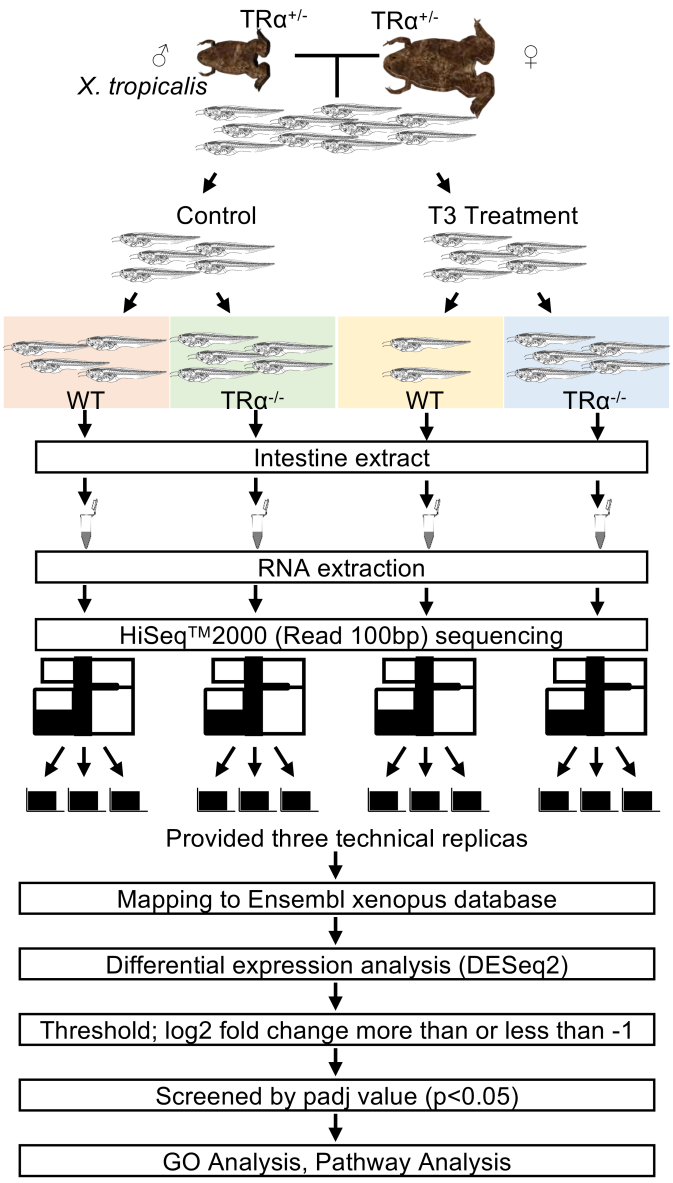
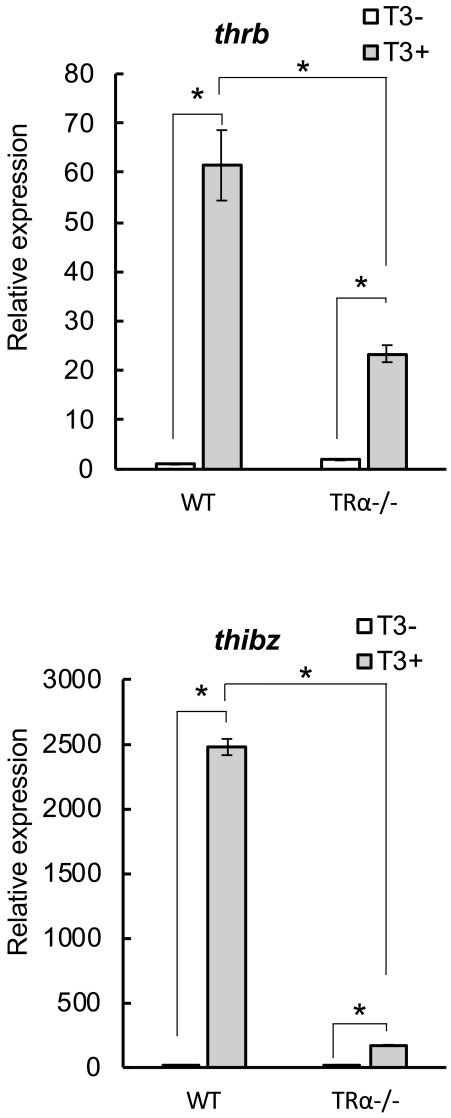
A



B



C

Database	Genes in database	Allingned genes	Convert to Human ID for GO analysis
NCBI	39860	8652	N. D
Ensemble	19921	16171	12592
Xenbase 9.0	34229	21069	10159

Supplemental Figure 2. Experimental design for RNA-seq.

(A) A pool of sibling tadpoles of different *TRα* genotypes at stage 52 to 54 were treated with or without T3. The intestine was isolated from each tadpole individually. After genotyping, intestine from individual tadpoles of same genotype and same T3 treated group were combined to obtain 4 samples. RNA was then extracted from each and quality was determined. To validate each sample, cDNA was synthesized and analyzed to confirm the regulation of some known T3-target genes such as *thrb*, *mmp11* and *thibz*. Then, the remaining RNA was subjected to RNA-seq. Sequence reads were mapped to Ensembl transcript data. Ensembl gene IDs of *Xenopus* genes were changed into the corresponding human Ensembl IDs, and analyzed for differential expression. Two-fold up- or down-regulated genes were subjected to GO and pathway analyses.

(B) The upregulation of two T3-direct target genes by T3 is reduced in $TR\alpha^{-/-}$ tadpoles. *X. toropicalis* tadpoles at stage 54 were treated with or without 10 nM T3 for 18 hours. RT-PCR analysis was carried out to confirm the regulation of *thrb* and *thibz* in the samples prior to RNA-seq analysis. Note that both of genes were upregulated by T3 treatment in wild-type tadpoles and this upregulation was drastically reduced in $TR\alpha^{-/-}$ tadpoles. The asterisk (*) indicates a significant difference between the T3− and T3+ or the $TR\alpha^{-/-}$ tadpoles and wild-type tadpoles ($P<0.05$). Each bar represents the mean plus S.E.