

Investigating the effects of DNA methylation on EE2 induction of Estrogen Receptor alpha gene expression in fathead minnows

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Background

- Exposure to exogenous estrogens such as 17 α -ethynylestradiol (EE2), is associated with reproductive problems in fish
 - Decreased fecundity
 - sperm production
 - feminization of males
- Estrogen receptors genes have important roles in reproduction
 - Encode nuclear receptors which allow binding of estrogens, or estrogen mimics
- Transcriptional regulation via epigenetic mechanisms is one method by which organisms adapt to changing conditions
 - DNA methylation

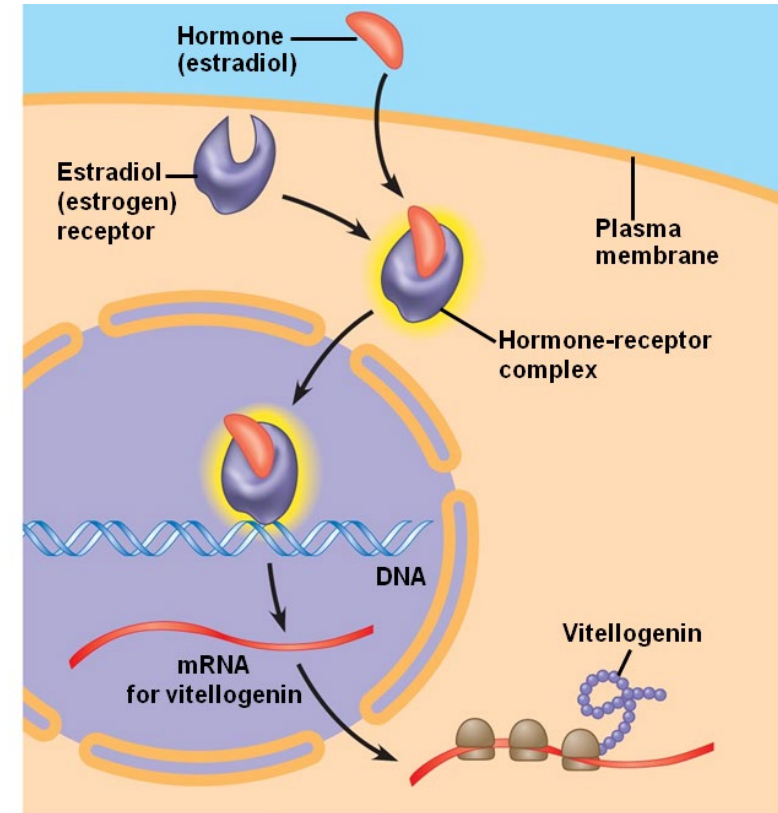


Image from Pearson Benjamin Cummings

What we know...

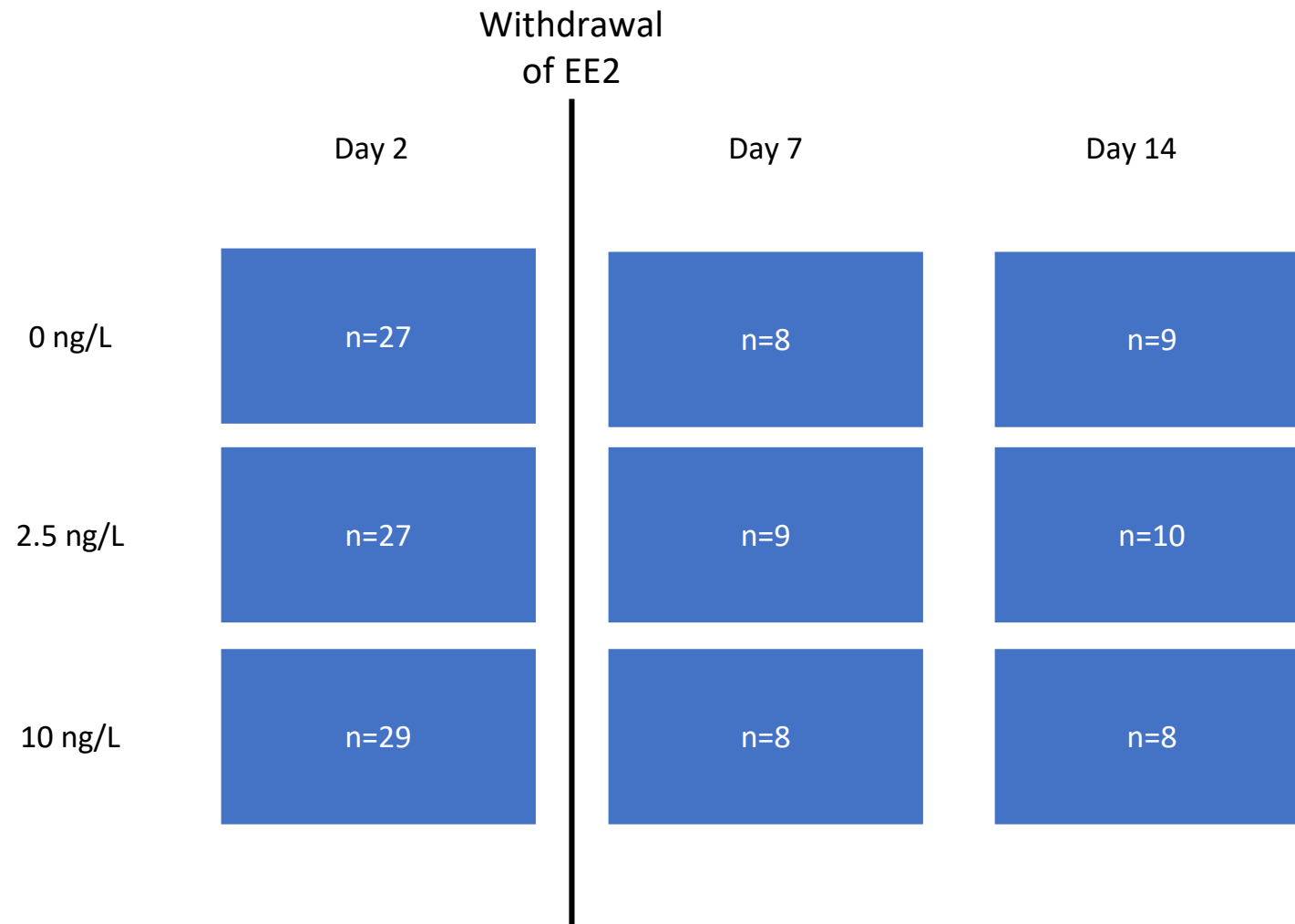
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- We hypothesize that an increase in ER α expression will be associated with demethylation of the promoter region of ER α

Research Objectives

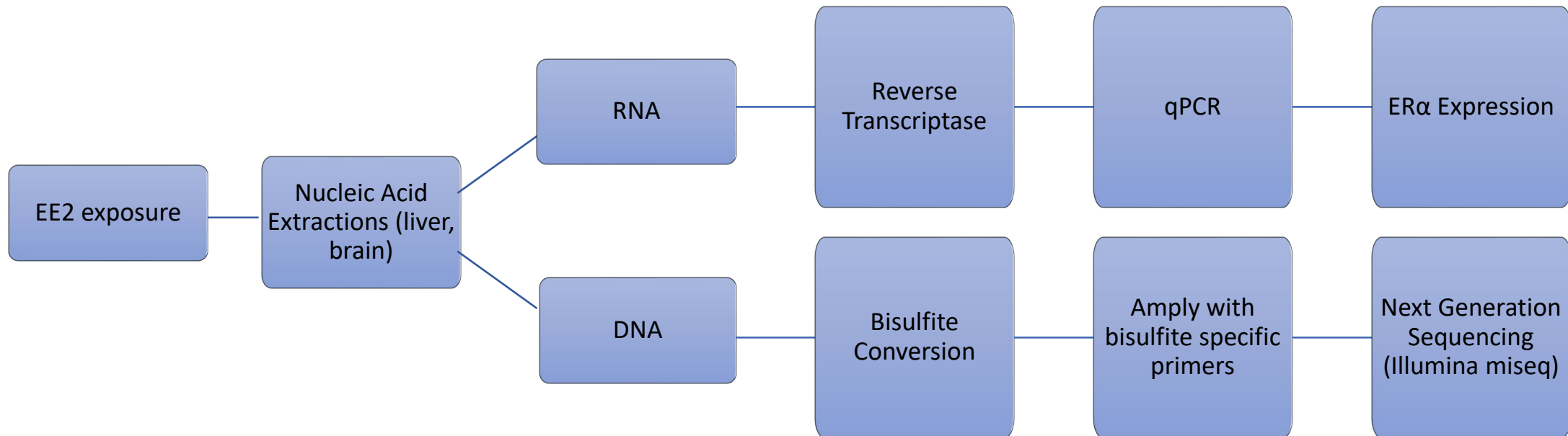


- Characterize the pattern and level of DNA methylation for ER α in liver and brain tissue of mature male fathead minnows (*Pimephales promelas*) exposed to EE2 for 48 hours
- Identify potential DNA methylation changes in brain and liver following a depuration period to determine whether potential changes persist post-exposure
- Determine whether DNA methylation changes are associated with gene expression

Study Design

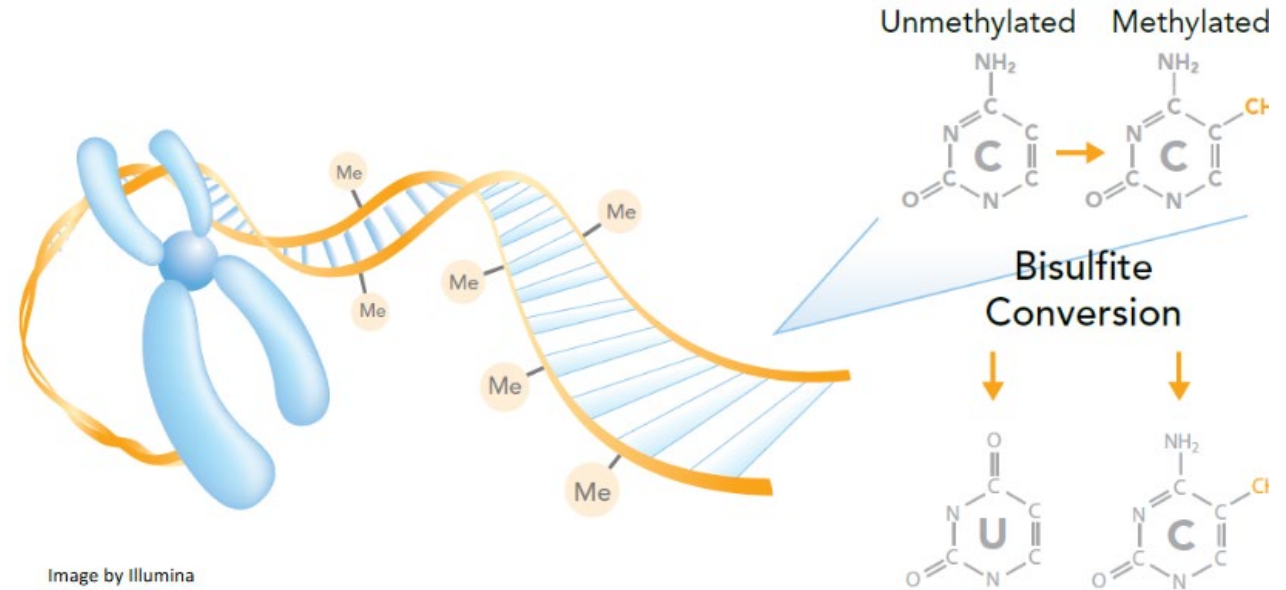


Methods

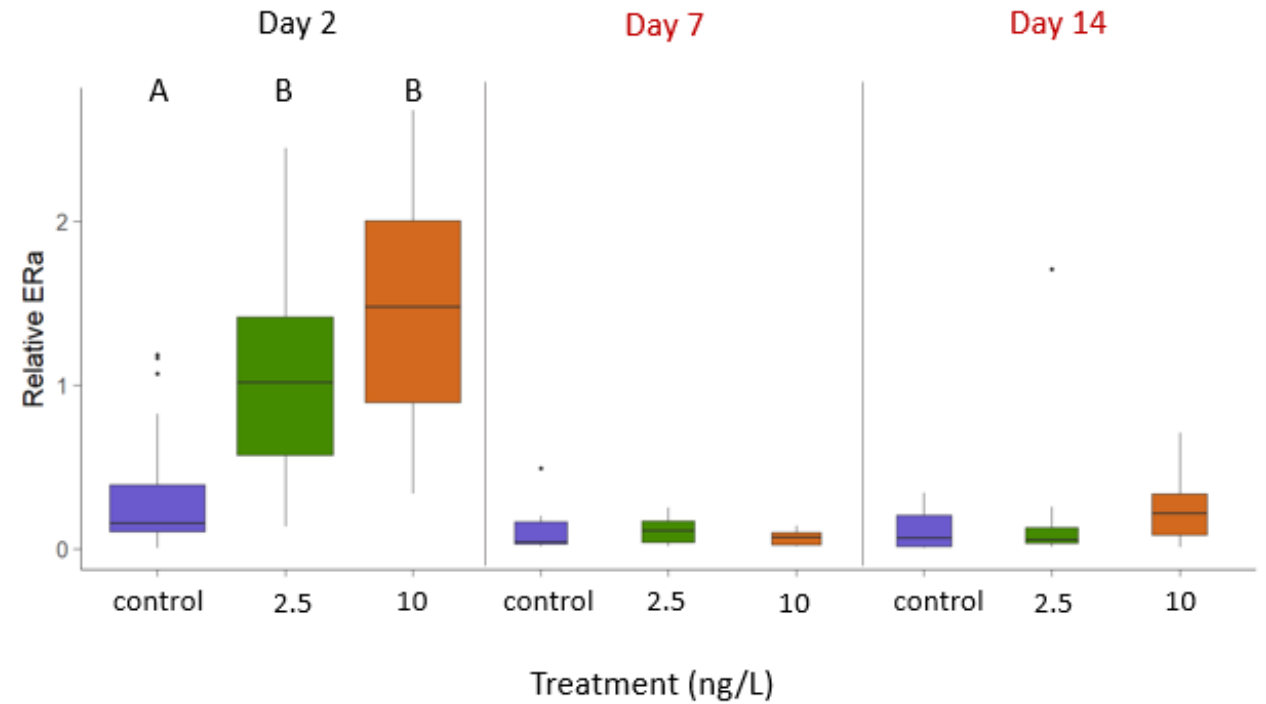
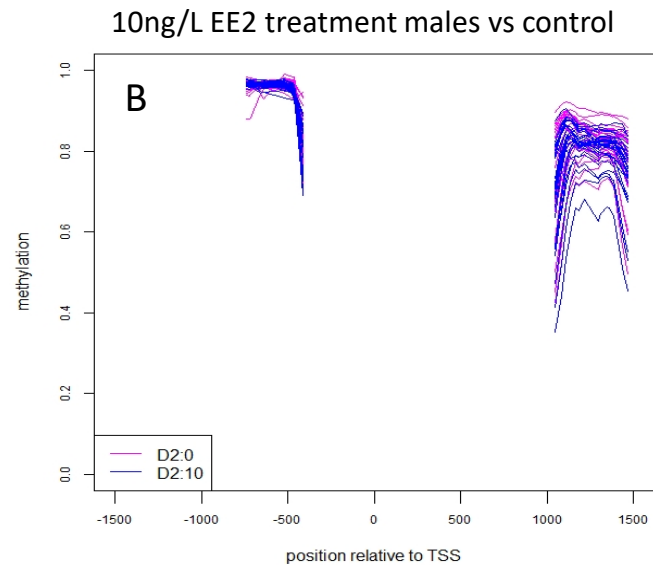
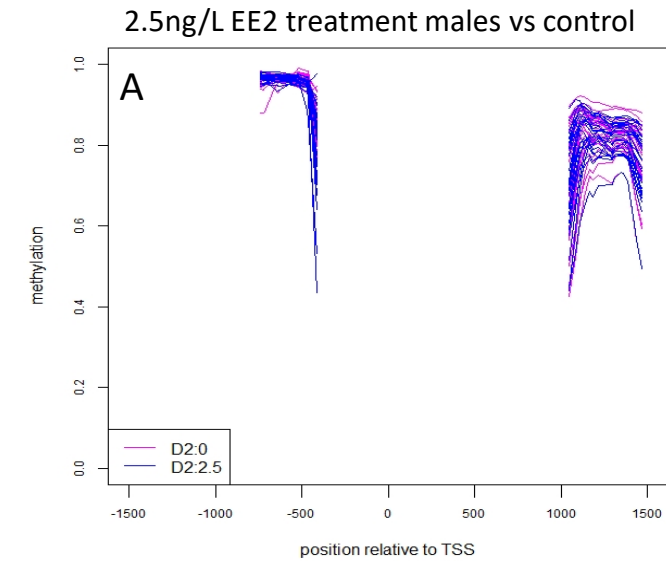


Bioinformatic Pipeline

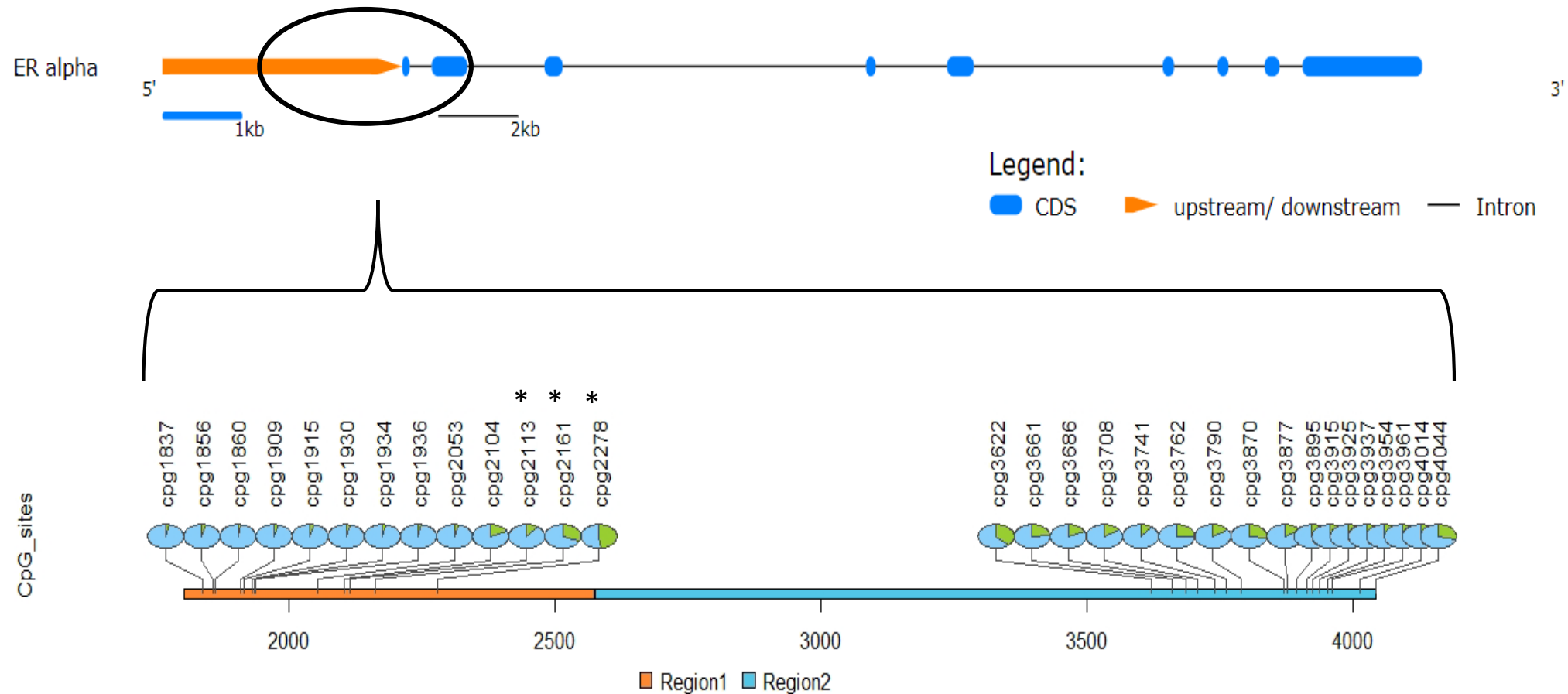
- Bioinformatics
 - QC of sequencing data: FastQC (v0.11.8)
 - Remove adapter sequences and primers: Cutadapt (v1.18)
 - Read alignment: Bismark (v0.19.0), Bowtie2 (v2.3.1)
- Statistics
 - Bisulfite analysis: BiSeq (v1.20.0), beta regression (5, 6)
 - Gene expression: ANOVA, Tukey multiple comparisons of means



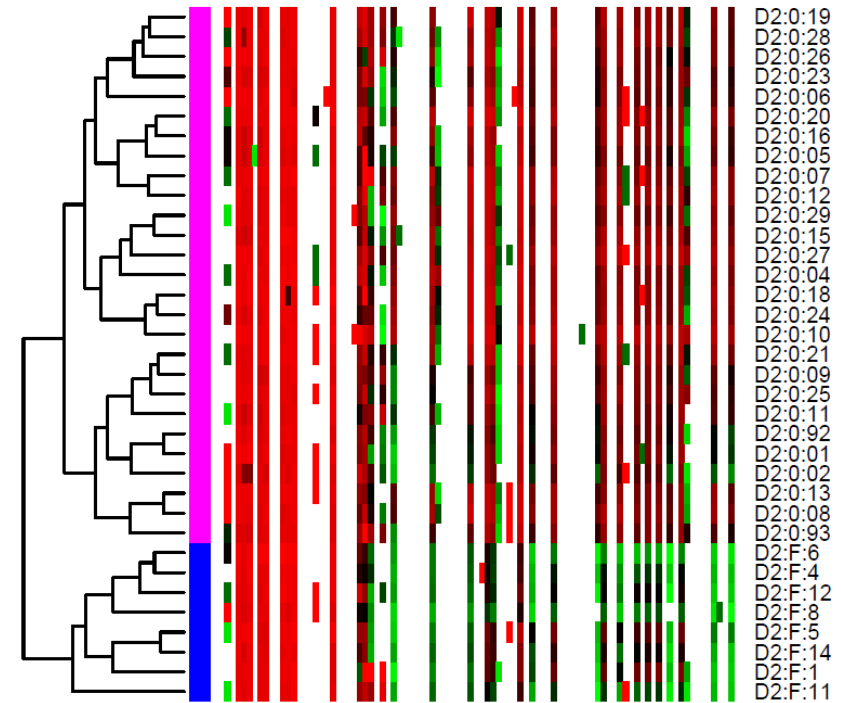
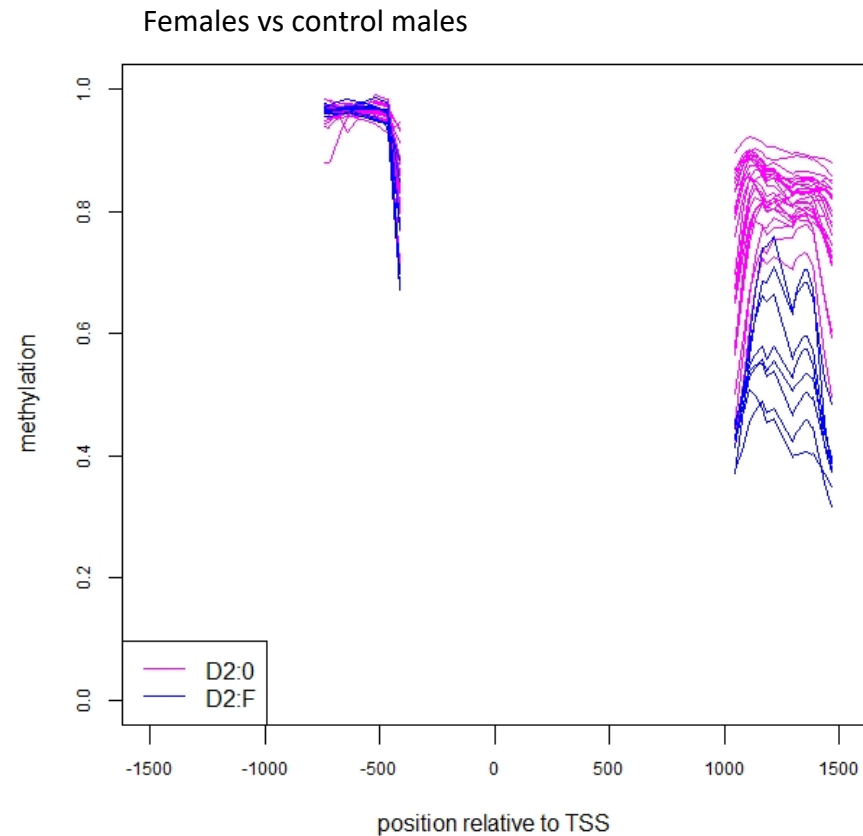
Methylation level and gene expression



DNA methylation of targeted regions of *ERα*



DNA methylation is different in female & male fish



Summary

- In liver, gene expression of treatment groups are significantly different from control at both doses of EE2 ($p < 0.001$)
- DNA methylation of upstream and coding regions of ER α is not correlated with gene expression (Pearson's correlation)
- 40 CpG sites were targeted in this study, 29 had enough sequence coverage for analysis (13 in the upstream region of the gene, 17 sites in the coding region)
- In female fish liver, all 17 CpG sites located in the coding region of ER α display significantly different DNA methylation from males (BH, $p < 0.001$). No significant difference is found in brain
- For liver, after the 2d EE2 exposure at CpG site 2161, DNA methylation is significantly different in females ($p < 0.005$) relative to control males
- After the 2d EE2 exposure, CpG sites 2113, 2161, and 2278 were found to be differentially methylated in the 10 ng/L EE2 treatment group (Figure 5; $p = 0.03, 0.006, 0.05$ respectively)