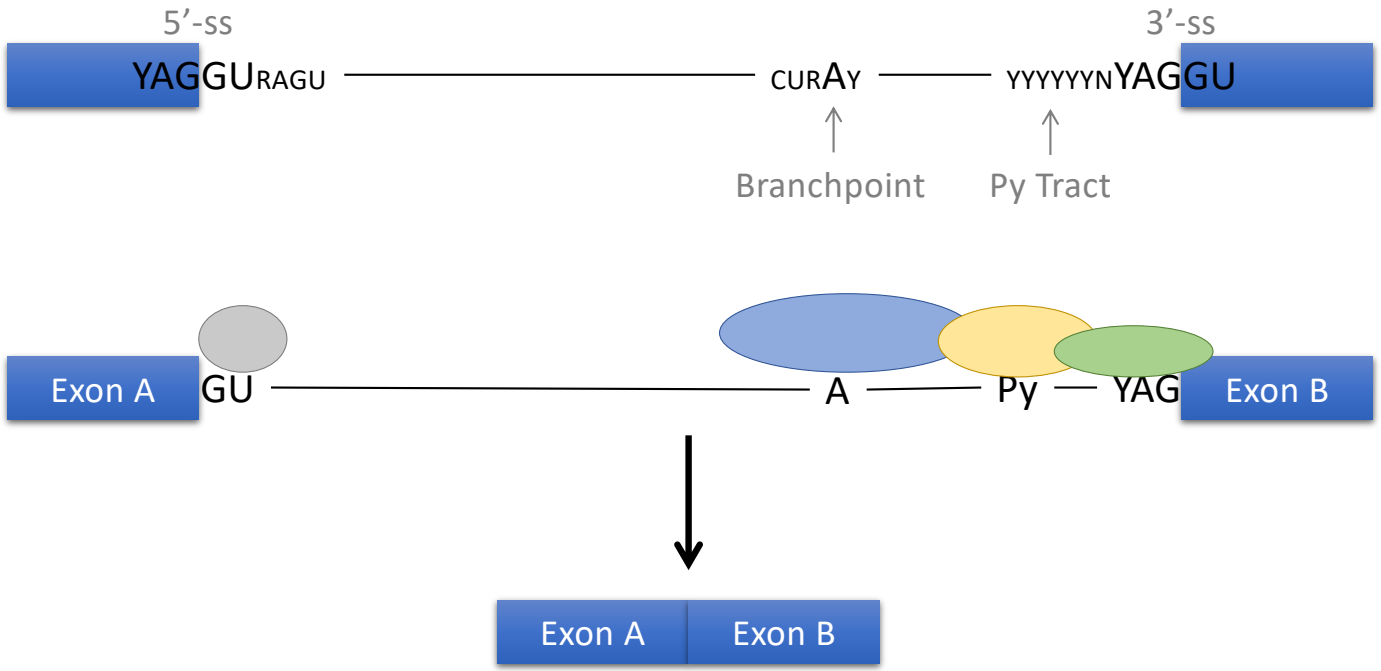
Splicing/Motif Mark

February 13, 2024

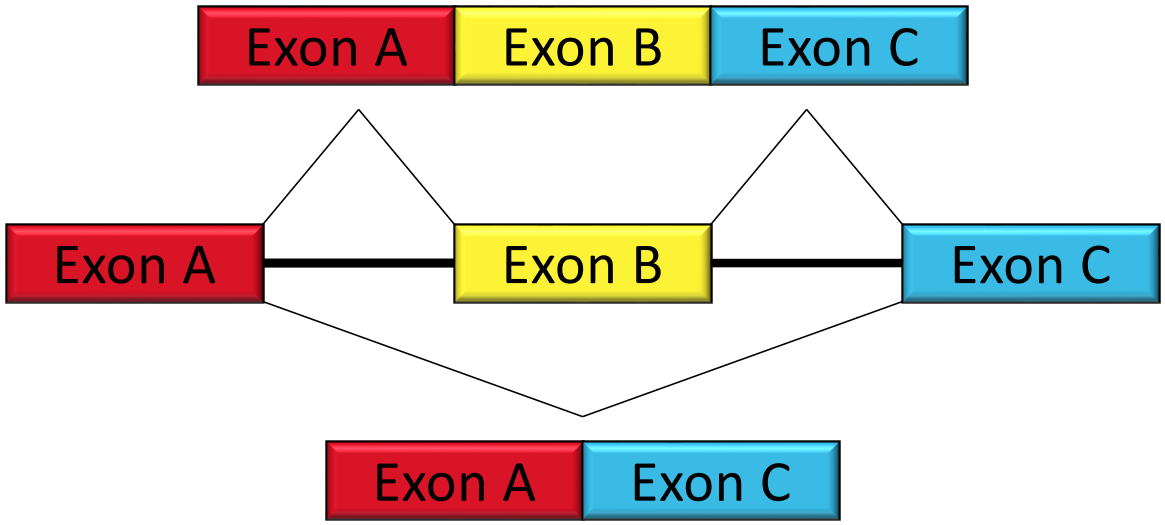
Observations

- The proteins MBNL1 and RBFOX1 are both regulators of alternative splicing

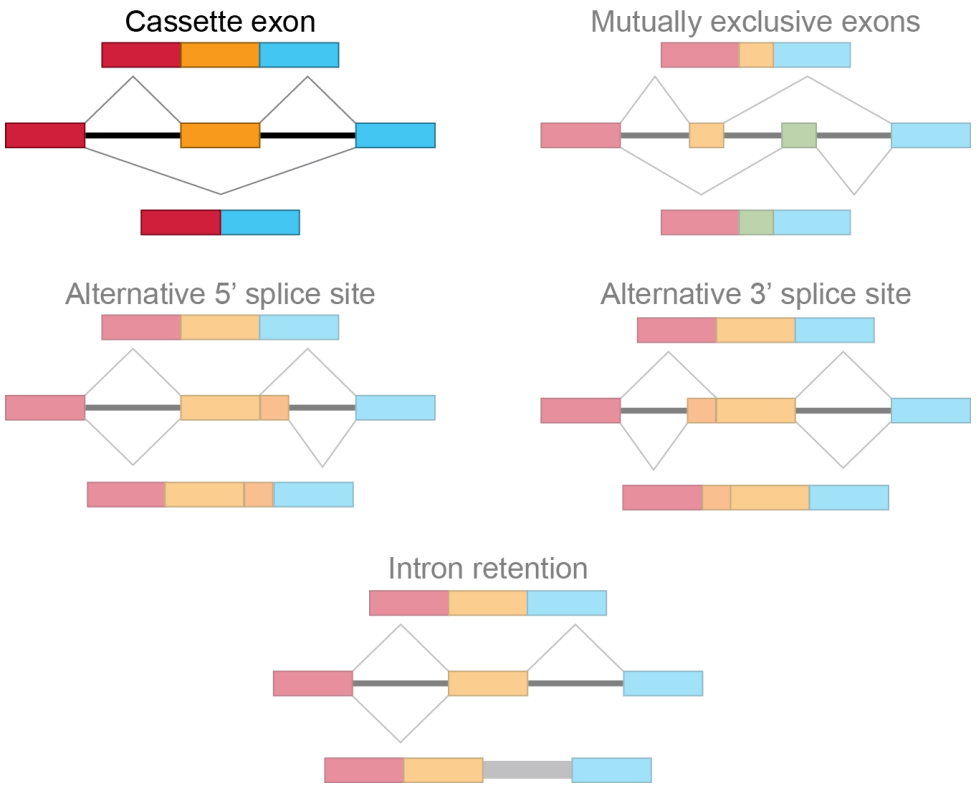
What is splicing?



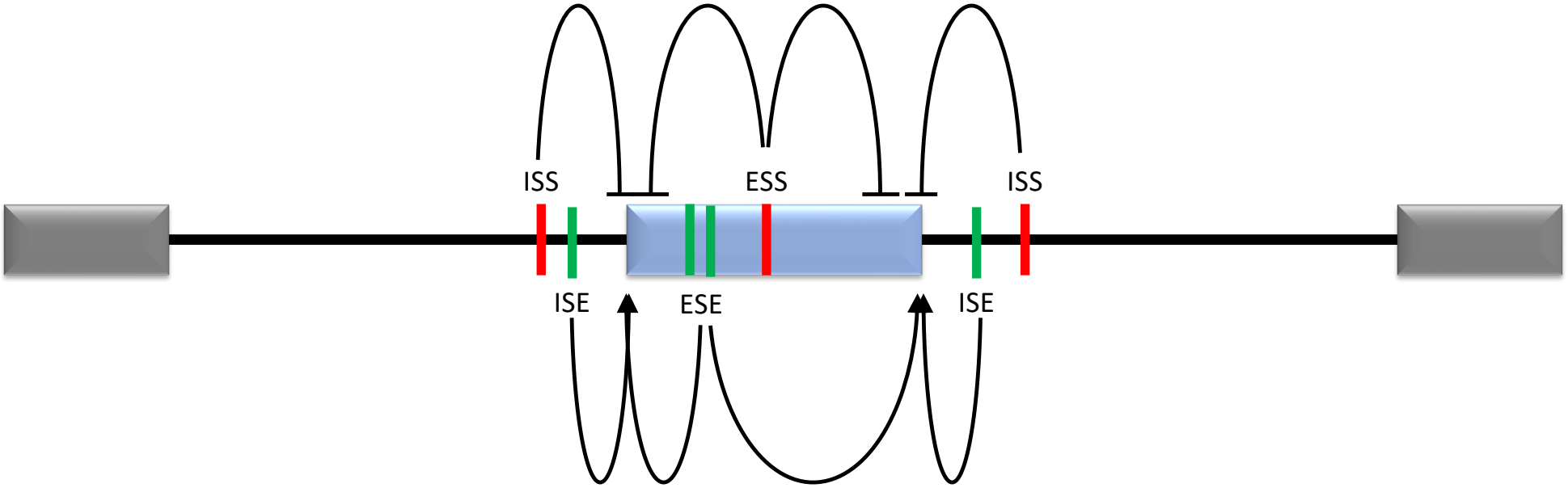
What is alternative splicing?



What is alternative splicing?

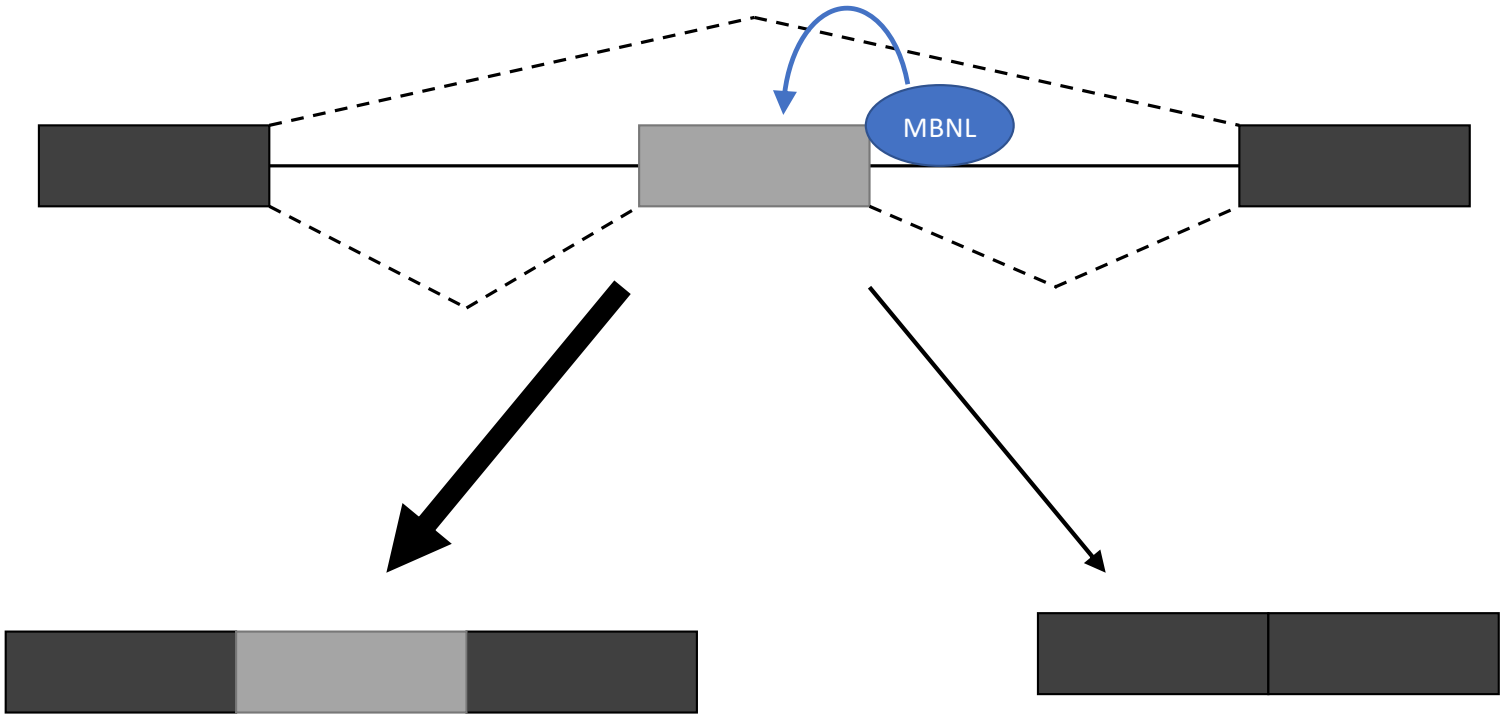


How is the splicing decision made?



- ISS – intron splicing silencer
- ISE – intron splicing enhancer
- ESS – exon splicing silencer
- ESE – exon splicing enhancer

MBNL is a splicing factor that is both an activator and suppressor of exon inclusion for pre-mRNA splicing



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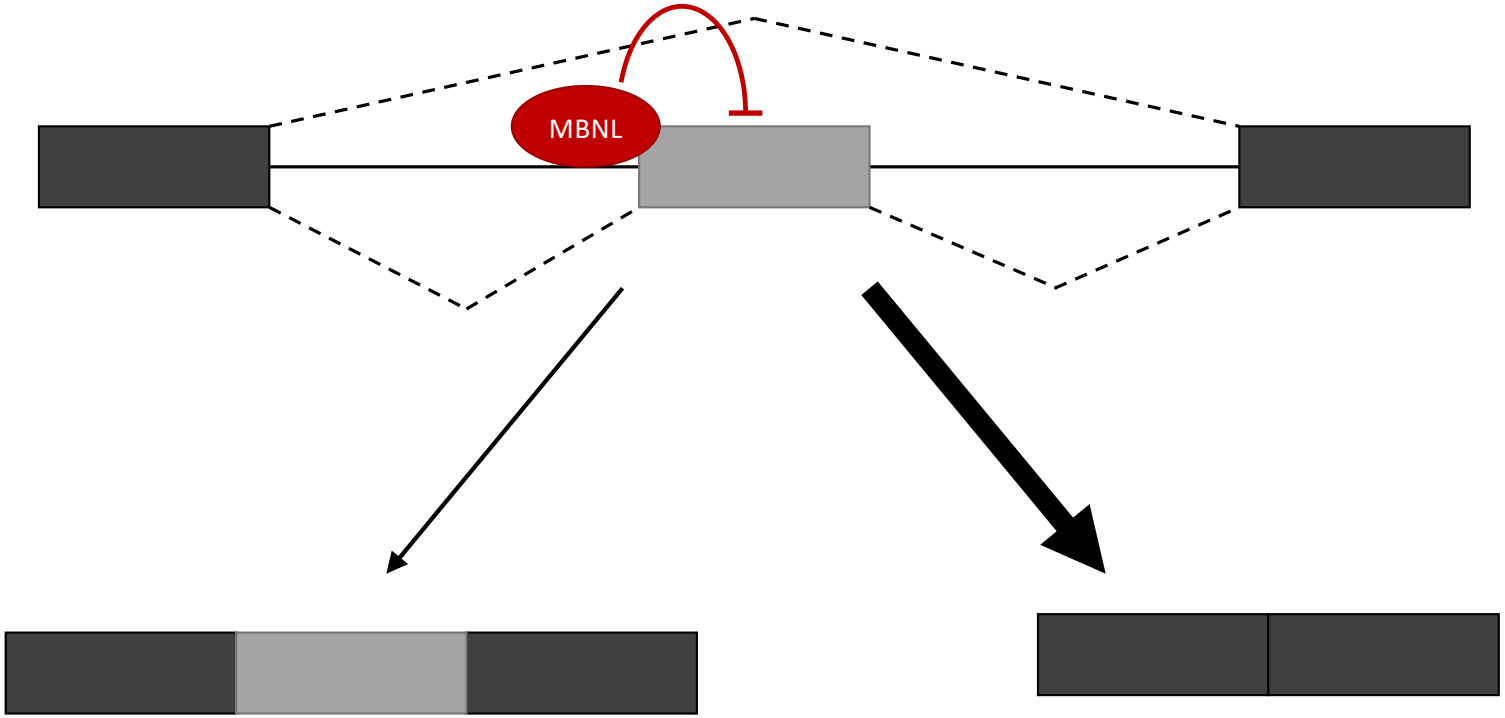
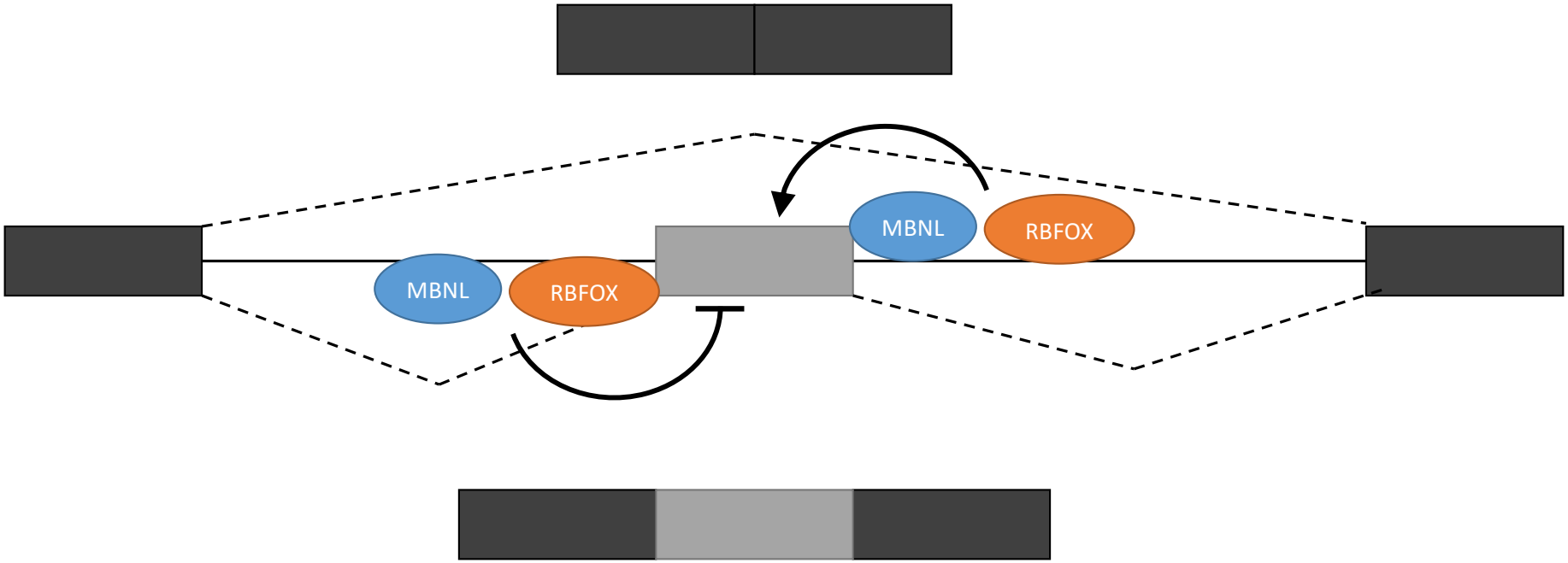


Observations

- The proteins MBNL1 and RBFOX1 are both regulators of alternative splicing
- MBNL1 and RBFOX1 regulate splicing in similar manners

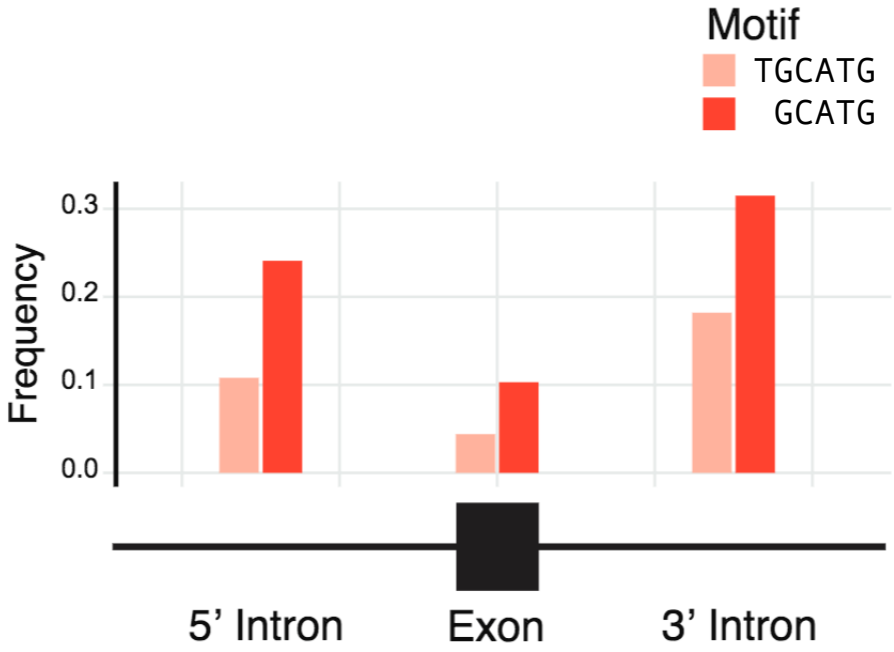
RBFOX1 regulates alternative splicing in a similar manner to MBNL1



Observations

- The proteins MBNL1 and RBFOX1 are both regulators of alternative splicing
- MBNL1 and RBFOX1 regulate splicing in similar manners
- MBNL1 and RBFOX1 have similar expression profiles
  - Both expressed in brain, heart, and skeletal muscle
  - Increased expression leads to alternative splicing changes critical for development
  - Depletions in expression have been associated with neurological disorders
- Some MBNL1 regulated splicing events have RBFOX1 binding sites

Analysis of 203 MBNL-regulated exons in mouse revealed the presence of RBFOX binding motifs



66% contain a GCAUG and
33% contain a UGCAUG
RBFOX motif in the exon or within 250 nucleotides for the flanking introns

Research question

- How do MBNL1 and RBFOX1 work together to regulate splicing events in transcripts that are regulated by both?

Our goal

- Develop a Python script to plot protein binding motifs on an image of an exon and flanking introns

>INSR chr19:7149896-7151209 (reverse complement)

MBNL binding site: YGCY

agaaagaaagaaagaaagaaagaaagaaagaaagaaagaaagagaaagaaagaaaggaaagagagaaagaaa
ggacagagaaaggaaggggcaagggagggagagaggagggaaggaaggaaggaaaaaaggaaaagaaagaaaaga
aagagaaagaaaatgaaaaaaagaaggaaggaaagagaaagaaagcaaatgaaggaaggaaagagggaaaaagaga
gaaagaaagtaaaagaggaaagaaggaaggaaggagagagagggagagagaaaagagaaagagaaaagaaaaggag
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gatctcttccttccttccttccttccttccttccttccttccttccttctttctttttttccttctttctttctttctttc

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agaaagaaagaaagaaagaaagaaagaaagaaagaaagaaagaaagagaaagaaagaaaggaaagagagaaagaaa
ggacagagaaaggaaggggggcaagggagggagagaggagggaaggaaggaaggaaaaaaggaaaagaaagaaaaga
aagagaaagaaaatgaaaaaaagaaggaaggaaagagaaagaaagcaaatgaaggaaggaaagagggaaaaagaga
gaaagaaagtaaaagaggaaagaaggaaggaaggagagagggagagagaaaagagaaagagaaaagaaaaggag
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Activity

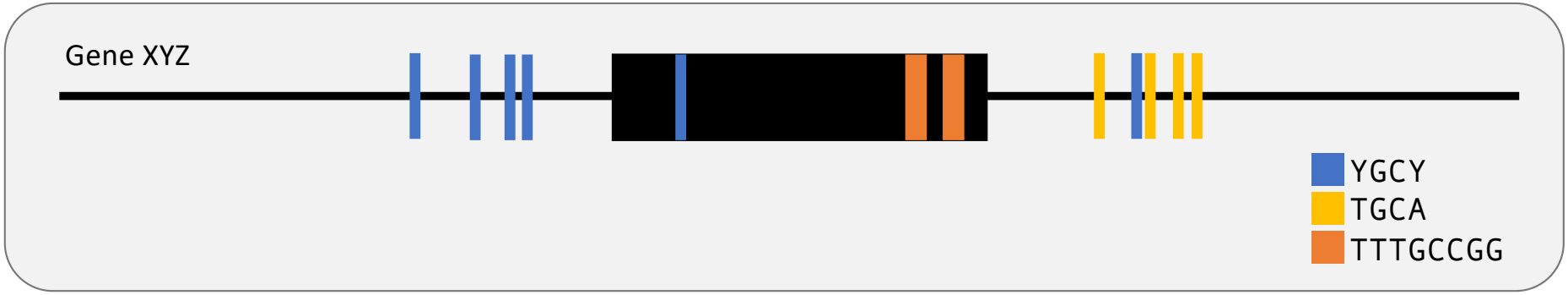
- Working in groups, discuss general strategy for the algorithm
  - What should the input look like?
  - What should the output look like?
  - What classes are you going to write?
  - What functions are you going to write?
  - How can you handle multiple motifs and multiple genes?
  - How are you going to identify motifs with ambiguity, i.e. YCGY?

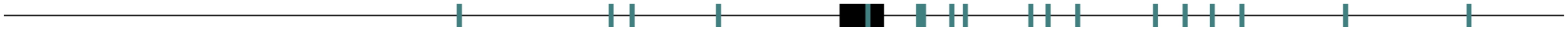
Input

- FASTA file
  - How denote introns vs exons?
- Coordinates
  - UCSC genome browser
  - Need assembly info
  - How interface with UCSC?
- Other?

Output

- Single image (vector-based)
- To scale!
- Introns vs exons
- Denote multiple motifs
- Key?





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agaaagaaagaaagaaagaaagaaagaaagaaagaaagaaagaaagagaaagaaagaaaggaaagagagaaagaaa
ggacagagaaaggaaggggcaagggagggagagaggagggaaggaaggaaggaaaaaaggaaaagaaagaaaaga
aagagaaagaaaatgaaaaaaagaaggaaggaaagagaaagaaagcaaatgaaggaaggaaagagggaaaaagaga
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Functions

- Parse FASTA
- Parse file with motifs
- Drawing function?

Activity

- Working in groups, discuss general strategy for the algorithm
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  - What should the output look like?
  - What classes are you going to write?
  - What functions are you going to write?
  - How can you handle multiple motifs and multiple genes?
  - How are you going to identify motifs with “flexibility”, i.e. YCGY?

Drawing with Pycairo


```
conda install -c conda-forge pycairo
```

```
import cairo
surface = cairo.SVGSurface("plot.svg", width, height)
context = cairo.Context(surface)
context.set_line_width(1)
context.move_to(50,25)
context.line_to(intron1+exon+intron2,25)
context.stroke()
surface.finish()
```

https://pycairo.readthedocs.io

https://cairographics.org/documentation/pycairo/3/

Your assignment: Motif Mark

- Create a repository in your GitHub profile called `motif-mark`
- Write a python script to visualize motifs on sequences
  - Minimum requirements:
    - Well commented Python3 compatible, object-oriented code, with CLEAR `readme.md` file
    - Use `argparse`
    - Output file has same prefix as input file (e.g., `Figure_1.fa` ➔ `Figure_1.png`)
    - Input FASTA file (seqs ≤1000 bases) and motifs file (≤10 bases each, one motif per line in a text file)
    - Ambiguous nucleotide motif handling (see https://en.wikipedia.org/wiki/Nucleic_acid_notation)
    - Capable of handling multiple sequences (max 10) and multiple motifs (max 5)
    - Consider how you will handle overlapping motifs and how you will denote introns/exons
    - All features (motifs, introns, exons) should be to scale
    - Output **single**, well-labeled png, per FASTA file
    - Key/labeling
- Turn in your code and figure(s) for the data that we will provide on Canvas