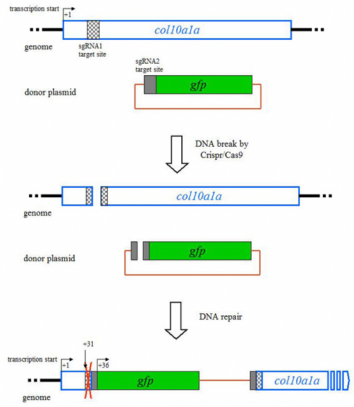
Abstract

Using the CRISP/Cas9 technology, we targeted the zebrafish *col10a1a* gene to insert the Gfp coding sequence into its genome, generating a transgenic zebrafish line expressing a secreted Gfp fusion protein in the developing skeleton that reveals the bone extracellular matrix.

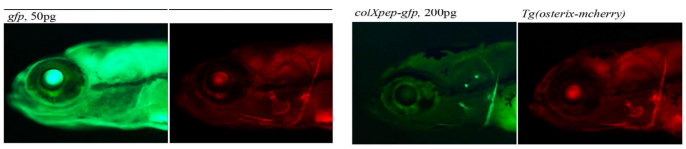
Introduction

The zebrafish is increasingly used as a model to study skeletal development and homeostasis. One advantage is the transparency of the larvae allowing live observation of internal structures. The zebrafish *col10a1a* gene is expressed in hypertrophic chondrocytes (cartilage) and in osteoblasts (forming bone) and thus is a perfect marker for the developing skeleton.

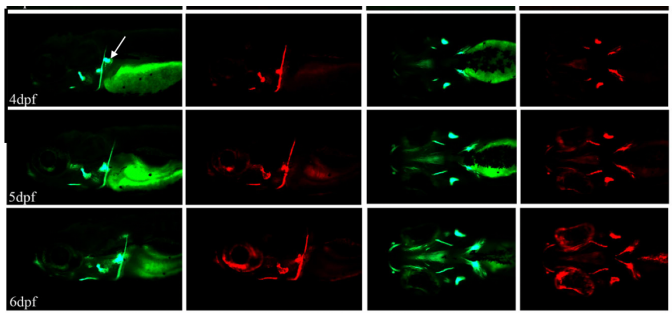
Results



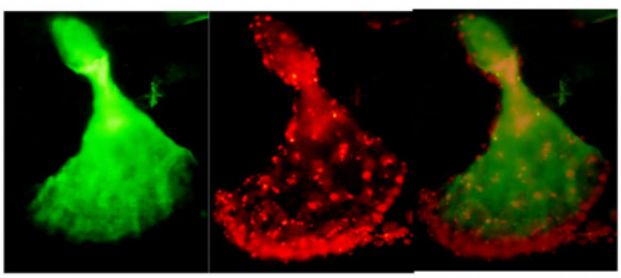
Schematic representation of the endogenous *col10a1a* gene (top line), the plasmid used for microinjection, and the two gRNAs (bait and col10a1a), the resulting cuts in the genomic DNA and plasmid, and the resulting reporter gene in the transgenic line.



Tg(Olasp7:mcherry) larvae (5dpf) after microinjection of mRNA coding for Gfp (left) or for the fusion protein Col10a1a-gfp (right). Gfp expression is strong in the entire body, while Col10a1a-gfp is located at bone elements (cleithrum and opercle) as visualized by the red fluorescence of mCherry.



Timeline of expression of Gfp (green fluorescence) and mCherry (red fluorescence) in the double transgenic larvae *Tg(col10a1a:col10a1a-gfp;Olasp7:mcherry-NTR).* mCherry is expressed in osteoblasts.



Close inspection of Gfp localization (green) compared to mCherry expression in osteoblasts in the opercle of 9dpf *Tg(col10a1a:col10a1a-gfp;Olasp7:mcherry-NTR)* zebrafish larvae.

Conclusion

We present a transgenic zebrafish zebrafish line expressing a secreted Gfp fusion protein in the developing skeleton that reveals the bone extracellular matrix.

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BIOMEDAQU

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