Sequence Alignment and Recognition of DNA Motifs

A Bioinformatics Approach

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Background

What are DNA sequence motifs?

A Broad Biological Phenomena

"Sequence motifs are short, recurring patterns in DNA that are presumed to have a biological function. Often they indicate sequence-specific binding sites for proteins… others are involved in important processes at the RNA level…"

- Patrik D'haeseleer

Motif Recognition and Representation

A Visual Pathway

1.
```
HEM13   CCCATTGTTCTC
HEM13   TTTCTGGTTCTC
HEM13   TCAATTGTTTAG
ANB1    CTCATTGTTGTC
ANB1    TCCATTGTTCTC
ANB1    CCTATTGTTCTC
ANB1    TCCATTGTTCGT
ROX1    CCAATTGTTTTG
```

2. YCHATTGTTCTC

3.
```
A 002700000010
C 464100000505
G 000001800112
T 422087088261
```

4. 

5. 

6. 

Inquiry and Approach

Research Inquiry

Discovering DNA Motifs

“If a data set of sequences is believed to possess a motif, how can we determine the composition and location of this motif?”

Addressing the Inquiry

A General Approach

1. Find a data set known to have the same, conserved motif in each sequence.
2. Select one sequence to be a reference.
3. Align all sequences to that reference at the motif location.
4. Determine the frequency and relative entropy of nucleotides at each position.

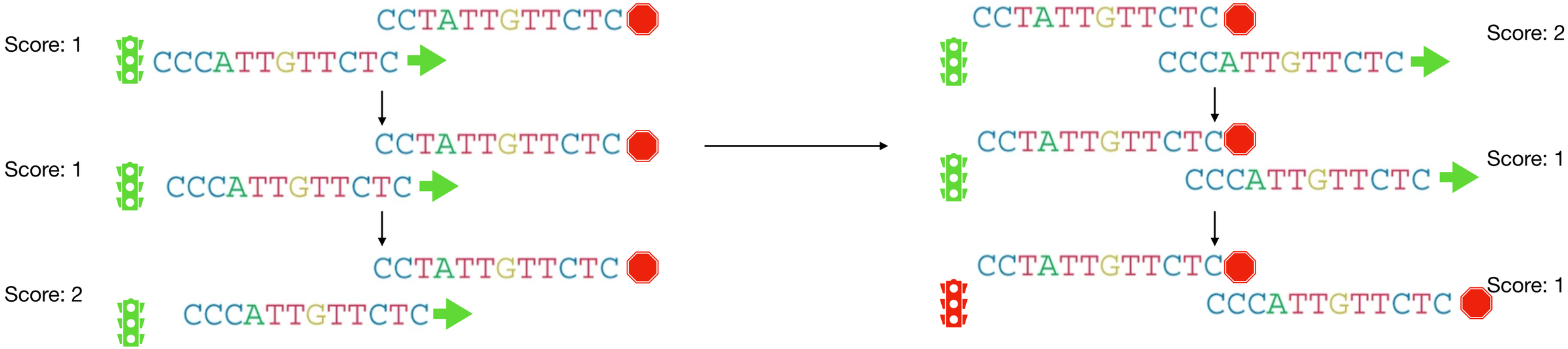
Addressing the Dilemma

Computational Concerns

- Without knowing the motifs beforehand, aligning sequences together at the motif becomes an algorithmically and computationally intensive affair.
    - The approach used for this pipeline involves finding a “sliding” score between a reference sequence and another.
        - The value for this score signifies at that specific shift of one sequence to the reference, how many nucleotides are similar at each position.
        - The higher the score, the more ideal alignment has been reached, but this may not be motif alignment.

Addressing the Dilemma

Computational Concerns



CCTATTGTTCTC

CCCATTGTTCTC

Score: 11

This is the maximum score.

Addressing the Dilemma

Computational Concerns

- Modeling and recognition of DNA sequence motifs often involves matrix encodement and mathematics.
    - It is simply quite difficult to run predictive complex models where the four nucleotides are represented as characters such as “A”, “C”, “G”, and “T.”
        - The approach for this pipeline uses an encoding method where each of the four nucleotides are represented in 3-dimensional space where the position of each nucleotide is equidistant to zero.

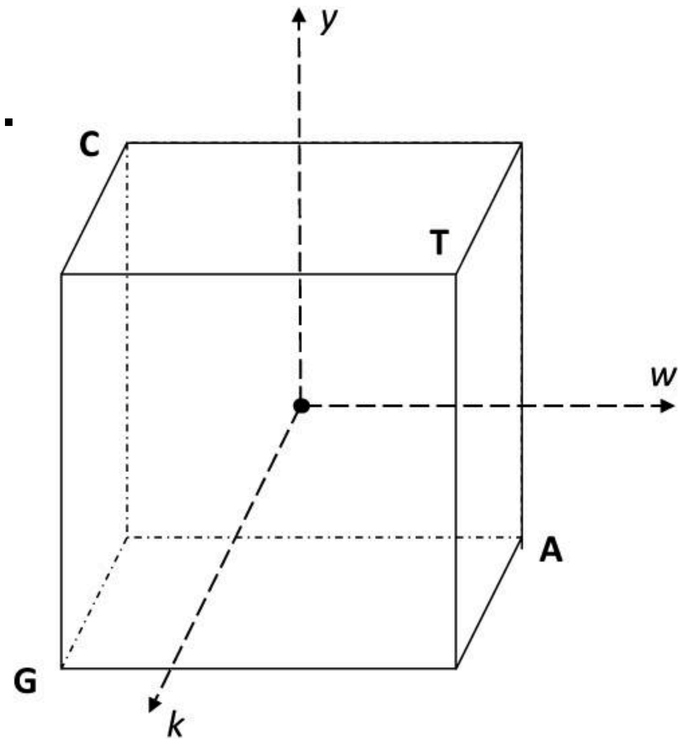
Addressing the Dilemma

Computational Concerns

1.

	A	C	G	T
w	1	-1	-1	1
y	-1	1	-1	1
k	-1	-1	1	1

2.



Pipeline

Pipeline Steps

Reproducible Computation

1. Encode all the desired data (as *.fasta* files) into *wyk* format.
2. Determine the shift value by calculating scores between an established reference *wyk* file and all remaining *wyk* files in the data set.
3. Shift the non-reference *wyk* files to align with the reference by adding 0's at the beginning or end of the file with the number of 0's obtained from the shift value.
4. Add by each index position (or line) the contents of the reference *wyk* file and all the non-reference, shifted *wyk* files to create a final sum file.
5. Convert the final sum *wyk* file into a nucleotide probability matrix.
6. Upload the contents of the nucleotide probability matrix to a DNA logo generator (http://www.benoslab.pitt.edu/cgi-bin/enologos/enologos.cgi) to visualize output.

Data, Results, and Analysis

Test 1 Data Set

Sequences With Identical Motifs

>test1a

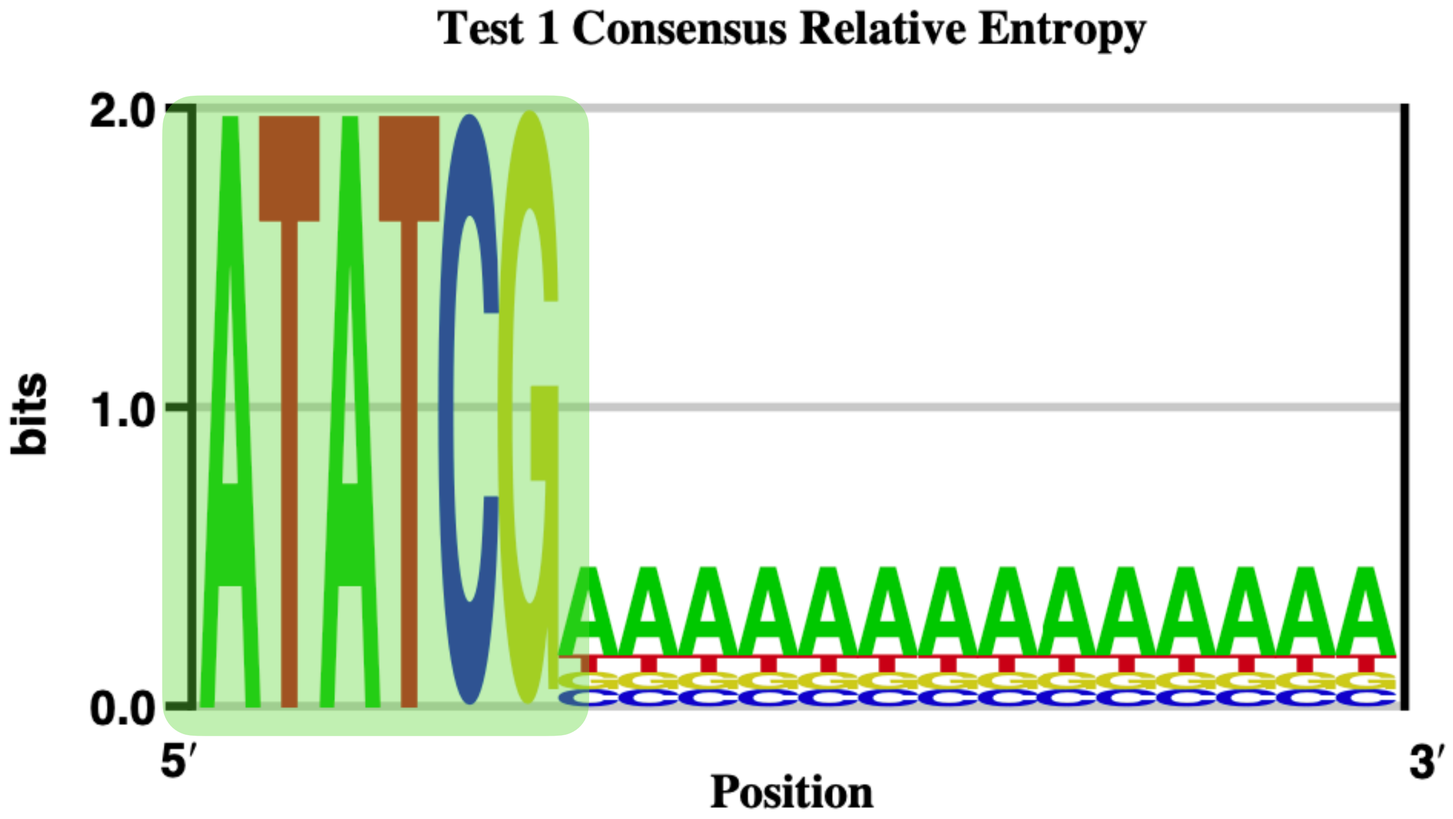
ATATCGAAAAAAAAAAAAAA

>test1b

TTTTTTTTTTTTTT**ATATCG**

Test 1 Data Set

DNA Logo Results



Test 2 Data Set

Sequences With No Similarities

>test2a

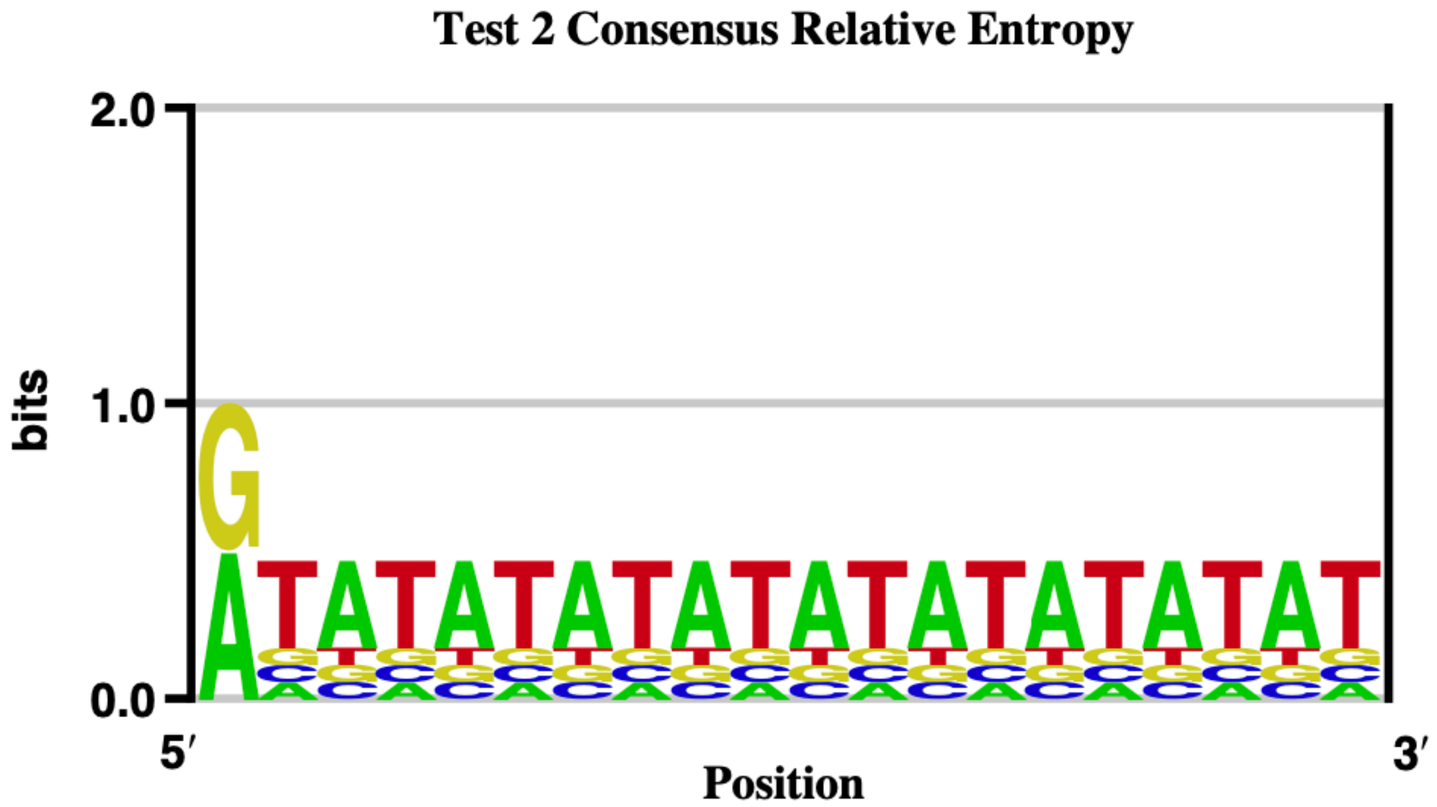
ATATATATATATATATATATAT

>test2b

CGCGCGCGCGCGCGCGCGCG

Test 2 Data Set

DNA Logo Results



Test 3 Data Set

Sequences That Are Identical

>test3a

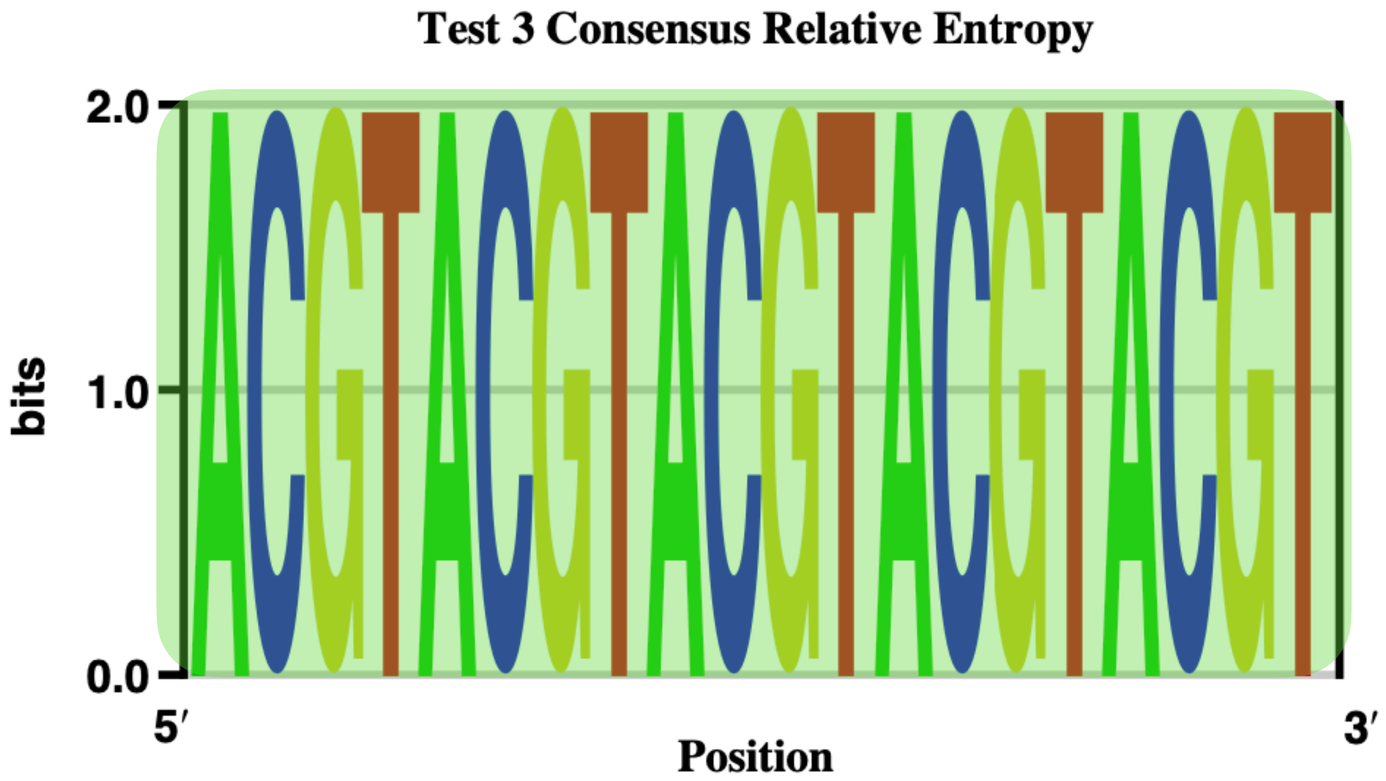
ACGTACGTACGTACGTACGTACGT

>test3b

ACGTACGTACGTACGTACGTACGT

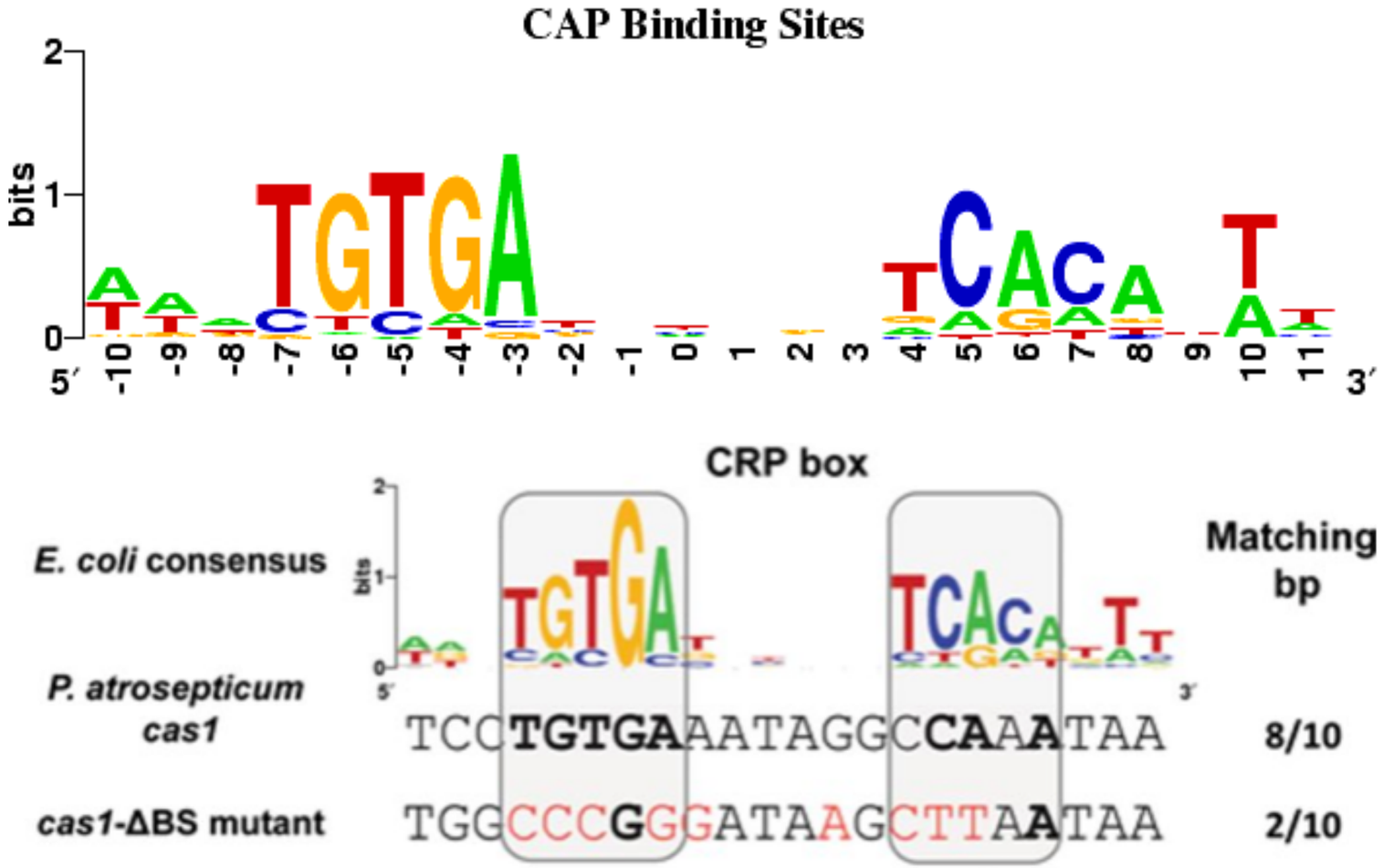
Test 3 Data Set

DNA Logo Results



Exploring An *E. coli* Motif

An Almost Palindromic Binding Site For Proteins



Dummy Data Set

Sequences With Same Motifs As *E.coli* CRP/CAP Binding Protein

>dummy1

TGTGAAAAAAA**TCACA**AAAAAAAAAAAAAAAA

>dummy2

CCCCCCCCCCCCCCCCC**TGTGA**CCCCCCC**TCACA**

>dummy3

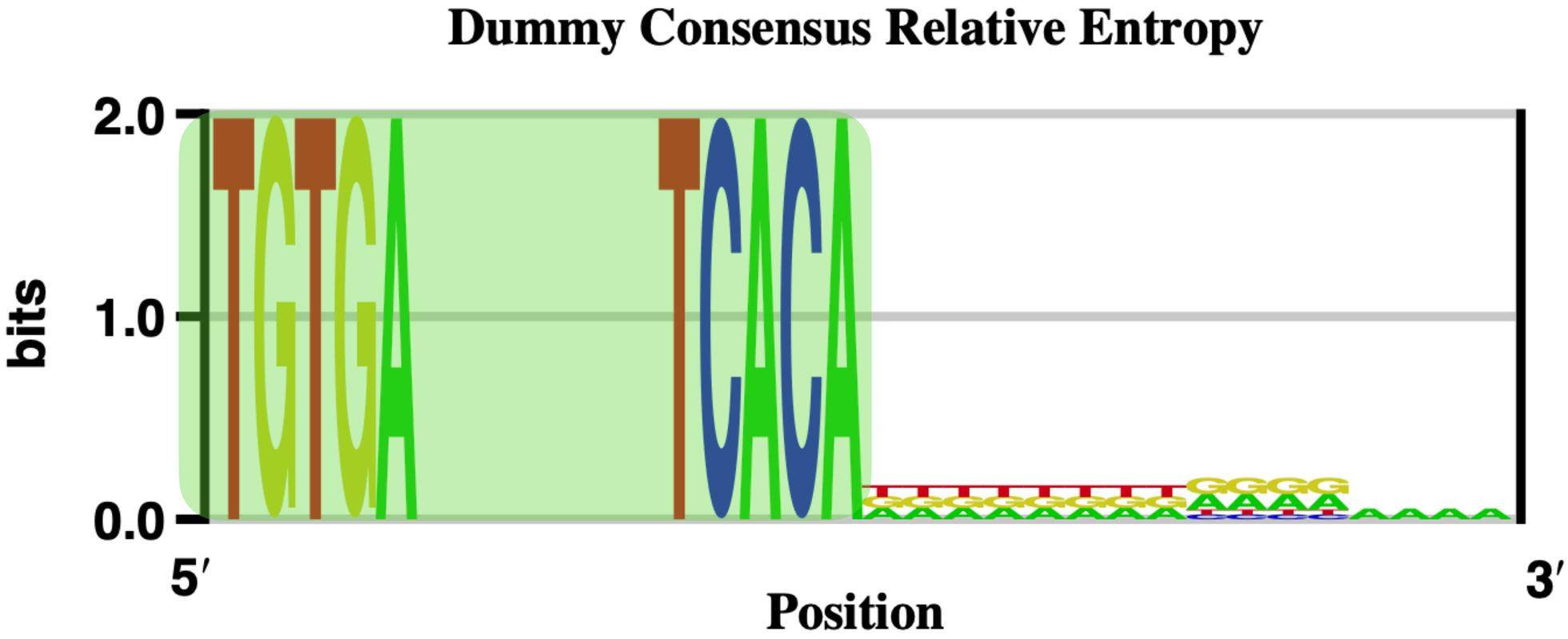
TTTTTTTT**TGTGA**TTTTTT**TCACA**TTTTTTTT

>dummy4

GGGG**TGTGA**GGGGGGG**TCACA**GGGGGGGGGGGGG

Dummy Data Set

DNA Logo Results



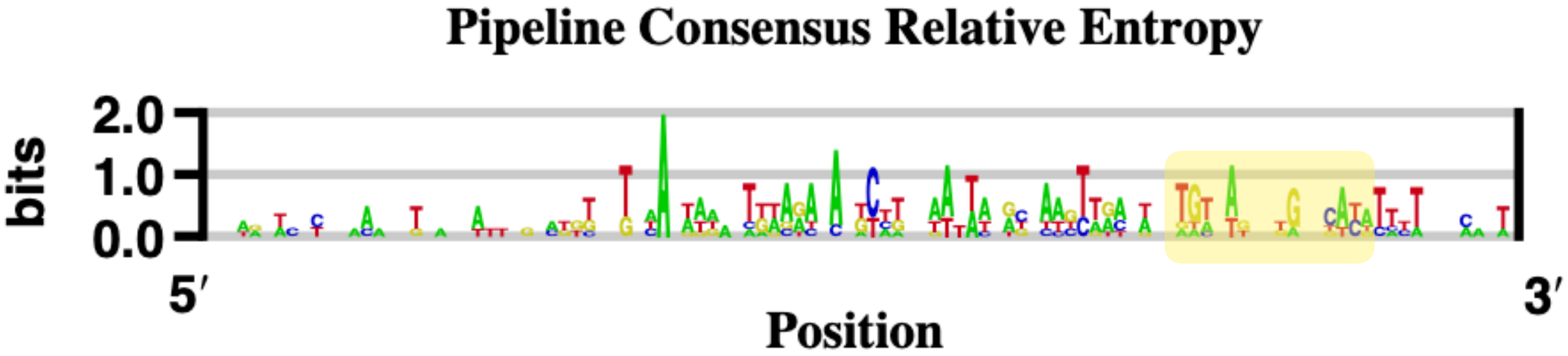
E. coli Data Set

Seven Genes With Common Motifs

>ECOBGLR1 (REFERENCE)
ACAAATCCCAATAACTTAATTATTGGGATTTGTTATATATAACTTTATAAATTCCTAAAA
TTACACAAAGTTAATAAC**TGTGA**GCATGG**TCATA**TTTTTATCAAT
>ECOCYA
ACGGTGCTACACTTGTATGTAGCGCATCTTTCTTTACGGTCAATCAGCAAGGTGTTAAAT
TGATCACGTTTTAGACCATTTTTTCGTCGTGAAACTAAAAAAACC
>ECOTNAA
TTTTTTAAACATTAAAATTCTTACGTAATTTATAATCTTTAAAAAAAGCATTTAATATTG
CTCCCCGAACGATTGTGATTCGATTCACATTTAAACAATTTCAGA
>TDC
GATTTTTATACTTTAACTTGTTGATATTTAAAGGTATTTAATTGTAATAACGATACTCTG
GAAAGTATTGAAAGTTAATTTGTGAGTGGTCGCACATATCCTGTT
>ECOOMPA
GCTGACAAAAAAAGATTAAACATACCTTATACAAGACTTTTTTTTCATATGCCTGACGGAG
TTCACACTTGTAAGTTTTCAACTACGTTGTAGACTTTACATCGCC
>ECOMALBA
ACATTACCGCCAATTCTGTAACAGAGATCACACAAAGCGACGGTGGGGCGTAGGGGCAAG
GAGGATGGAAAGAGGTTGCCGTATAAAGAAACTAGAGTCCGTTTA
>ECOMALBA2
GGAGGAGGCGGGAGGATGAGAACACGGCTTCTGTGAACTAAACCGAGGTCATGTAAGGAA
TTTCGTGATGTTGCTTGCAAAAATCGTGGCGATTTTATGTGCGCAA

E. coli Data Set

Pipeline DNA Logo Results



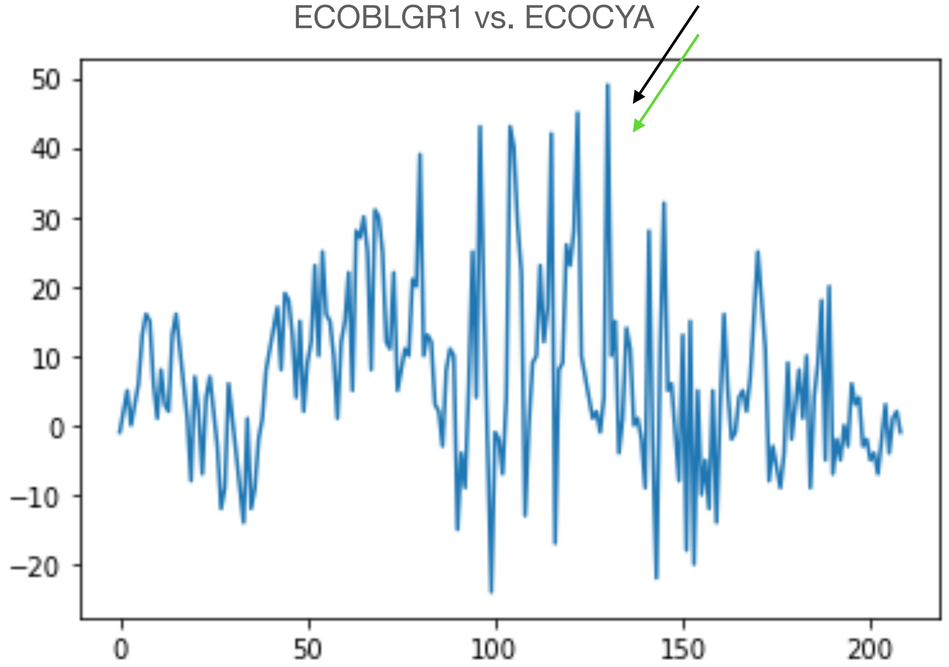
E. coli Data Set

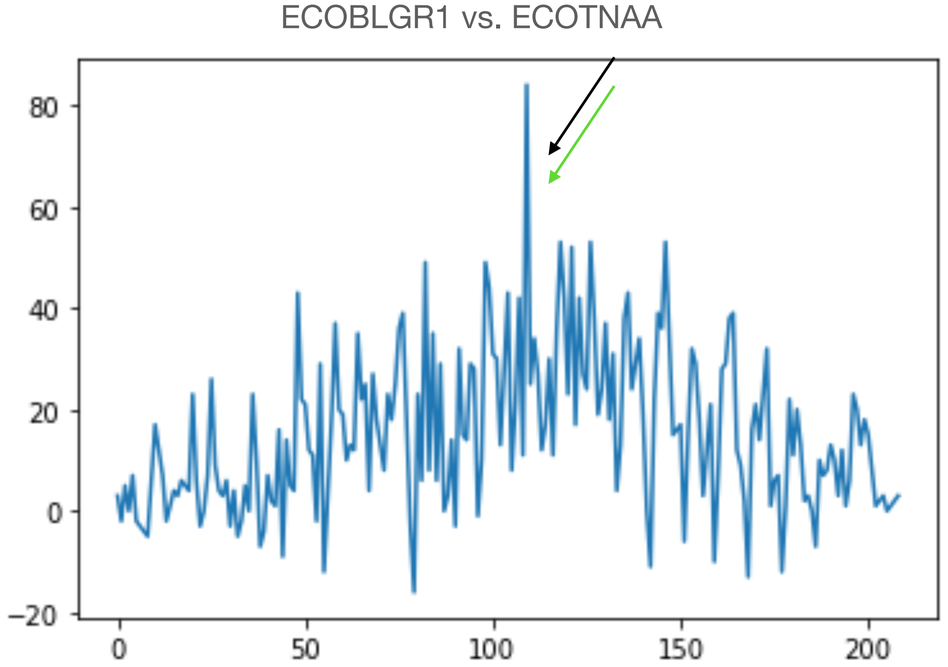
Revisiting the Data Set

Sequence	Reference	Hand-Counted Distance from One End to Motif	Relative Distance from Reference
ECOBLGR1	Yes	8	N/A
ECOCYA	No	34	26
ECOTNAA	No	13	5
TDC	No	6	-2
ECOOMPA	No	36	28
ECOMALBA	No	62	54
ECOMALBA2	No	47	39

E. coli Data Set

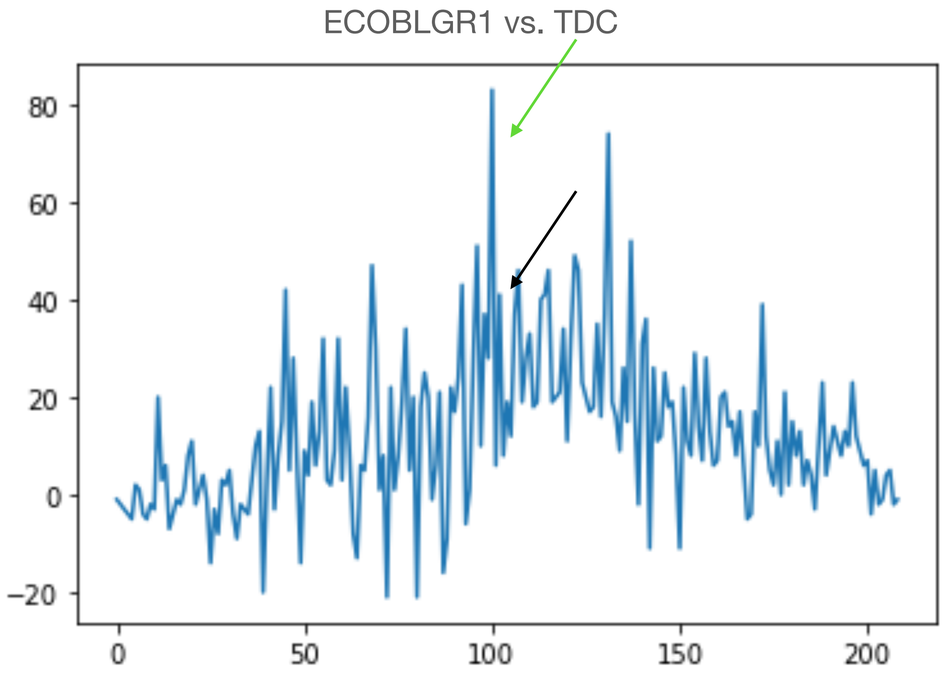
A Graphical Troubleshoot

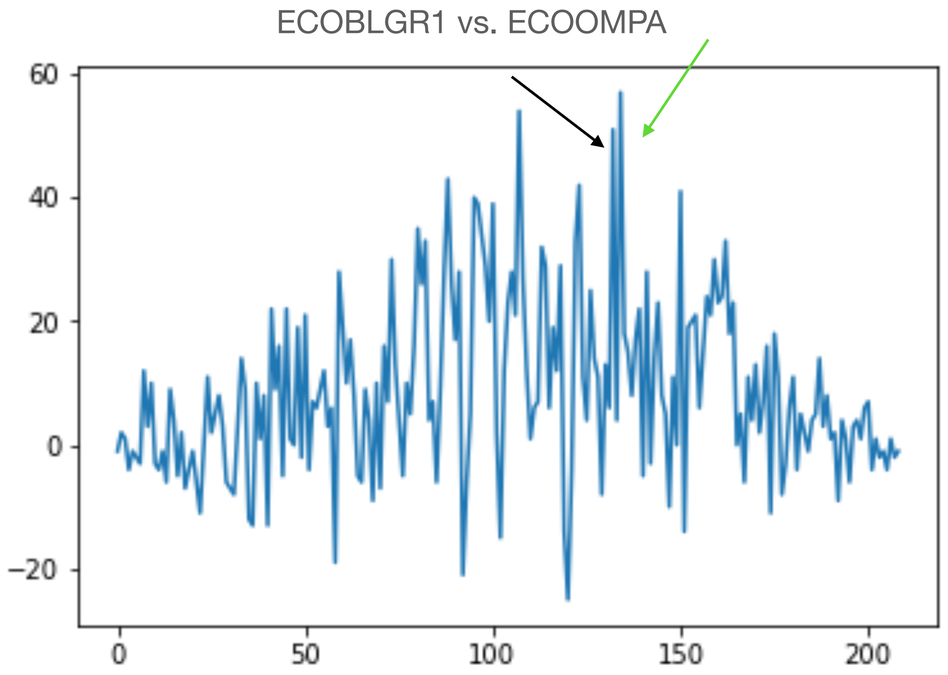




E. coli Data Set

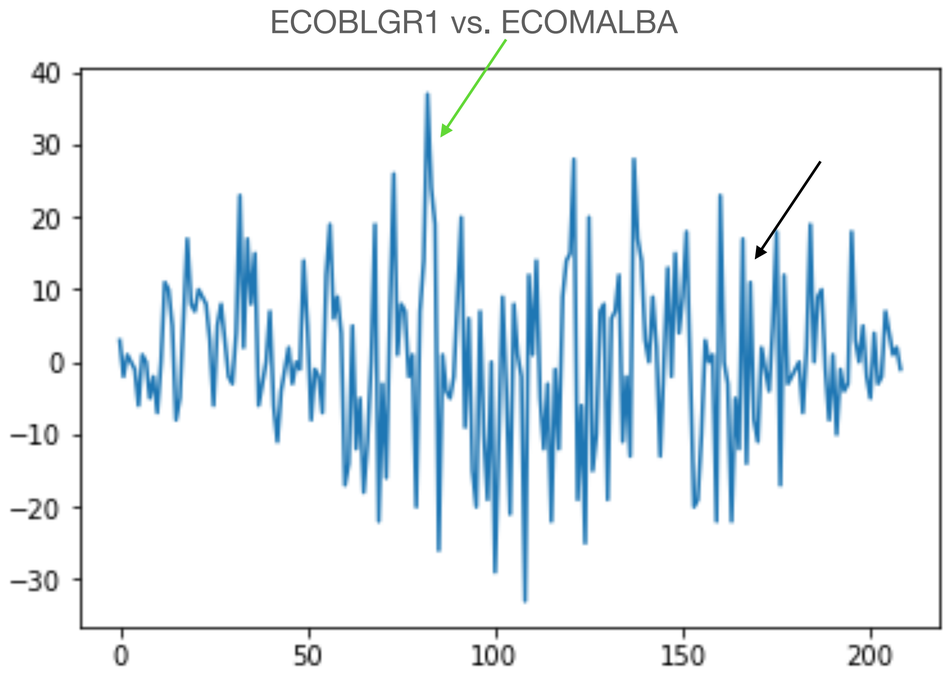
A Graphical Troubleshoot

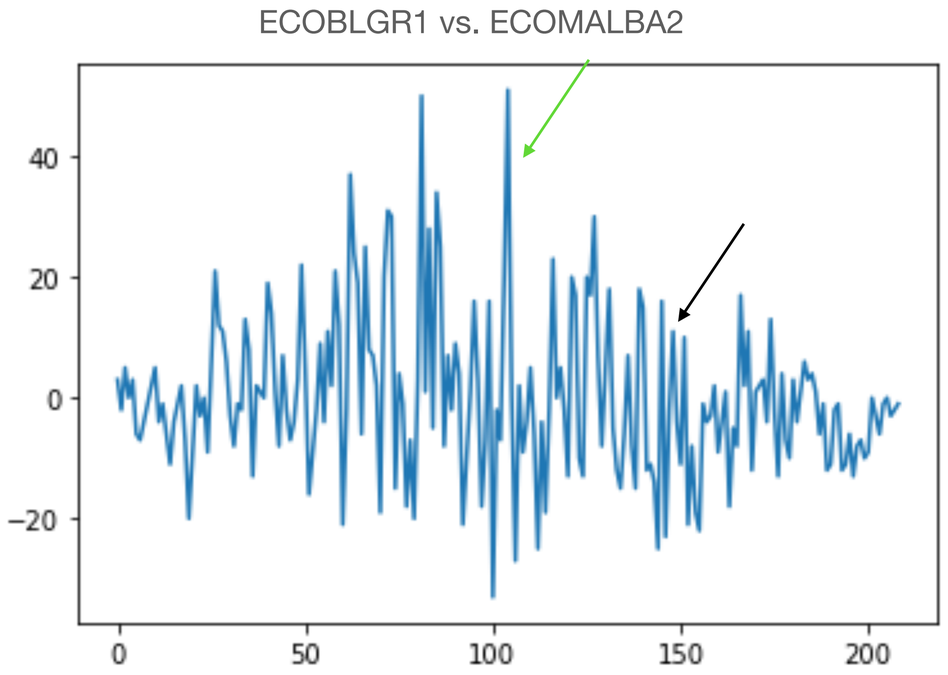




E. coli Data Set

A Graphical Troubleshoot





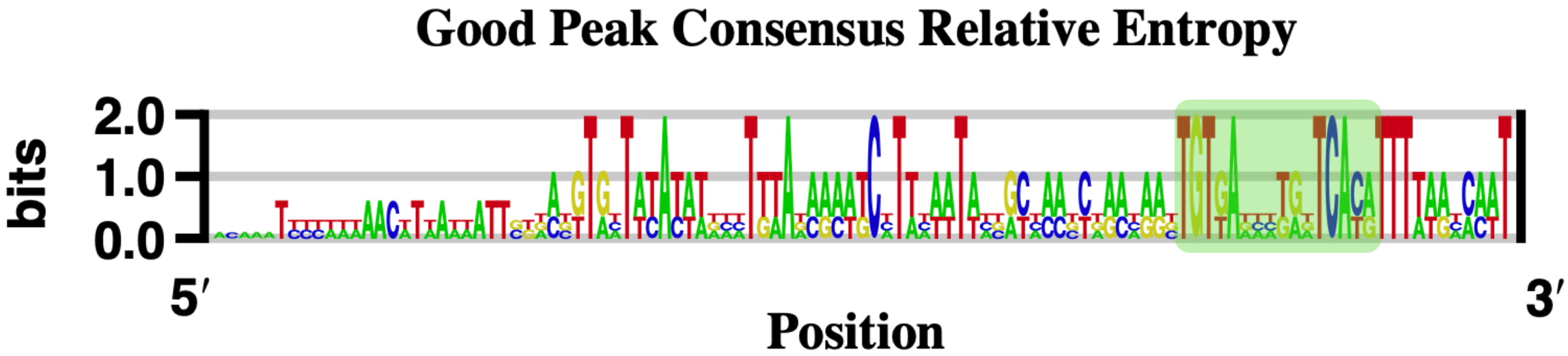
E. coli Data Set

Further Data Analysis

Sequence	Reference	Hand-Counted Distance from One End to Motif	Relative Distance from Reference	Obtained Shift/ Relative Distance	Consensus
ECOBLGR1	Yes	8	N/A	N/A	N/A
ECOCYA	No	34	26	26	Good
ECOTNAA	No	13	5	5	Good
TDC	No	6	-2	-4	Close
ECOOMPA	No	36	28	30	Close
ECOMALBA	No	62	54	-22	Little to None
ECOMALBA 2	No	47	39	0	Little to None

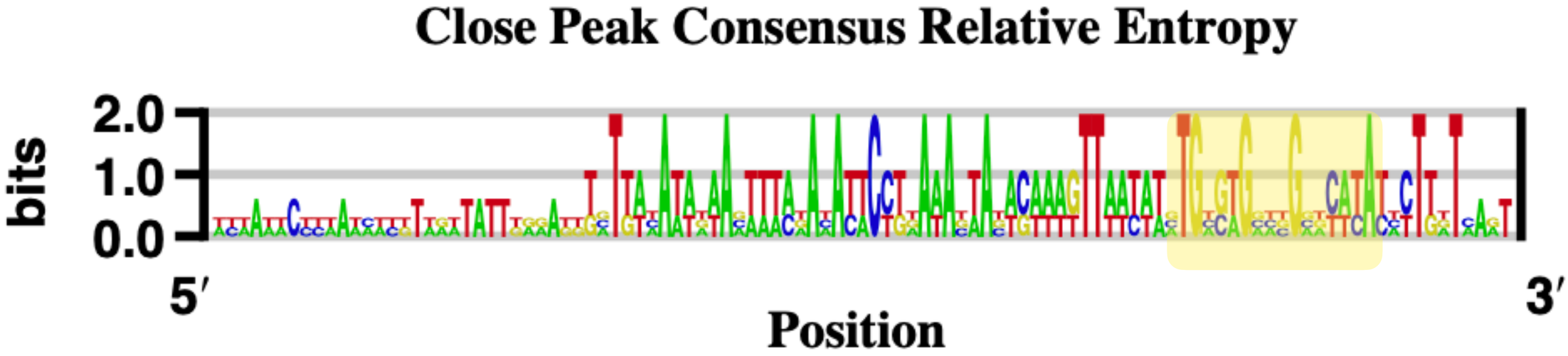
E. coli Data Set

Good Consensus DNA Logo Results



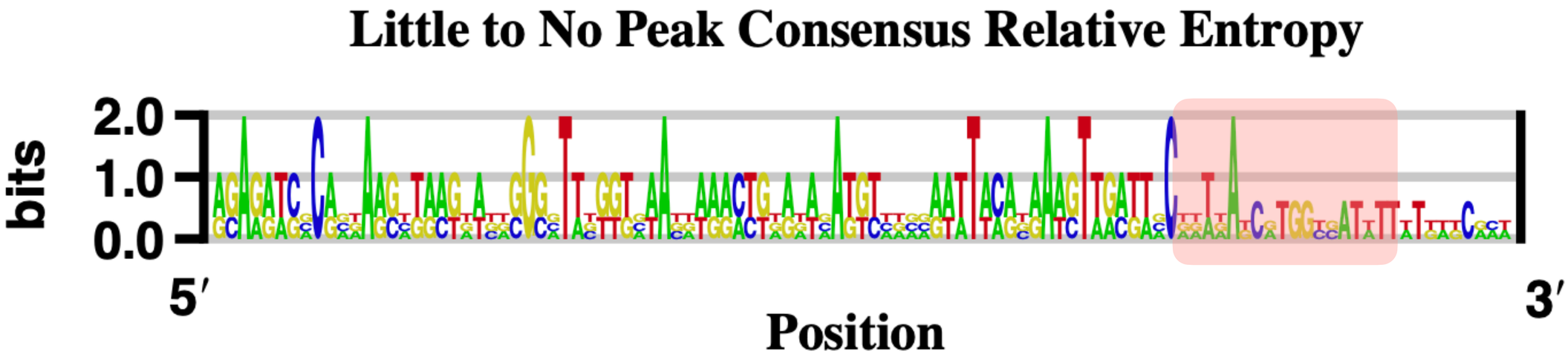
E. coli Data Set

Close Consensus DNA Logo Results



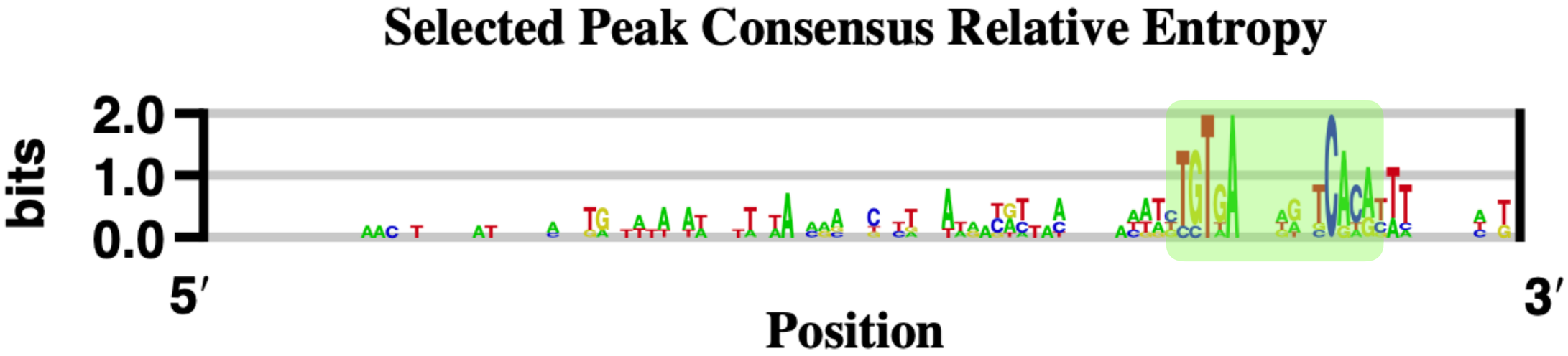
E. coli Data Set

Little to No Consensus DNA Logo Results



E. coli Data Set

Manually Selected Peak Consensus DNA Logo Results



Citations and Conclusion

Sources & Works Cited

Special Thanks to the Gary D. Stormo, *PhD* Lab

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