Generating Zebrafish Transgenics using Mouse Tendon and Ligament Enhancers

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Abstract

Tendons and Ligaments are fibrous connective tissues that are specialized, with tendons connecting muscle to bone and ligaments connecting bone to bone. A lot remains unknown about tendon and ligament development, but we do know that these connective tissues have stayed relatively similar throughout evolution. This suggests that the development of tendons and ligaments, just like bones, could have a universal regulation between species.

To test this idea I worked on determining whether Mouse tendon and ligament enhancers can drive expression in zebrafish tendon and ligament cells. By taking regions of open chromatin in Mouse Scleraxis GFP (Msg) ATAC data, potential tendon and ligament enhancers were identified. There were 3 specific clusters with possible tendon and ligament enhancers from which highly enriched peaks were chosen. These clusters are; Cluster-13, Cluster-12, and Cluster-2, and I worked with Peak-1 (P1) from each of these clusters. The enhancers from these peaks/clusters are then brought together with CFP (cyan fluorescent protein) and GFP (green fluorescent protein) and injected into zebrafish embryos.

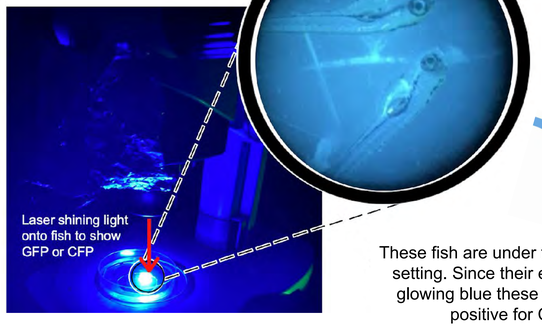
After taking either injected fish (P0) or fish from crossing previously injected fish (F1), I would screen them at 5 days old looking for expression of CFP in the eye and GFP in facial tendons/ligaments. After crossing C-12 injected zebrafish, I found 8 fish with eye CFP, and in the C-2 cross I found 3 fish with eye CFP. Also, after injecting embryos with Msg C-13, 5 were positive for CFP and had possible GFP.

The data showed that the C-13, C-12, and C-2 enhancers did not drive expression in the tendons and ligaments. This could mean that they need to be injected again, especially since we are not clear on whether or not a given enchanter will work with another species because of the difference between animal's regulatory sequences.

Screening for GFP and CFP

This zebrafish eye is glowing from CFP which can be seen in the GFP channel. This tells us that the injection Solution was successfully integrated into the genome.

The screening room stays dark so you can see real fluorescence



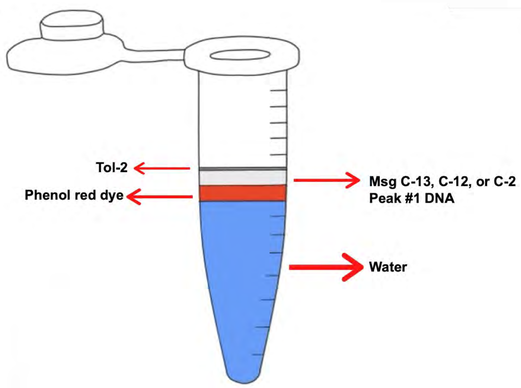
These fish are under the CFP laser setting. Since their eyes are not glowing blue these fish are not positive for CFP.

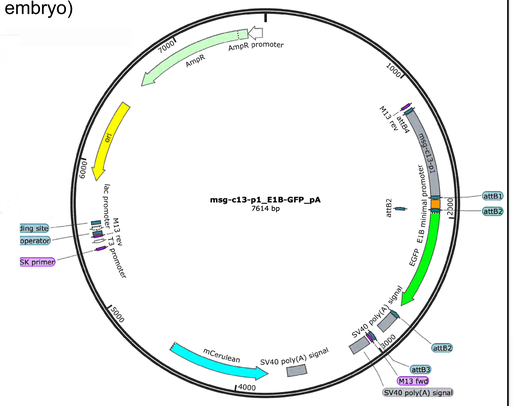
Injection Solution and Plasmid

Injection Solution Parts:

- 1 microliter of diluted Msg Cluster-13, Cluster-12, or Cluster-2 Peak #1 DNA
- 8 microliters of water
- 1 microliter of phenol red dye
- 0.3 microliters of Tol-2 (helps to reinsert the DNA into the embryo)

You get 30 Nano grams of the DNA per microliter because of the dilution





Imaging Zebrafish with Confocal Microscope

The confocal is a microscope that uses light from a laser to image a specimen, and show fluorescence.

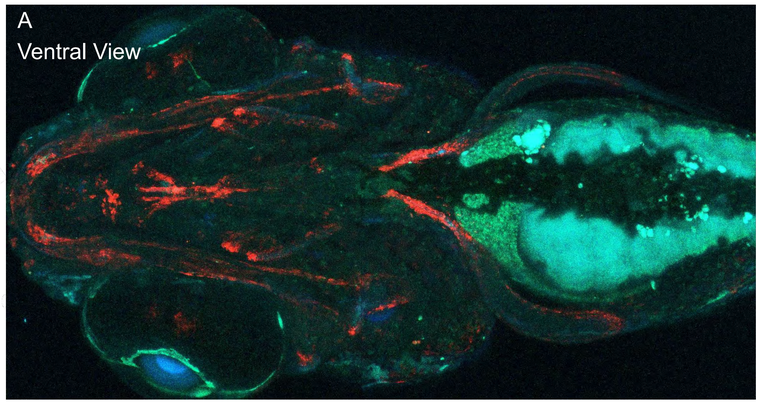
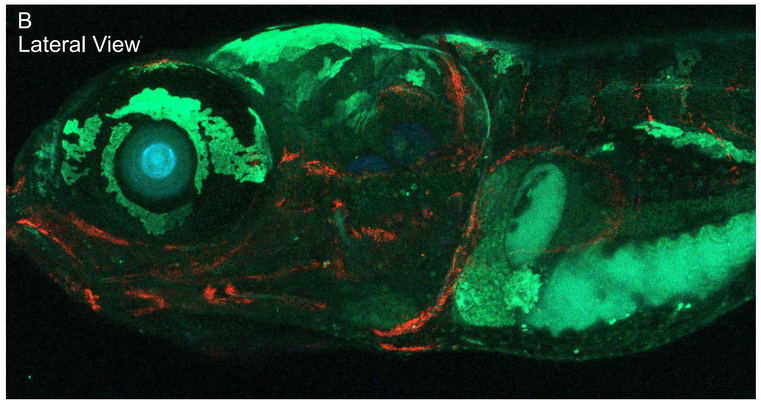
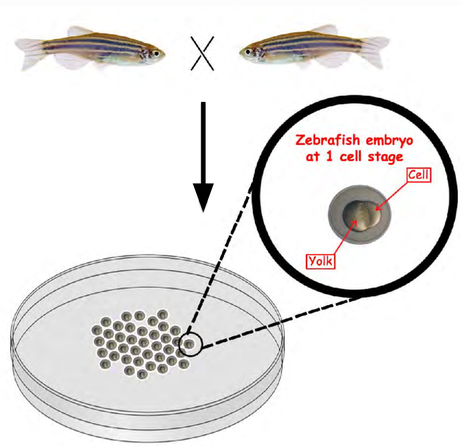


Figure 3 A and B. Ventral and Lateral View. C-12 Peak-1 GFP F1 fish with Scleraxis (red), and eye CFP.

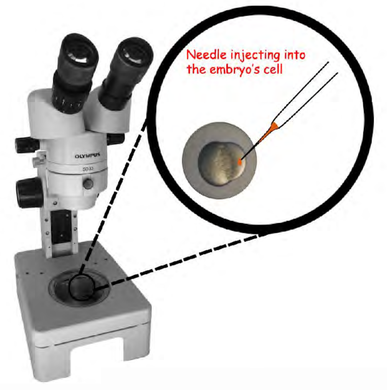
You can see CFP in the eye meaning it was integrated into the genome but since there is no GFP the enhancers did not work.



Micro Injections



When injecting the embryos with C-13, C-12, & C-2 enhancer GFP/CFP reporter DNA, you want to do it at the single cell stage. This is the time period in which the embryos only have one cell, and so by injecting during this time, the enhancers have a greater chance of integrating into the genome.



By injecting into the cell and not the yolk there is also a better chance of integration into the genome.

Summary

After screening F1 fish I found that while the injections did generate transgenics, the enhancers (C-13, C-12, C-2) did not show GFP expression in the tendons and ligaments. This possibly suggests that new Clusters from the Mouse Scleraxis GFP (Msg) ATAC data need to be chosen because the others aren't sufficient and don't drive expression in zebrafish tendons and ligaments. However, another possibility for these results is that since the plasmid randomly inserts itself, it might have been silenced causing no tendon and ligament expression. In this case you would need to keep injecting and hope that the plasmid inserts in a good spot for expression and transgenics.

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