

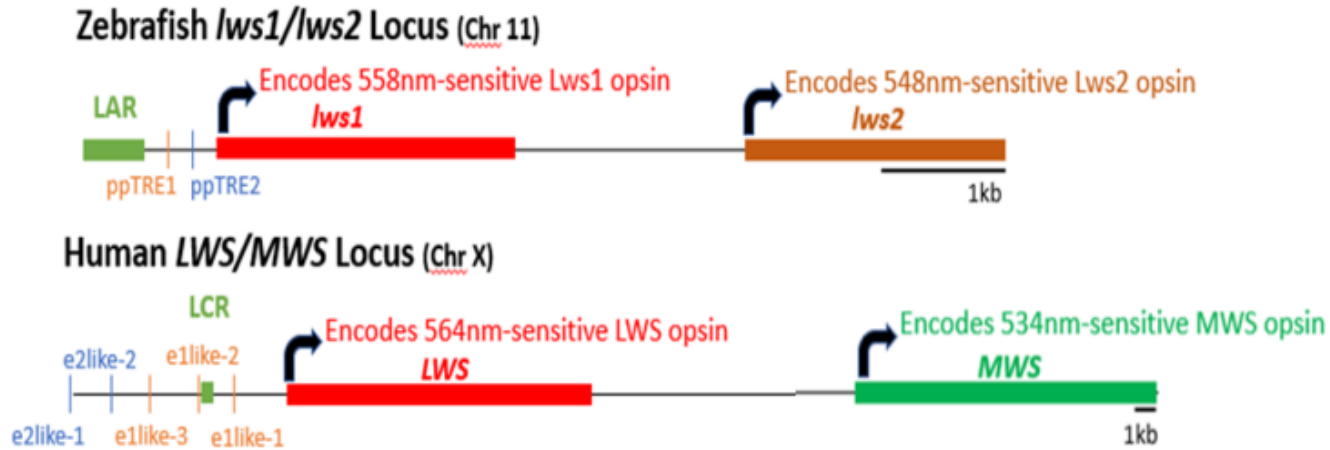
Predicted palindromic thyroid hormone  
response elements affect the thyroid  
hormone regulation of opsin expression in  
zebrafish

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# Introduction

## Figure 1. Zebrafish *lws1/2* and human *LWS/MWS* loci.



**Fig. 1:** The known and predicted regulatory sequences for *lws1* and *lws2* in zebrafish and human *LWS* loci include LAR (*lws* activating region)<sup>6</sup>, ppTRE1, and ppTRE2. The ppTRE elements are hypothesized to regulate *lws1/2* through TH. Additionally, for *LWS* and *MWS*, the regulatory sequences include LCR (locus control region)<sup>7</sup> and predicted ppTRE1/2-like elements (e1like and e2like) identified via genome alignment tools.

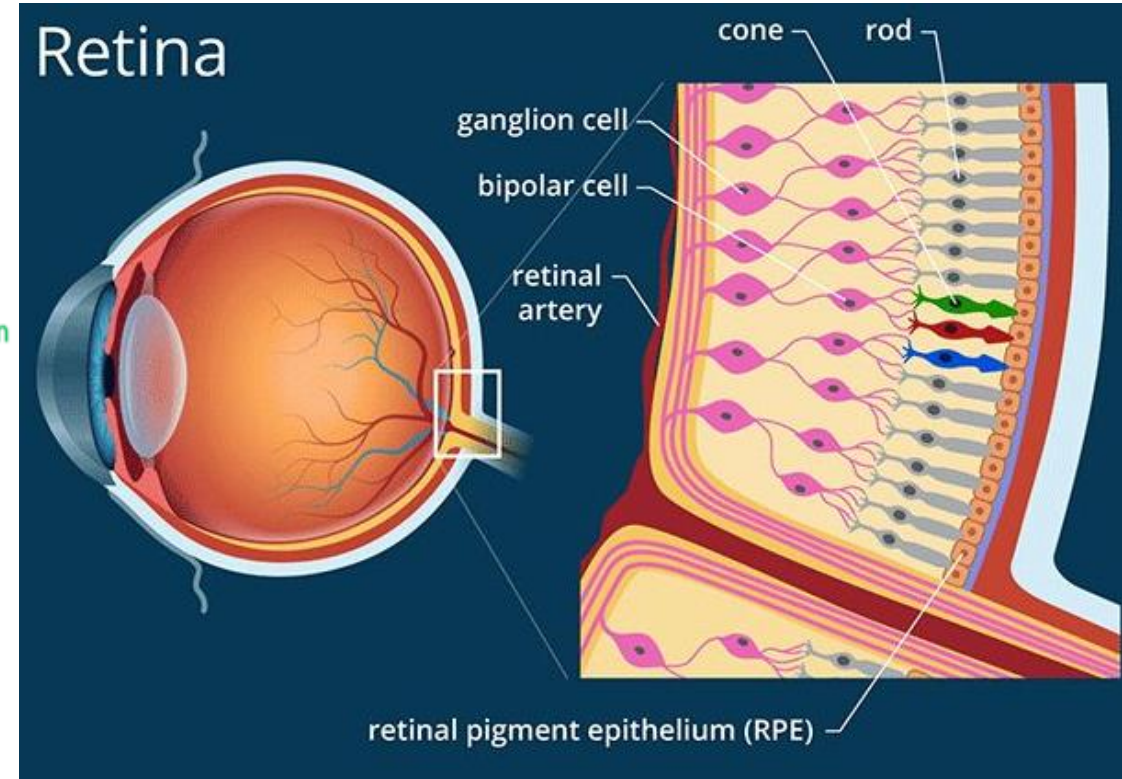
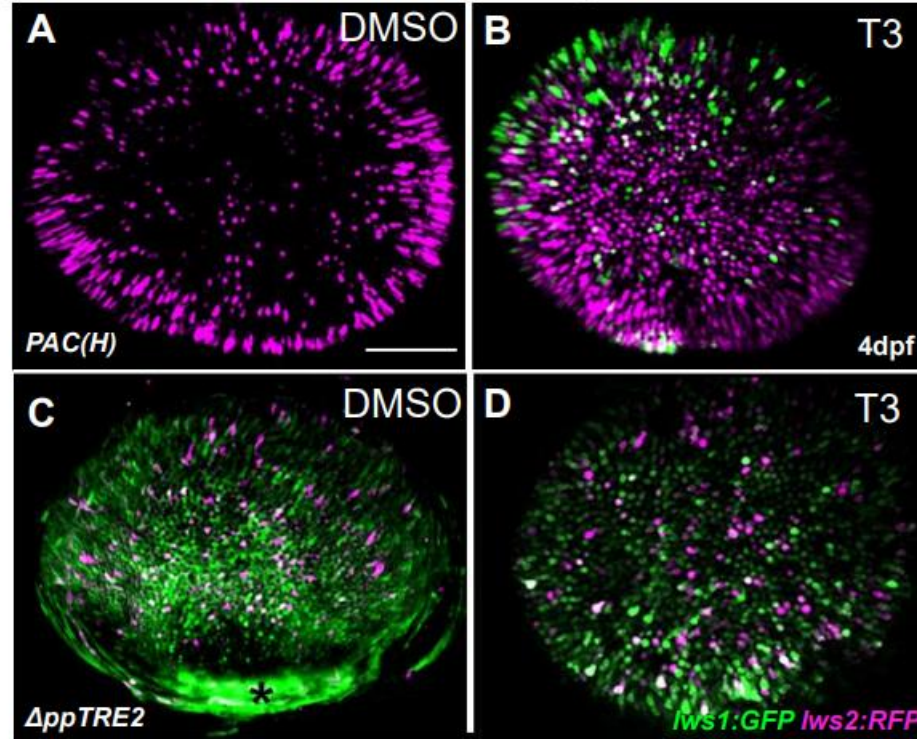


Image Credit: All About Vision

# Background

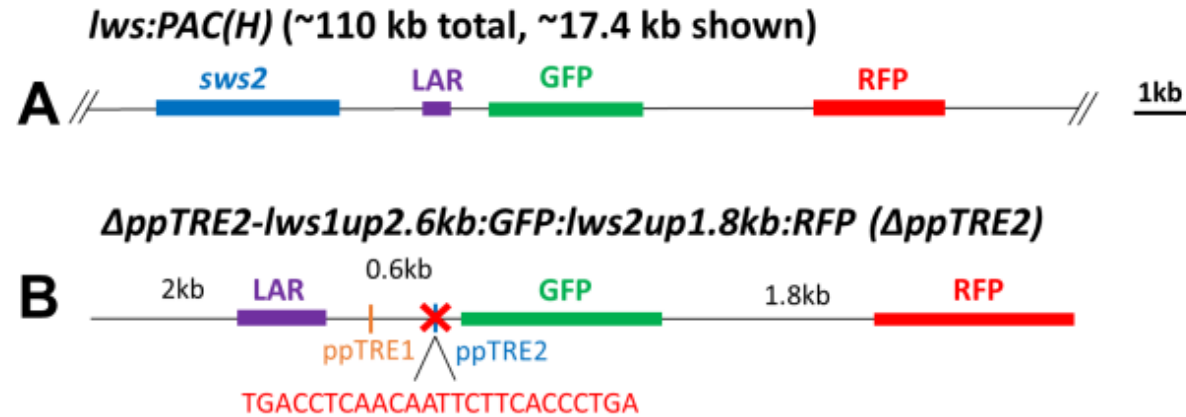
Figure 2. *lws:PAC(H)* larvae treated with increased T3 show an upregulation in *lws1* (GFP) and  $\Delta ppTRE2$  larvae show no significant difference in expression of *lws1* and *lws2* reporters.



**Fig. 2:** (A&B) 4 days post-fertilization (4dpf) *PAC(H)* zebrafish larval eyes under DMSO (A) or T3 (B) treatment. \*, region of autofluorescence from undissected sclera. Scale bars = 50 $\mu$ m. *lws1* is reported by GFP, and *lws2* is reported by RFP (pseudocolored magenta). (C&D)  $\Delta ppTRE2$  zebrafish larvae eyes under DMSO (C) or T3 (D) treatment. *lws1* is reported by GFP, and *lws2* is reported by RFP (pseudocolored magenta).

# Methods

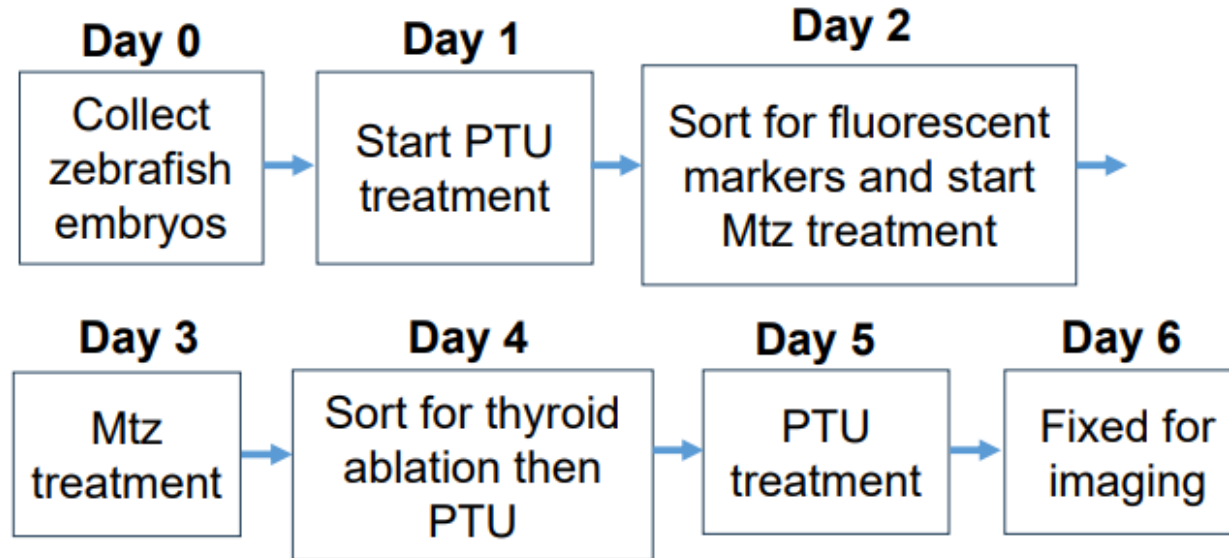
**Figure 3. Schematics of transgenic constructs.**



**Fig. 3: (A)** *lws:PAC(H)* which includes 110kb of zebrafish chromosome 11 with GFP-polyA inserted into exon 1 of *lws1* and RFP-polyA inserted into exon 1 of *lws2*.<sup>6</sup> **(B)** *ΔppTRE2-lws1up2.6kb:GFP:lws2up1.8kb:RFP* (*ΔppTRE2*) which includes the 2.6kb region upstream of *lws1* and the 1.8kb intergenic region, but with a 25bp region deleted which includes the ppTRE2. We crossed these lines with *Tg(tg:nVenus-2a-nfsB)wp.rt8*<sup>8</sup> to allow for thyroid ablation.

# Methods

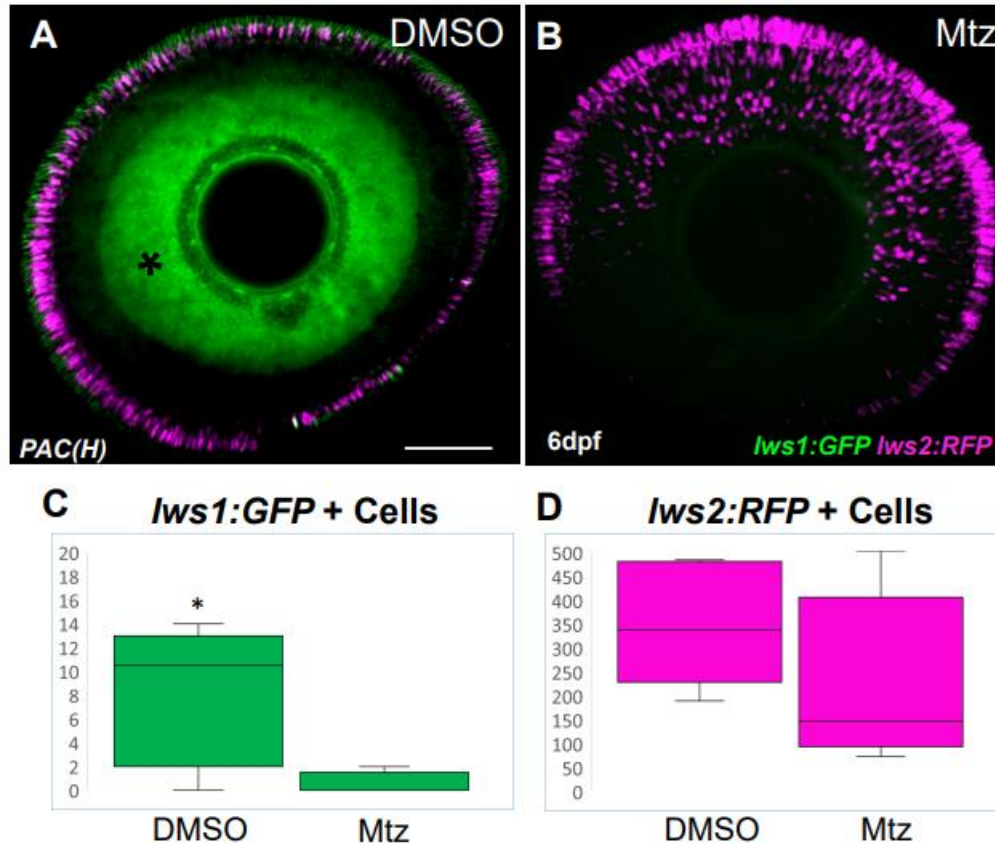
**Figure 4. Flowchart of larval zebrafish metronidazole (Mtz) treatment protocol.**



**Fig. 4:** 7-day procedure for full ablation of the thyroid. Samples were either treated with Mtz or DMSO to observe under athyroid and euthyroid conditions. Phenylthiourea (PTU) is a tyrosine inhibitor that blocks the formation of pigmentation. This enables a clearer observation of zebrafish embryos under the microscope.

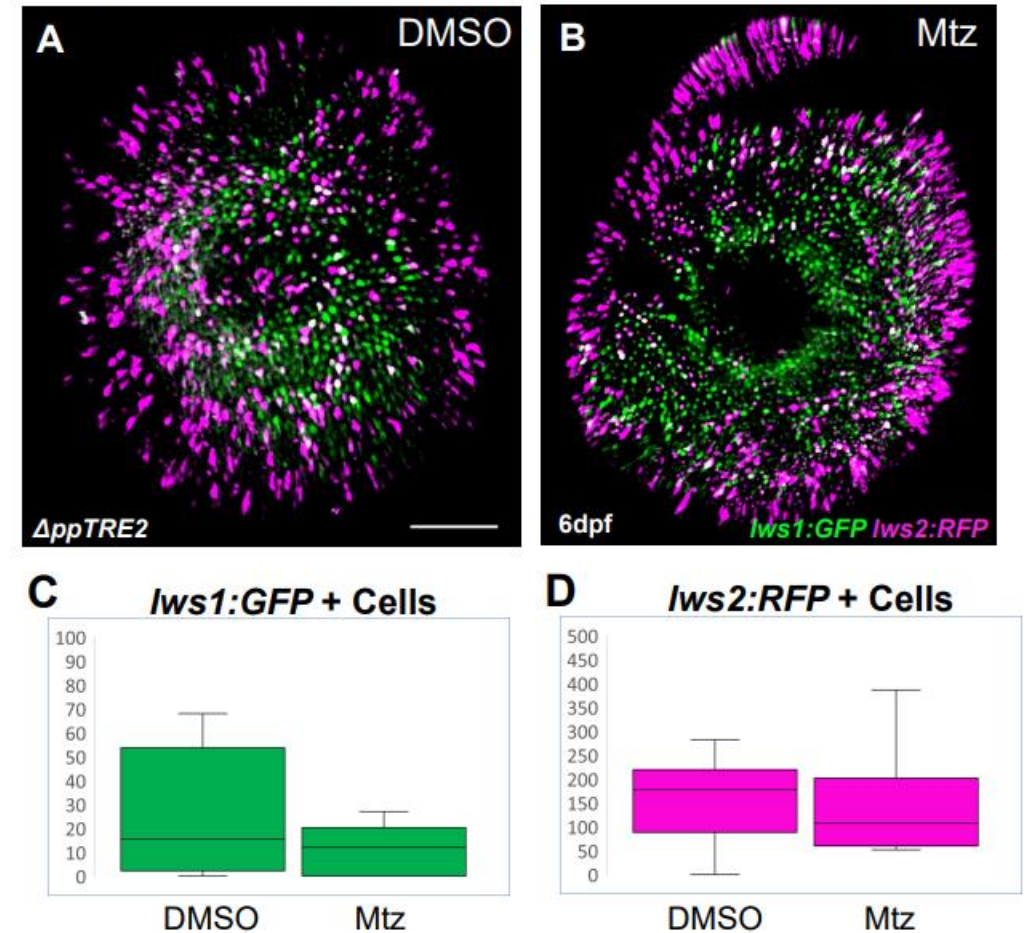
# Results

**Figure 5.** *lws:PAC(H)* larvae under Mtz (athyroid) and DMSO treatment show a downregulation in *lws1* (GFP) when endogenous TH was removed.



**Fig. 5:** (A&B) 6dpf zebrafish larval eyes under DMSO (A) or Mtz (B) treatment. \*, region of background fluorescence. Scale bars = 50 $\mu$ m. *lws1* is reported by GFP, and *lws2* is reported by RFP (pseudocolored magenta). Cell counts of *lws1* (C) and *lws2* (D) under control (n=5) and Mtz (n=4) treatments. Kruskal-Wallis p-value for *lws1* expression was 0.024257. \*p<0.05.

**Figure 6.**  $\Delta ppTRE2$  larvae under DMSO and Mtz (athyroid) conditions reveal no significant difference in expression between *lws1* and *lws2* reporters.



**Fig. 6:** (A&B) 6dpf zebrafish larvae eyes under DMSO (A) or Mtz (B) treatment. Scale bars = 50 $\mu$ m. *lws1* is reported by GFP, and *lws2* is reported by RFP (pseudocolored magenta). Cell counts of *lws1* (C) *lws2* (D) under control (n=6) and Mtz (n=5) treatments.

# Future Direction

- ChIP-PCR and ChIP-seq methods to determine whether a TH receptor binds within the 0.6Kbp region.

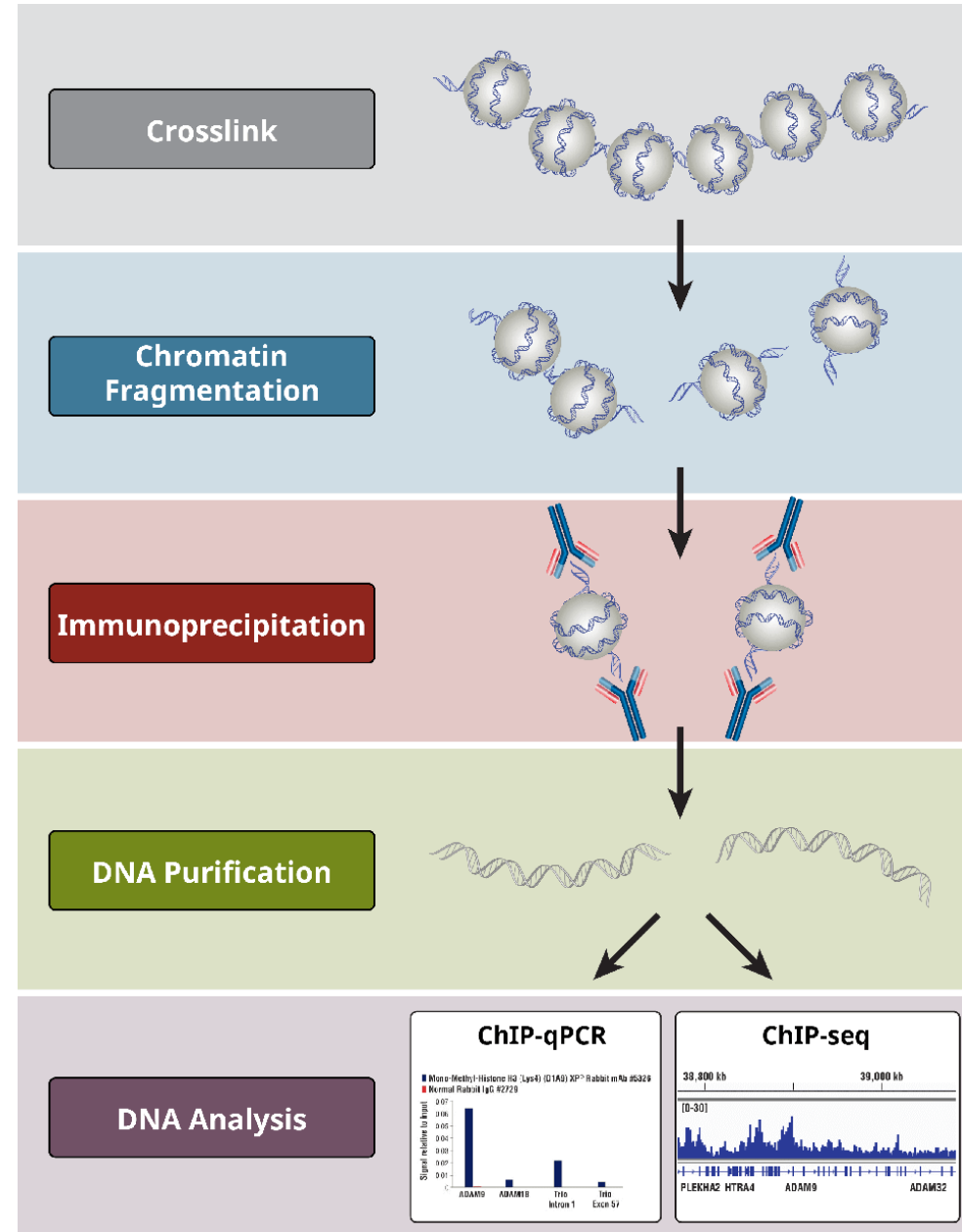


Image Credit: Cell Signaling Technology

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**Thanks for listening!**