Establishing a Modular Pipeline for Identifying the Cis-Regulatory Elements and Transcription Factor Binding Sites in *C.elegans*

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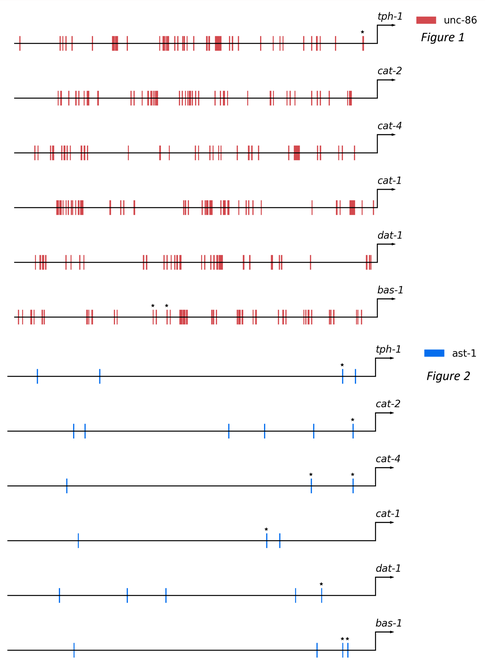
Introduction

The long-term goal of this project is to perform a comparative analysis of how regulatory mechanisms of neuron type specification have evolved across the animal phylogeny. As a first step, we created a modular computational pipeline that would identify cis-regulatory sequences and transcription factor binding sites that regulate the expression of *C. elegans* neuron type–specific genes.



Figures 1 and 2 show examples of individual transcription factor binding sites for the marker genes. Investigating why these differences between transcription factors arise would be an ideal next step in this project.

We used to database Wormbase to find all of the transcription factors for each marker gene.

For the TFs with no PWMs found in CisBP we searched for putative homologues using the HMMER tool and found their corresponding PWMs.

The inputs to our pipeline are the effector genes you want the CREs and TF binding sites for. In this example, we are inputting the TPH-1, CAT-2, and CAT-4 marker genes

Input Genes

Transcription Factors

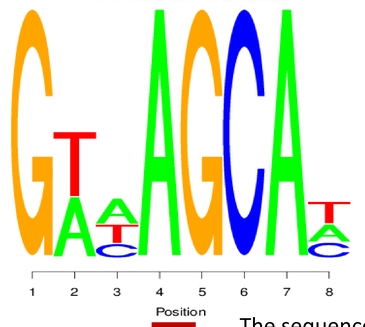
We searched CisBP for the Position Weight Matrices to find candidate binding sites along the sequence.

The sequences 2000 bp upstream of the start point of each effector gene in question.

DNA Binding Domain Alignment

```
HUMAN KKASKPKKAASKAPTKKPKATPVKKAKKKLAATPKKAKKPKTVKAK
MOUSE KKAAKPKKAASKAPSKKPKATPVKKAKKKPAATPKKAKKPKVVKVK
RAT   KKAAKPKKAASKAPSKKPKATPVKKAKKKPAATPKKAKKPKIVKVK
COW   KKAAKPKKAASKAPSKKPKATPVKKAKKKPAATPKKTKKPKTVKAK
CHIMP KKASKPKKAASKAPTKKPKATPVKKAKKKLAATPKKAKKPKTVKAK
```

Position Weight Matrices



Cis-Regulatory Sequences

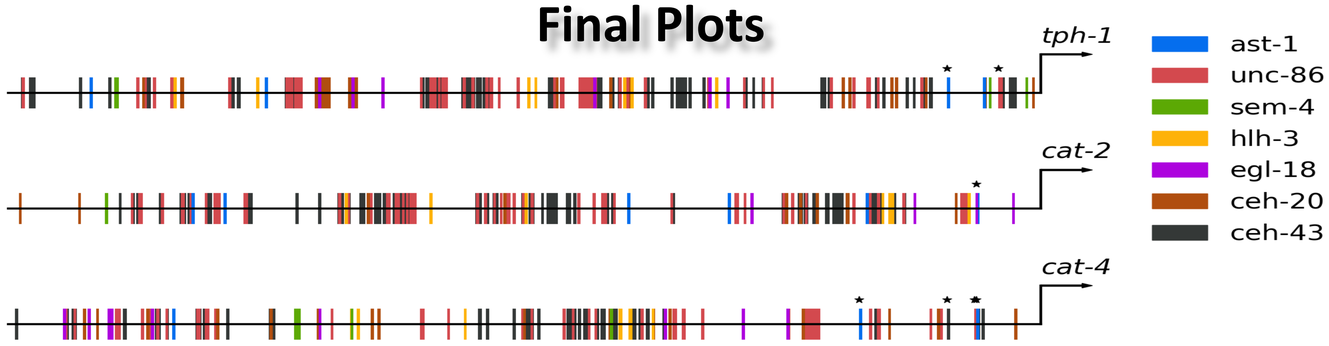
```
>dat-1 CRE
AGATCTGATTTGATCTCCTACTCTA
CAGTTTACTACCCAGGTTACCTAGA
GGTTATCTAGCAACTACAAAAATTG
>cat-1 CRE
GGATATAGGGATACACATCAAAACT
AAACTACAGCATACAGCATCAGATT
GGGAGTCCAGGTCATATTGTACAGT
CAGTACGATAATTATCCGGTCTAAG
```

We can now obtain PWMs for the putative homologues of the DNA binding domains for the TFs of interest and find candidate binding sites to plot.

The sequences corresponding to the effector genes in question are searched for high affinity binding sites using the PWMs obtained from CisBP and using the HMMER alignment tool.

The PWMs were used to search the sequence for high affinity binding sites to plot.

Final Plots



What's Next?

The immediate goal in the future would be to use this pipeline to compare the binding profiles of these genes to those of negative control genes, along with examining the question of cooperativity: which pairs of transcription factors appear to co-bind more often than expected by chance?

Acknowledgements

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References