

RNA structure and genetic variation in introns: the case for the RiboSPLitch

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33rd TBI Winterseminar in Bled

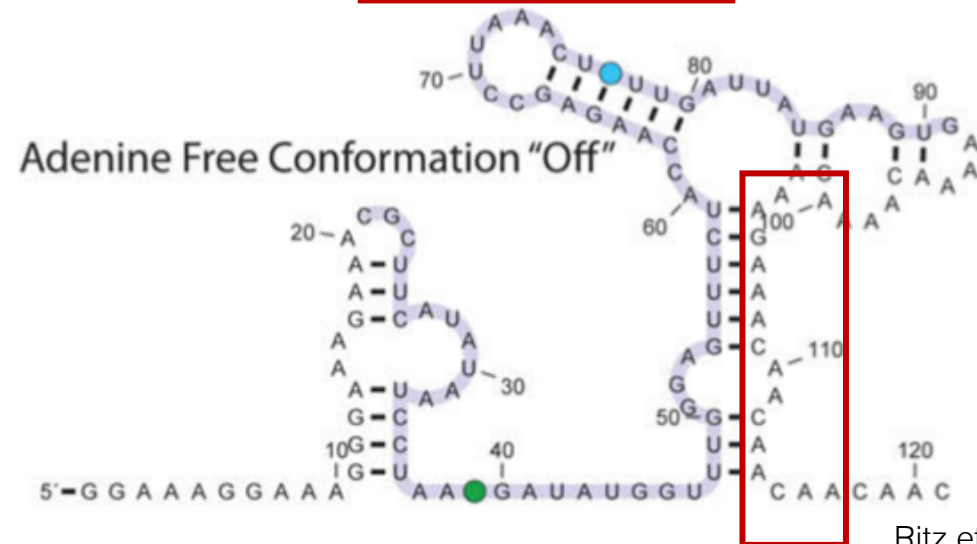
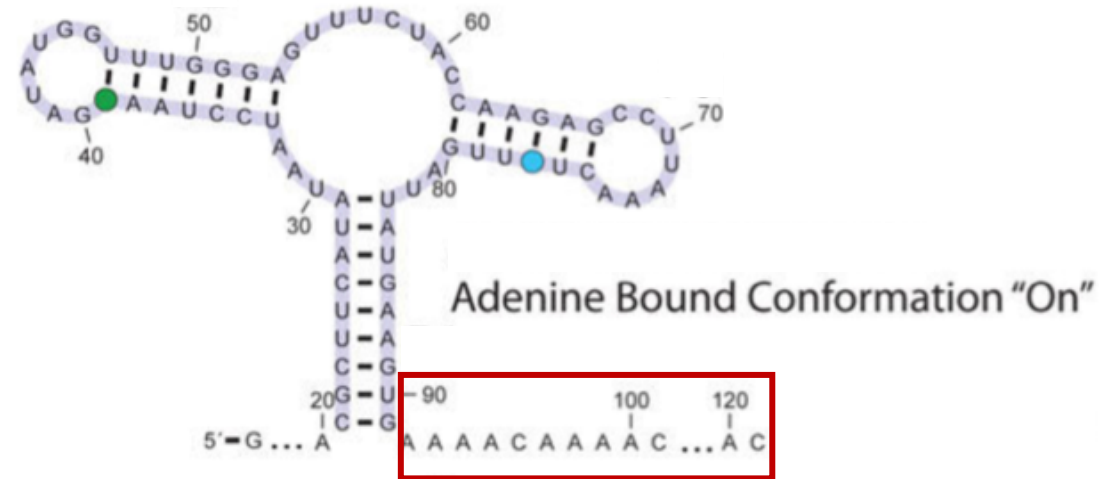
02/14/18

Let's get this straight

- Riboswitch
- Ribo**SN**itch (SNP)
- Ribo**SPL**itch (Splice)

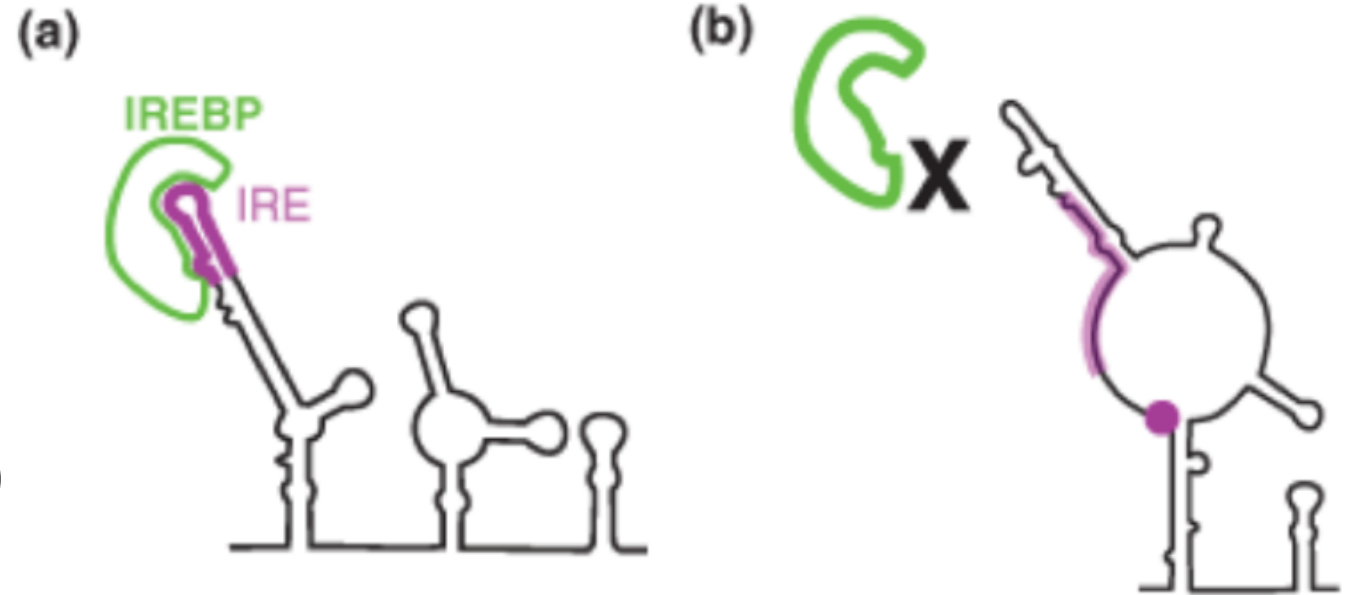
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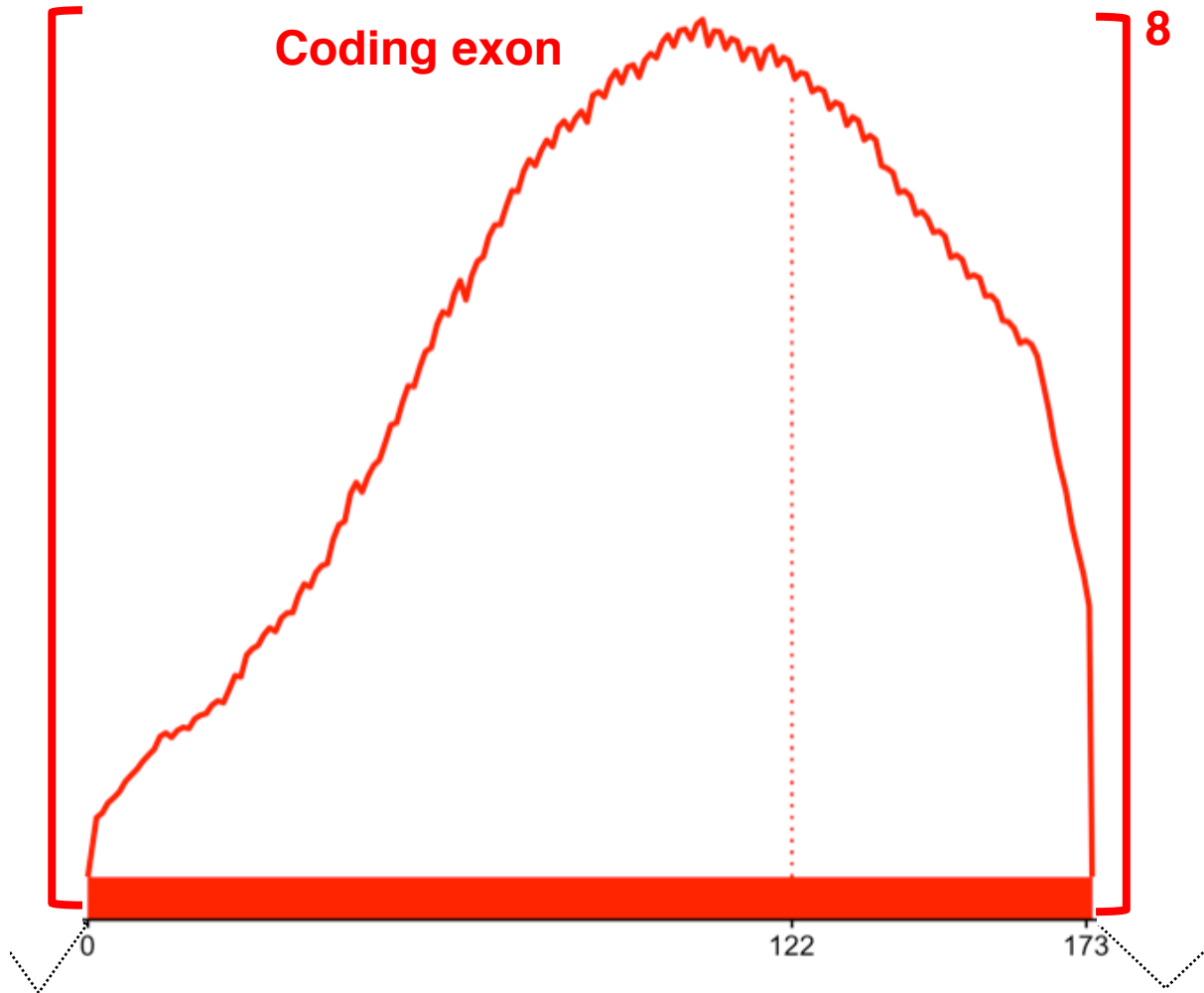
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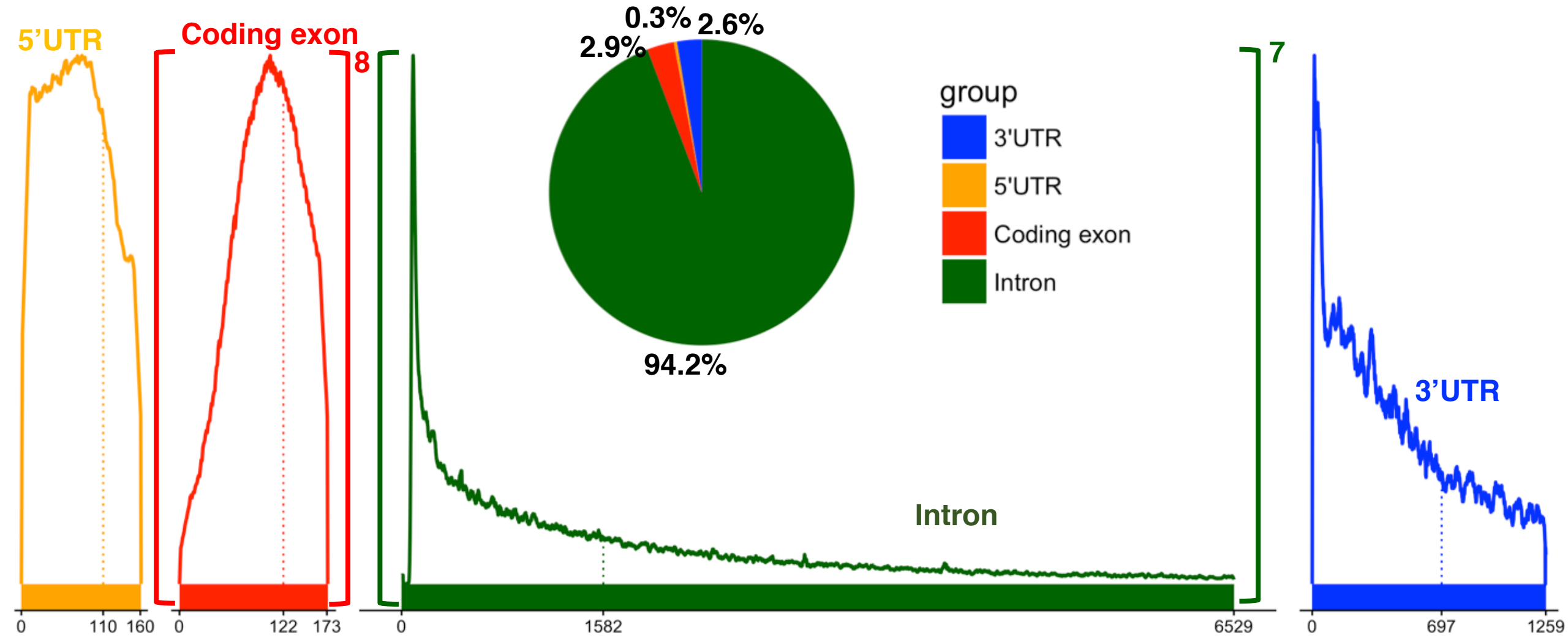
Typical messenger RNA (mRNA)



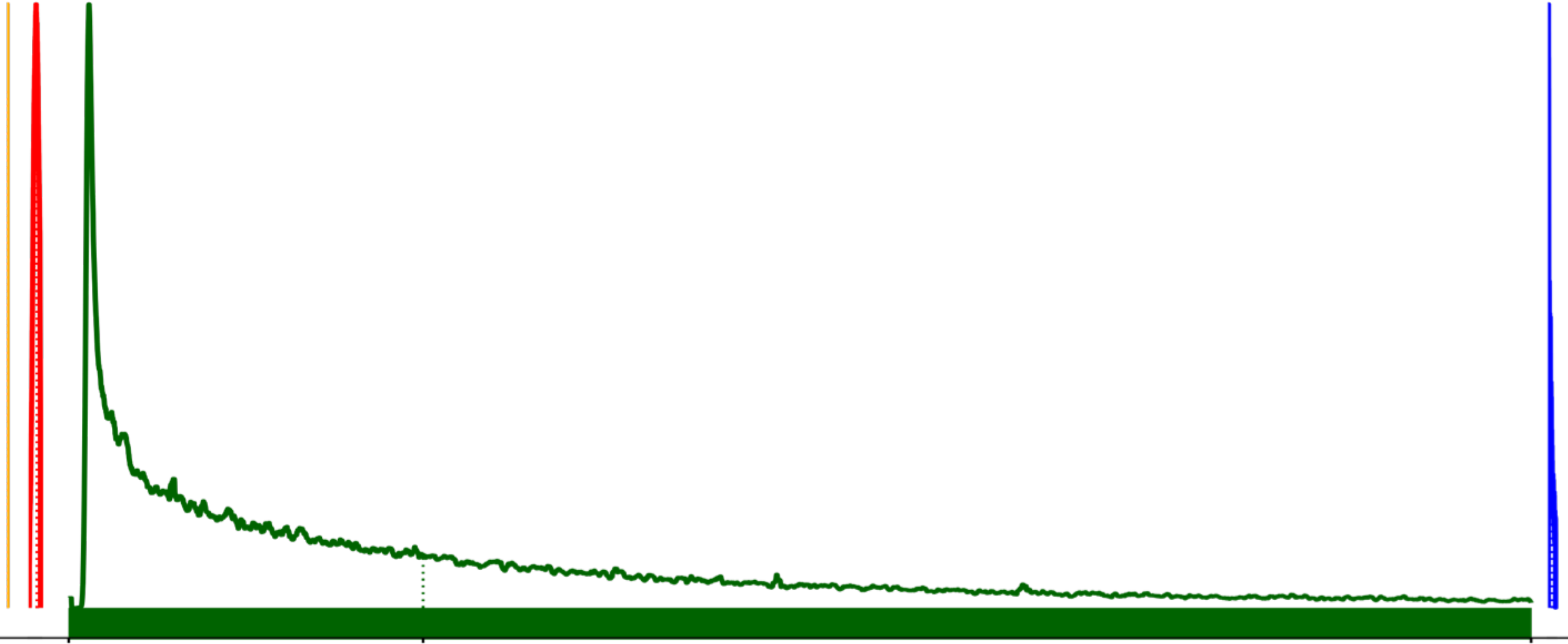
Typical mRNA: Length Distribution



What actually gets transcribed?



What actually gets transcribed?

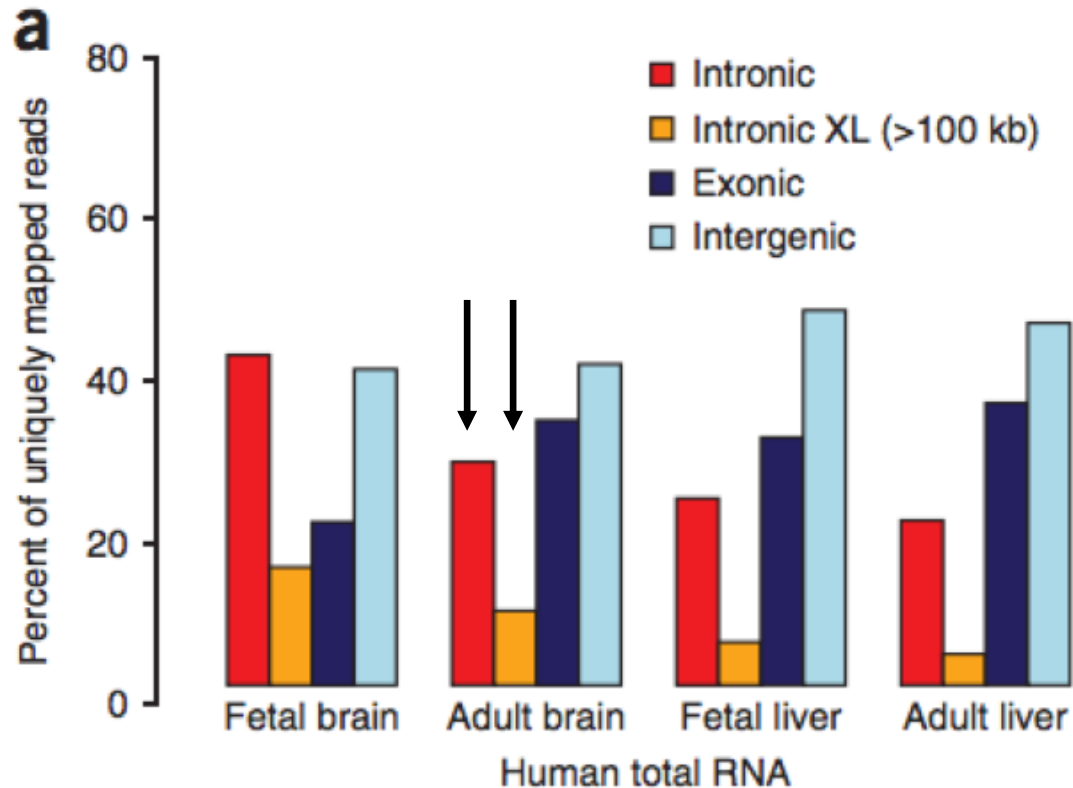


Are we able to detect introns?

- Introns are spliced out by the spliceosome
- Co-transcriptional splicing genome-wide in eukaryotic tissue
- Typical RNA techniques don't always measure introns

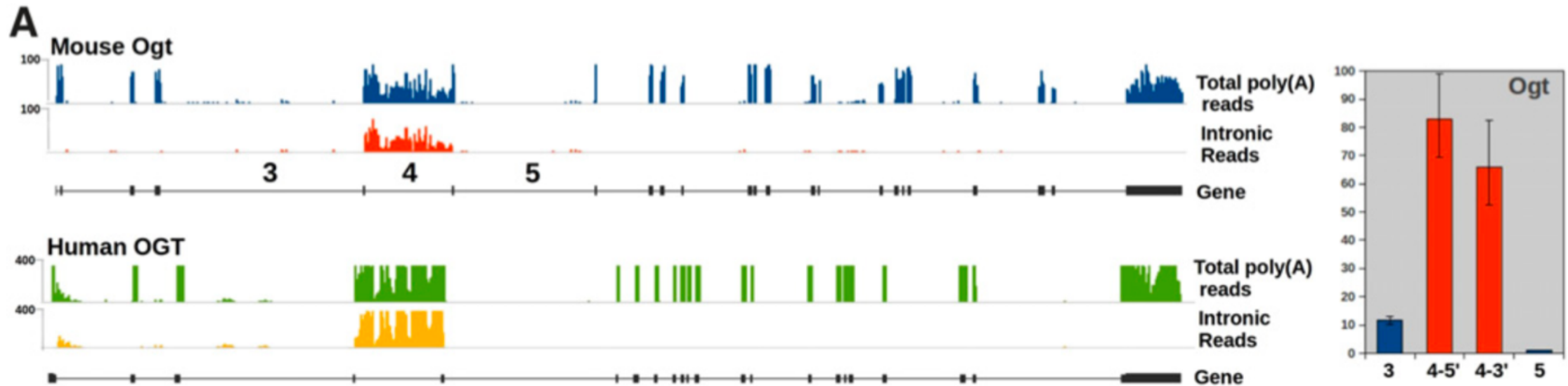
Are we able to detect introns?

- Introns detected in Total RNA-sequencing

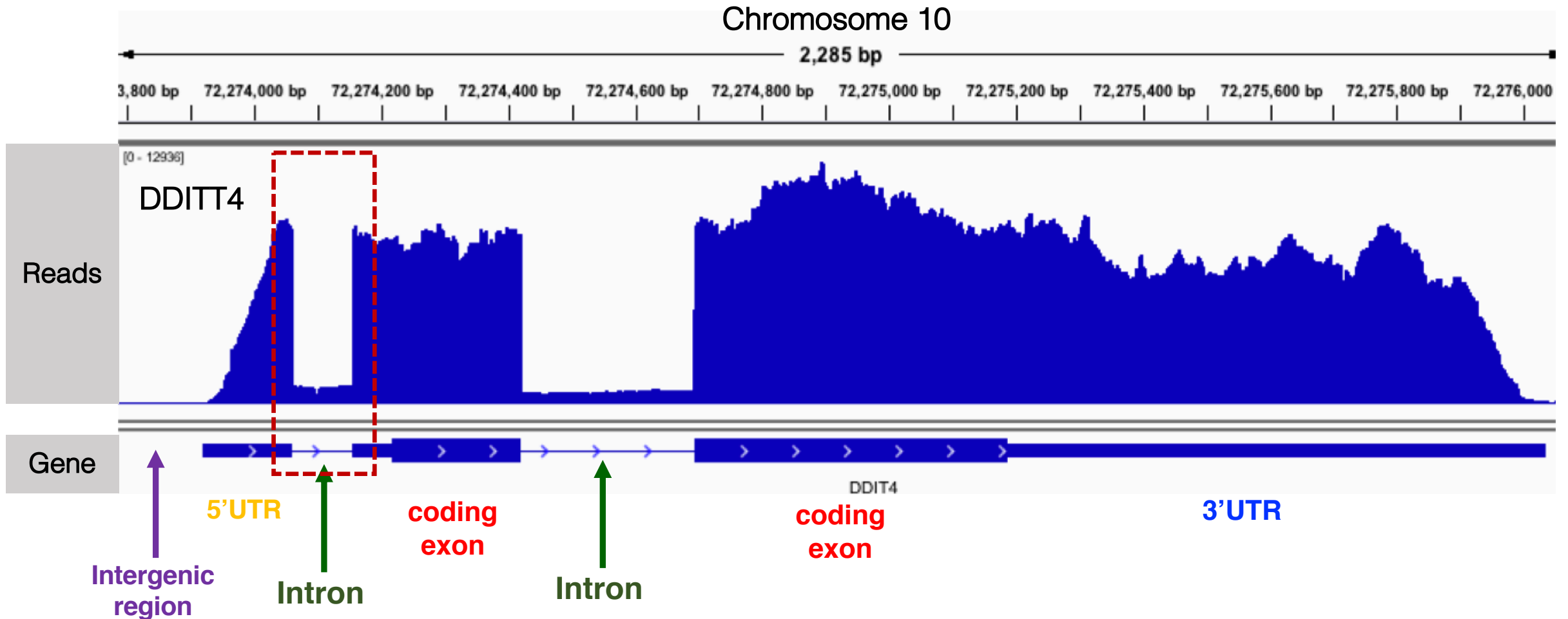


Are we able to detect introns?

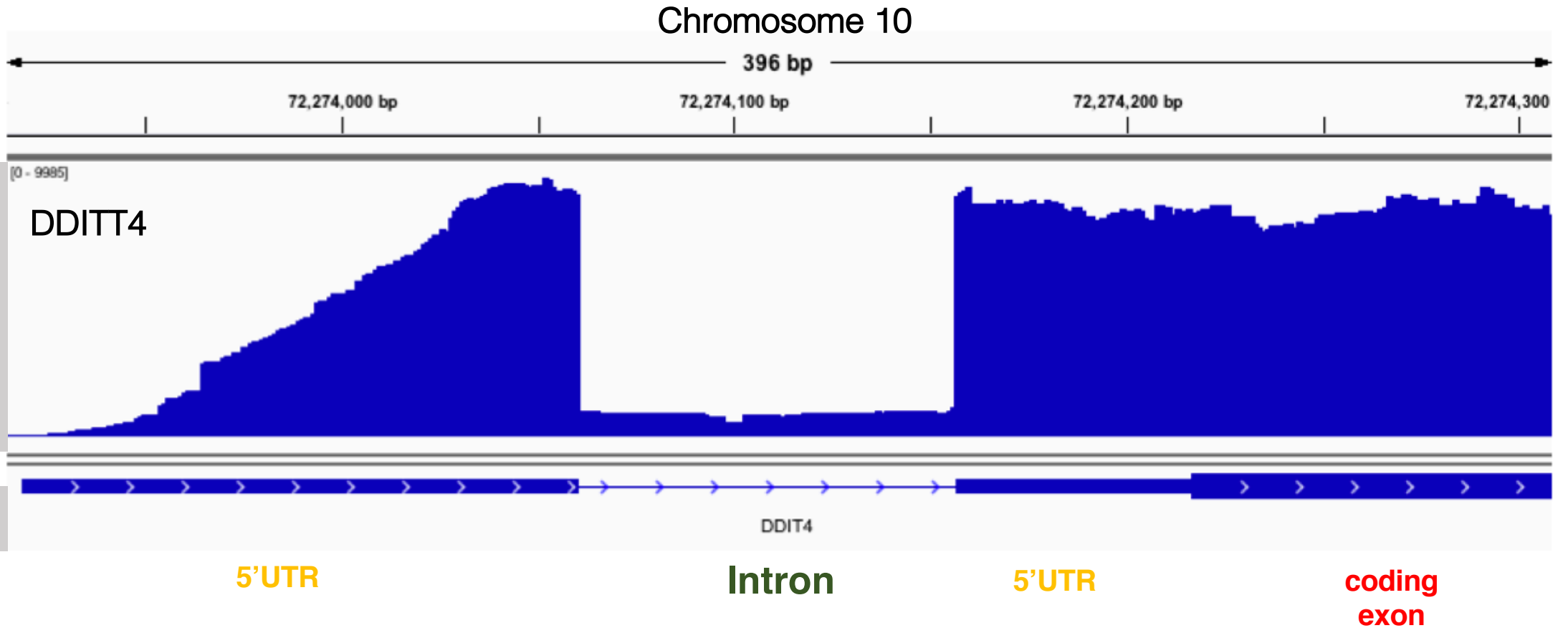
- Introns in poly(A) primed RNA-seq



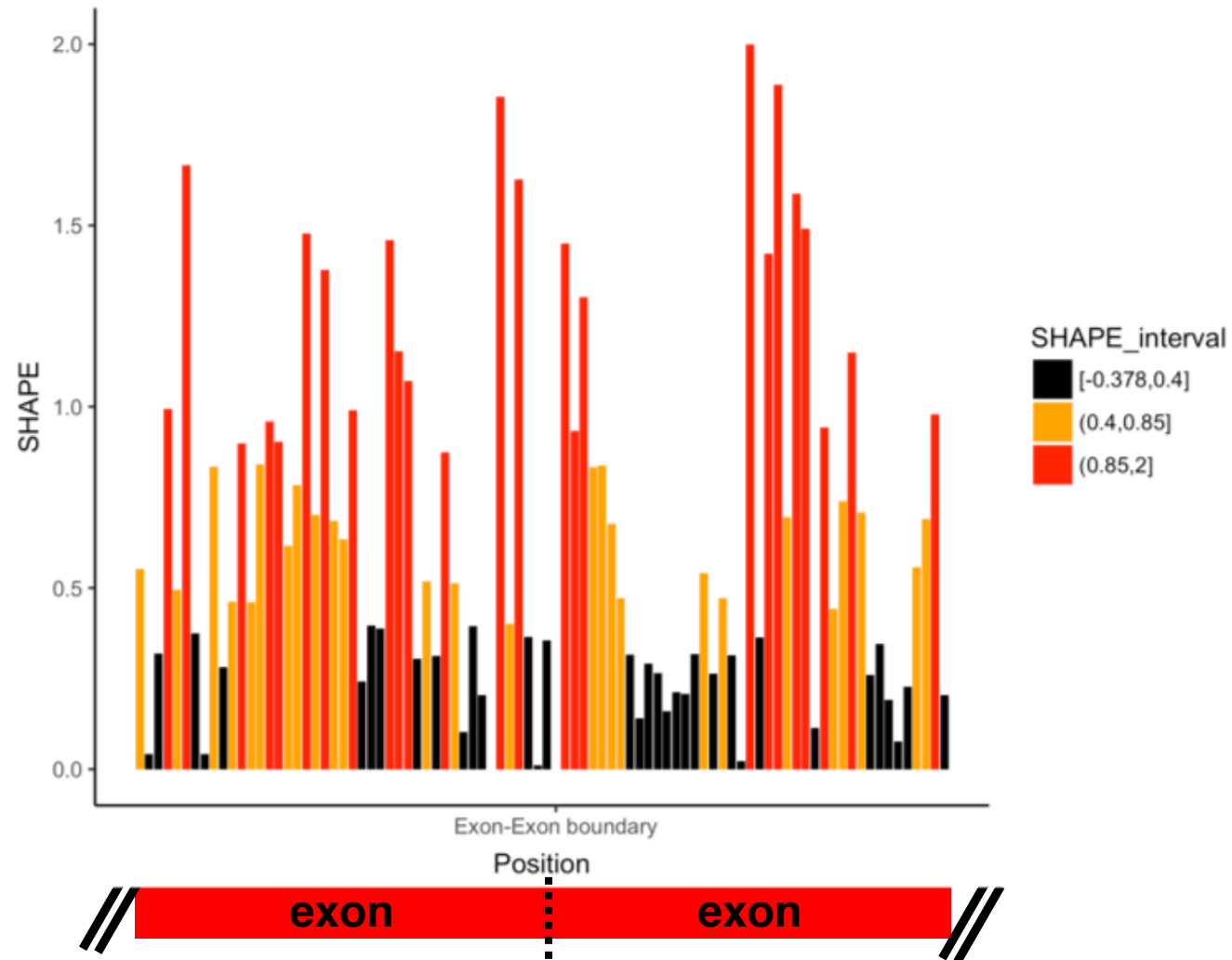
rRNA depleted RNA-sequencing



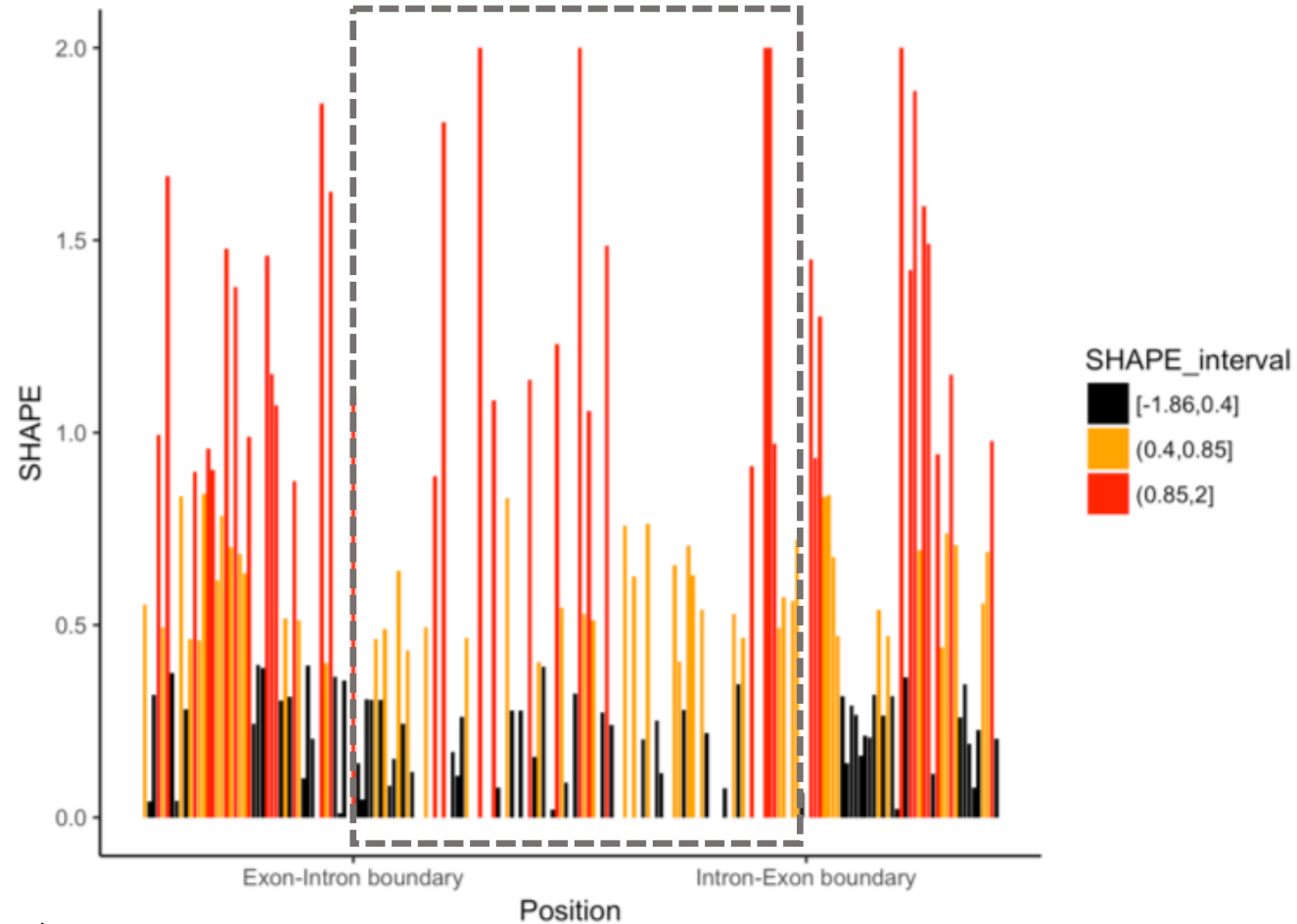
Are we able to detect introns?



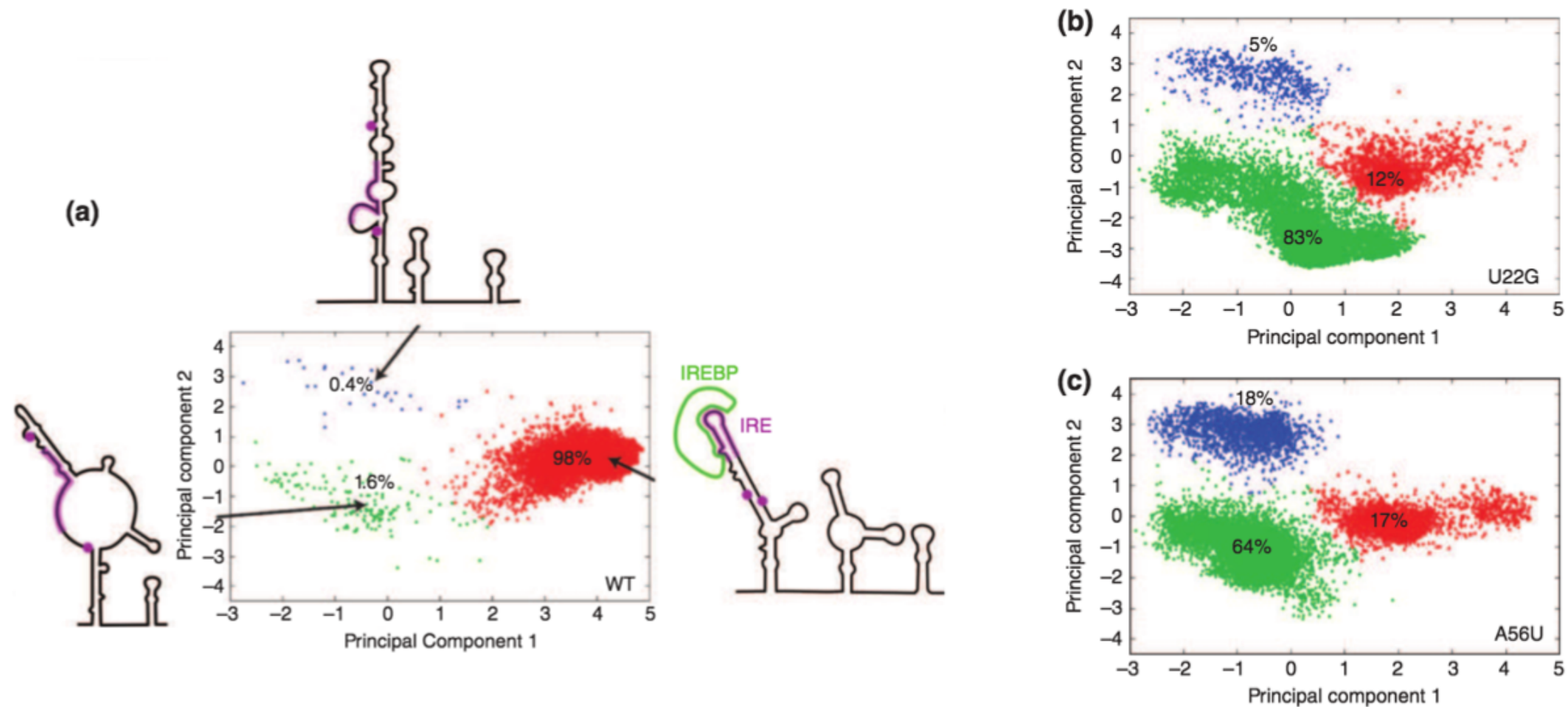
Are introns structured?



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RiboSNitch: 5'UTR of Ferritin Light Chain (FTL) gene

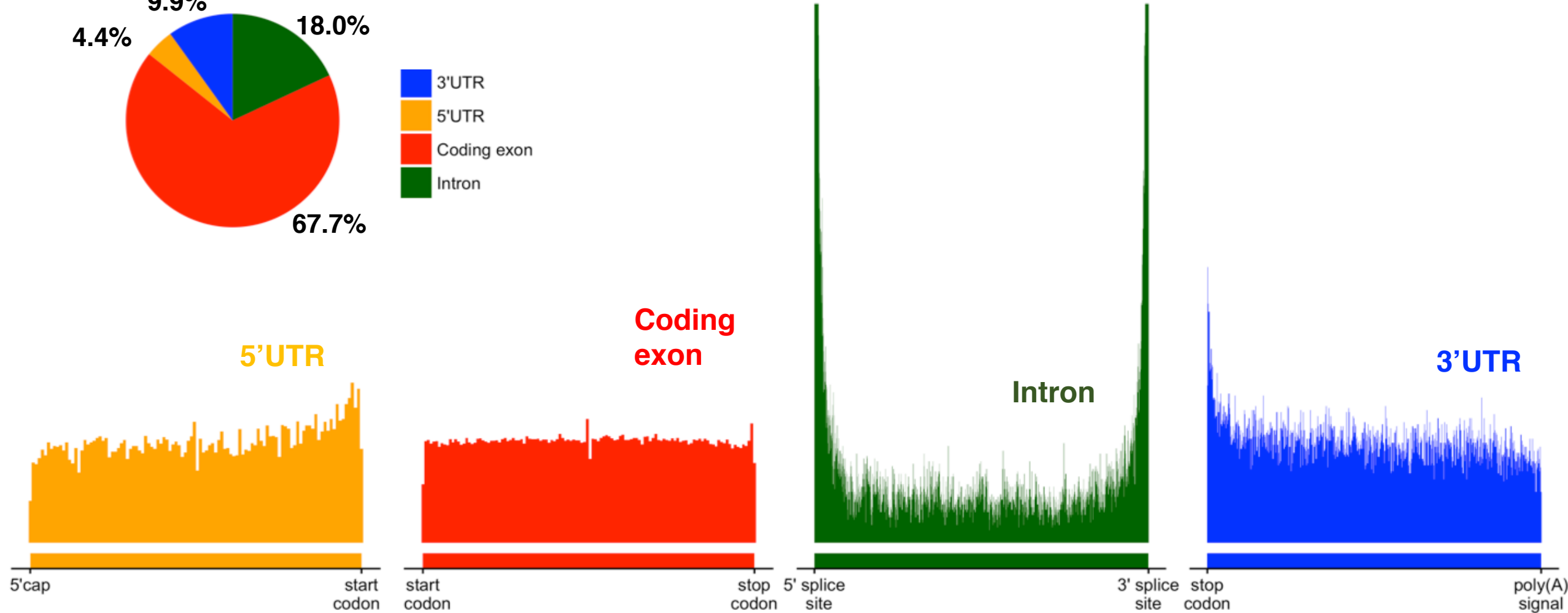
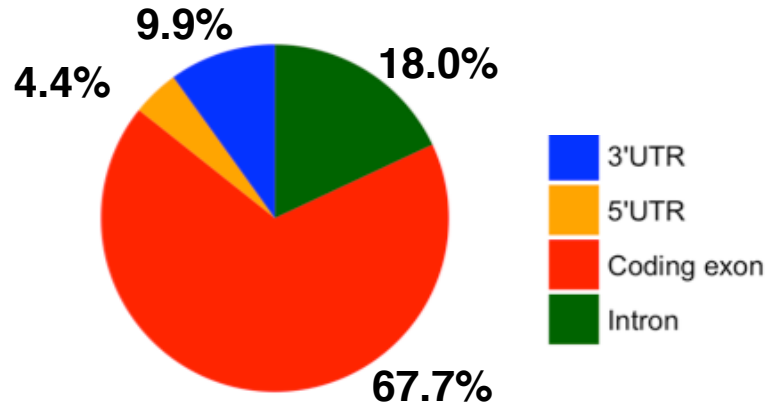


Disease-associated variants (DAVs)

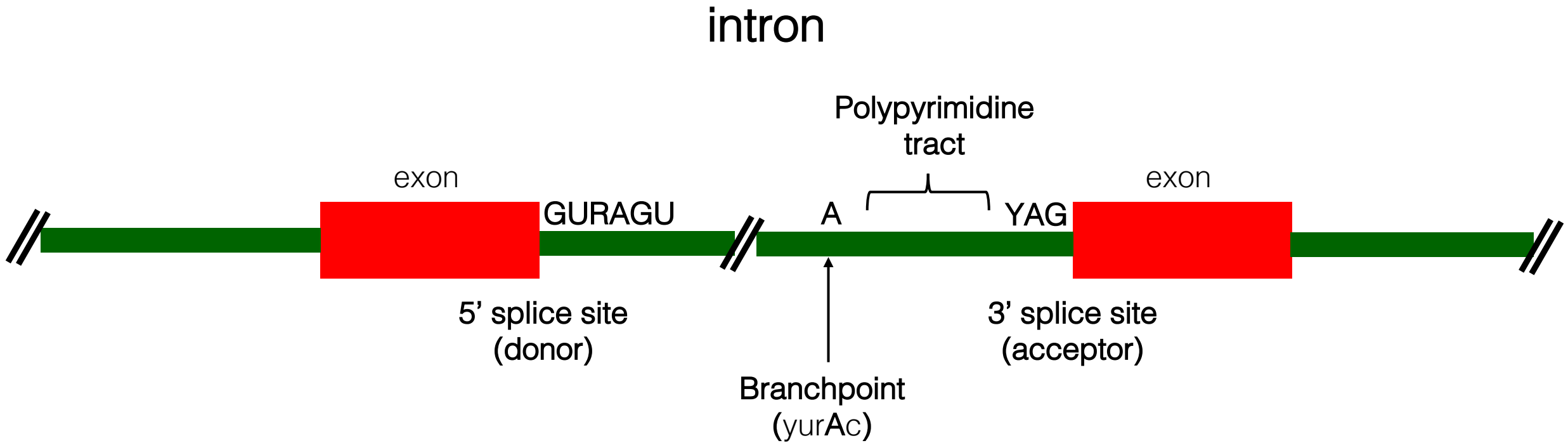
- Rare and penetrant variants associated with a disease
- Curated in Human Gene Mutation Database (HGMD) & NCBI ClinVar: 360,646
- Hypothesis: there are DAVs that will alter structure in introns that in turn affects splicing ability

RiboSPLitches

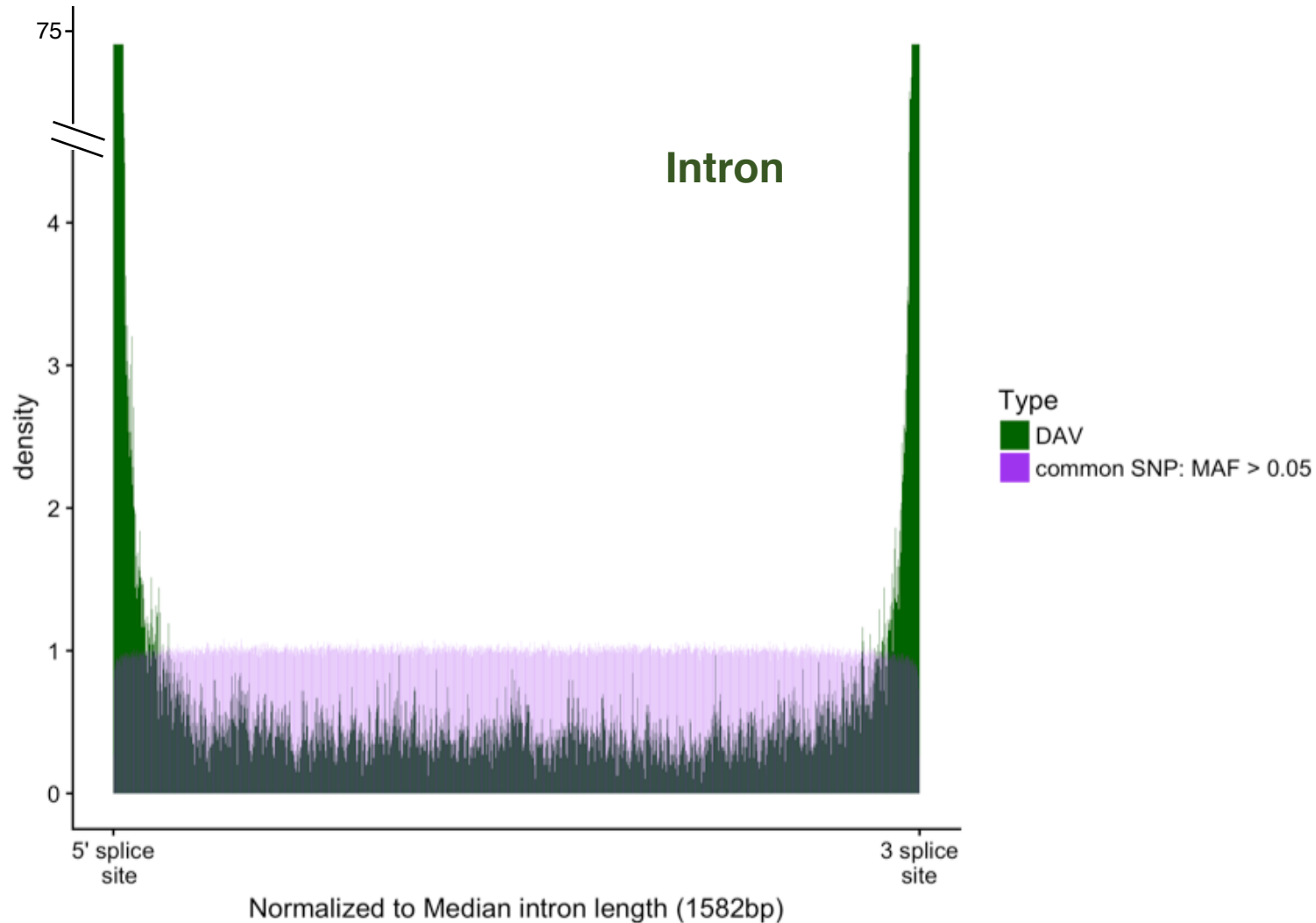
DAV distribution: precursor-mRNA



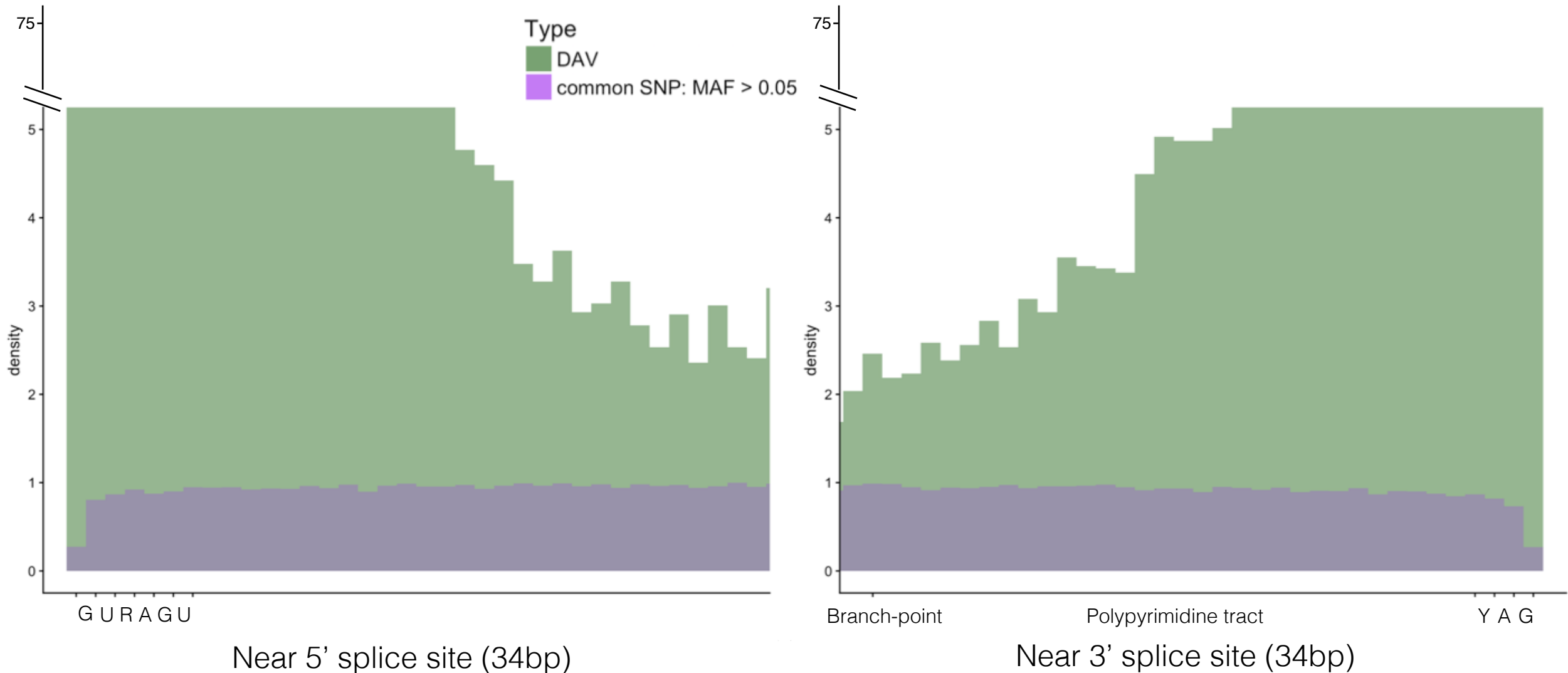
Splicing code



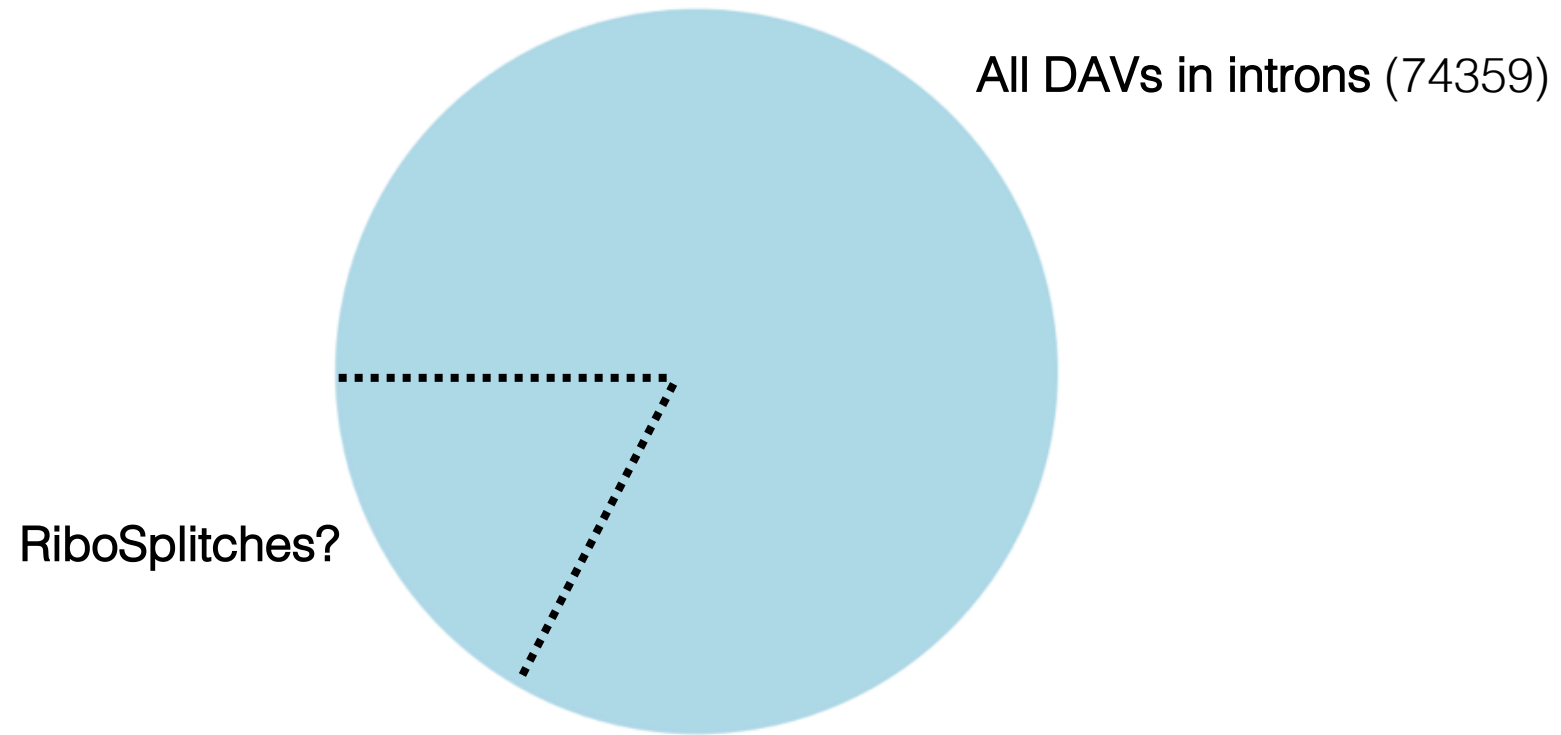
DAVs in intronic regions of precursor-mRNAs



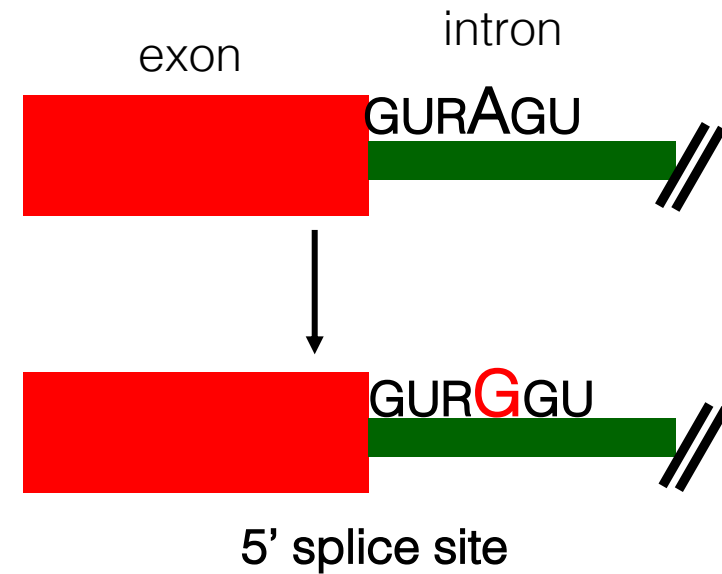
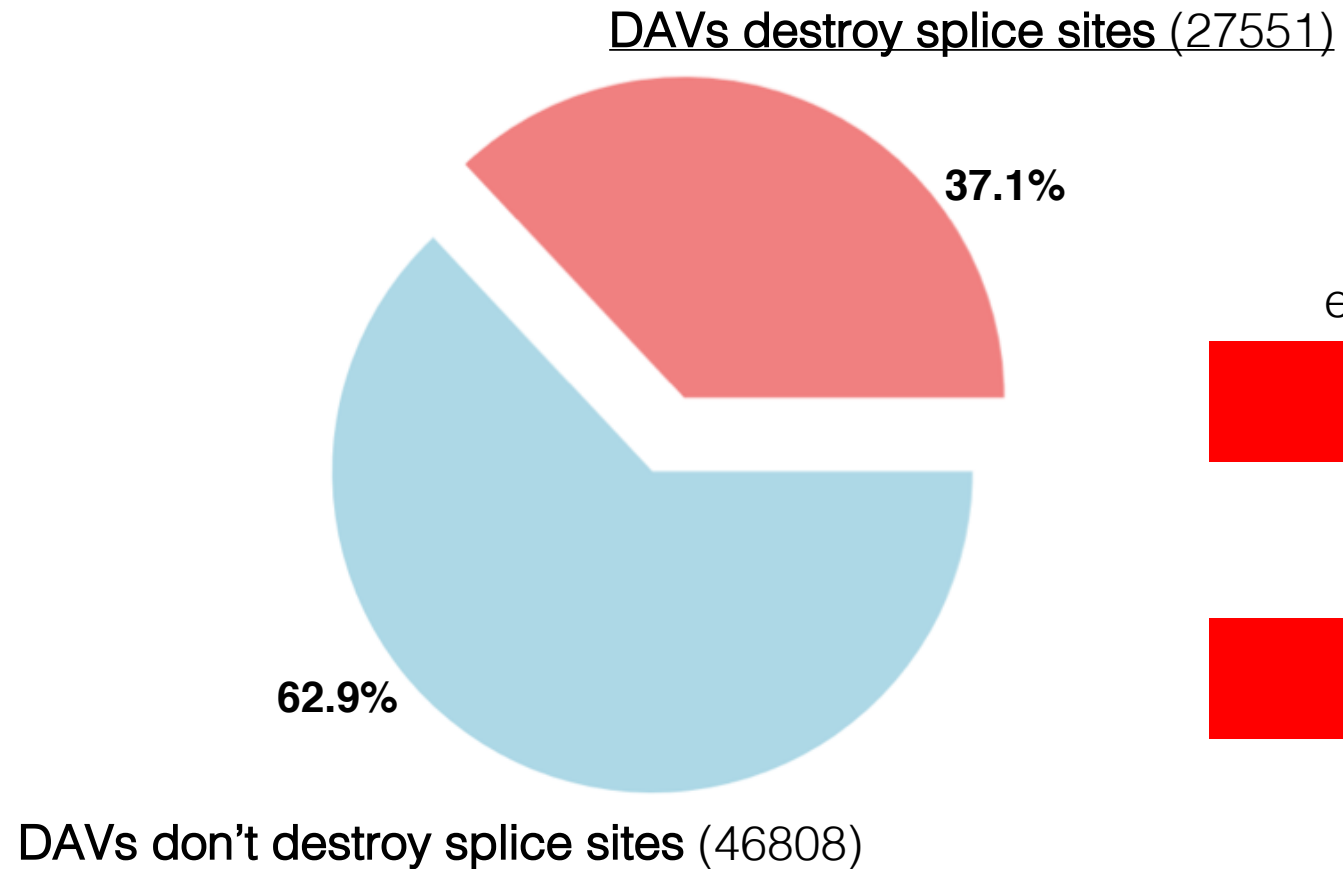
DAVs in intronic regions of precursor-mRNAs



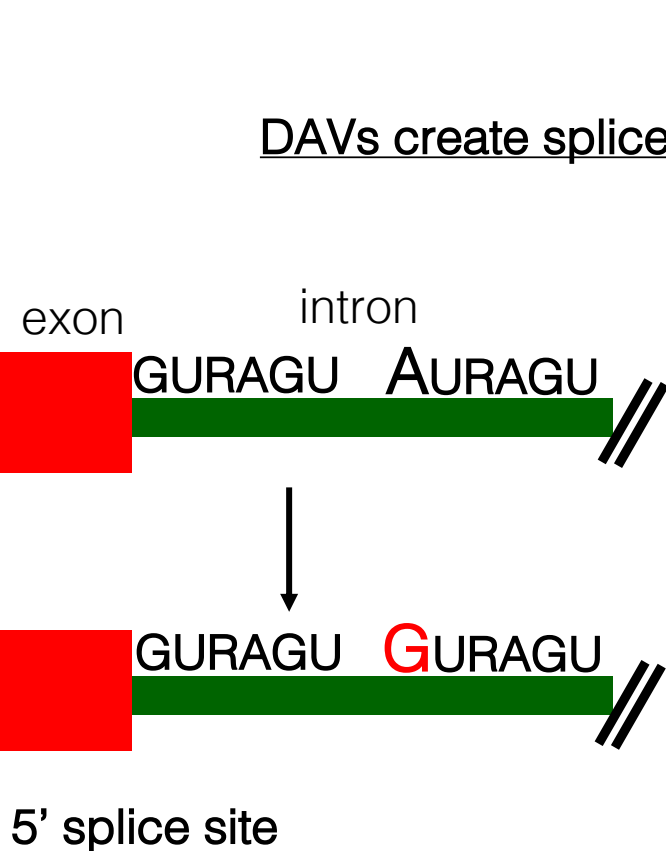
Impact of DAVs within introns



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Impact of DAVs within introns



DAVs create splice sites (1474)

2.0%

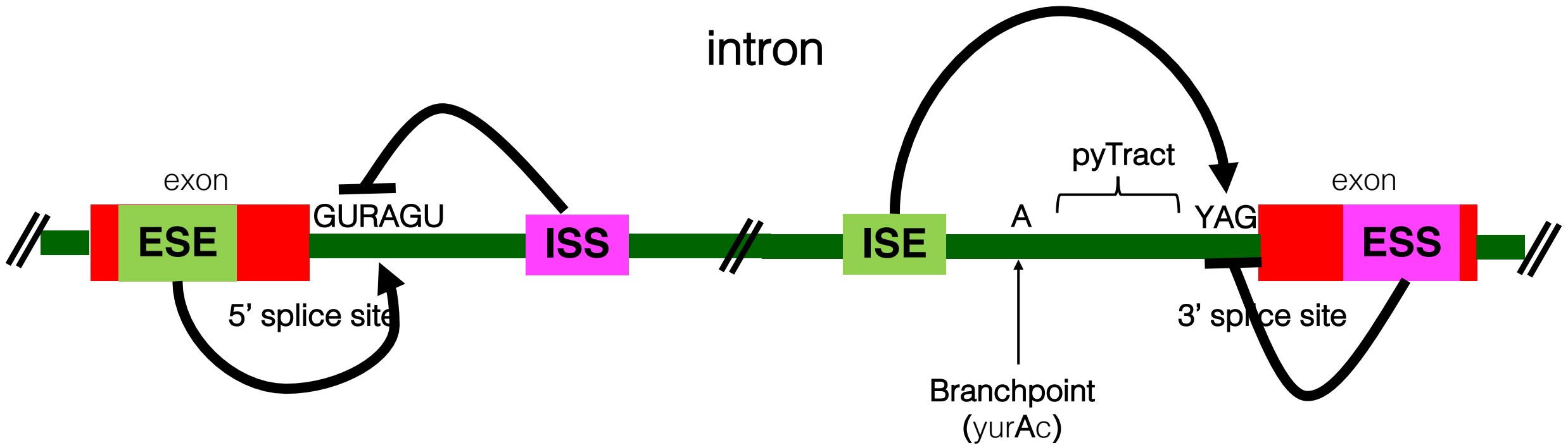
DAVs destroy splice sites (27551)

37.1%

60.9%

DAVs don't destroy or create splice sites (45334)

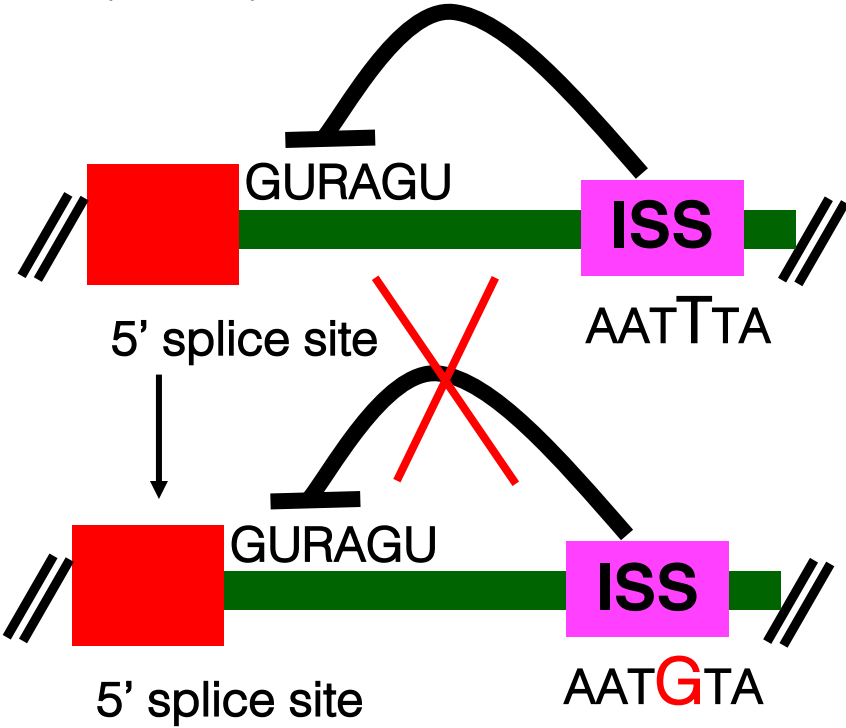
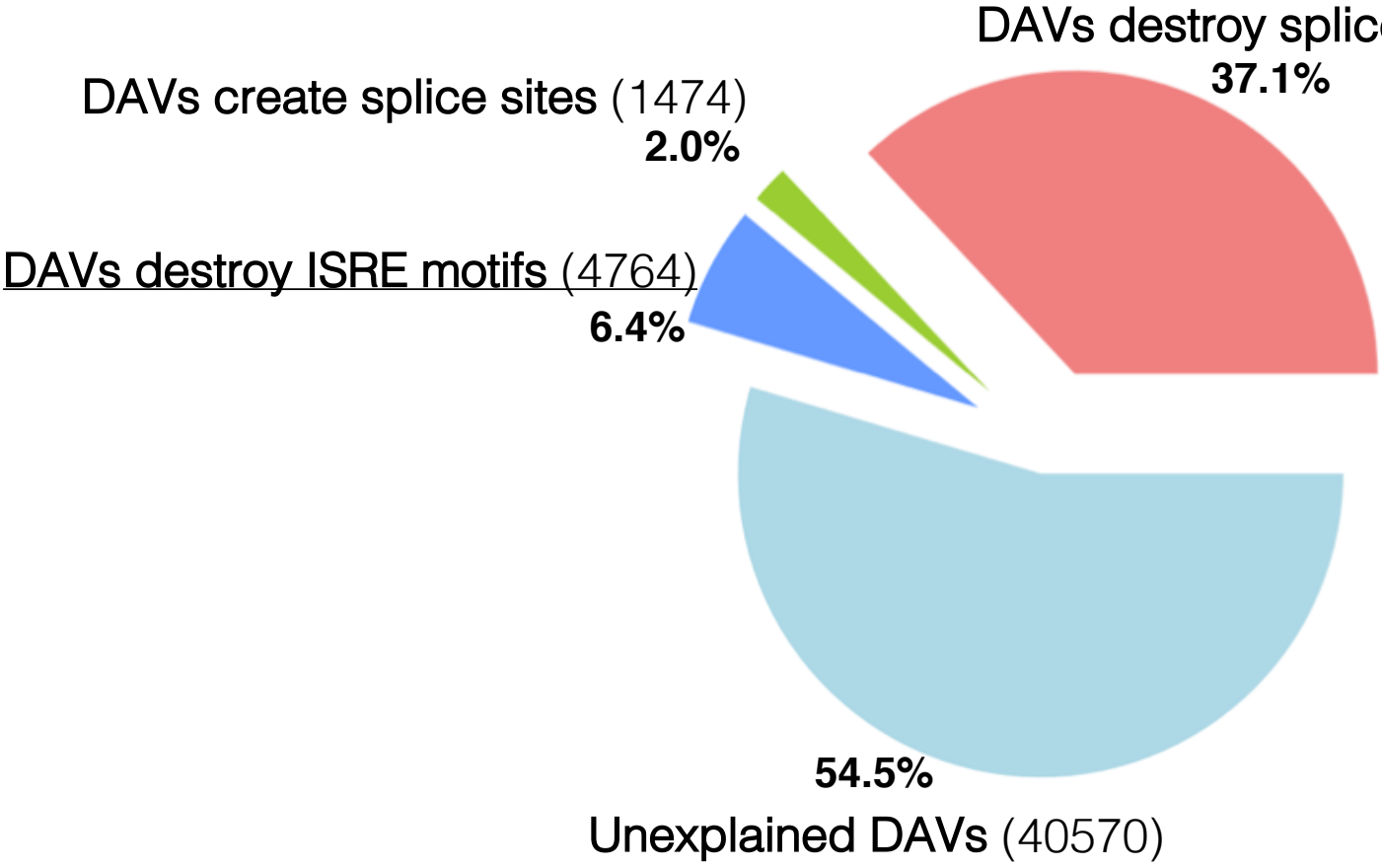
Splicing code 2.0



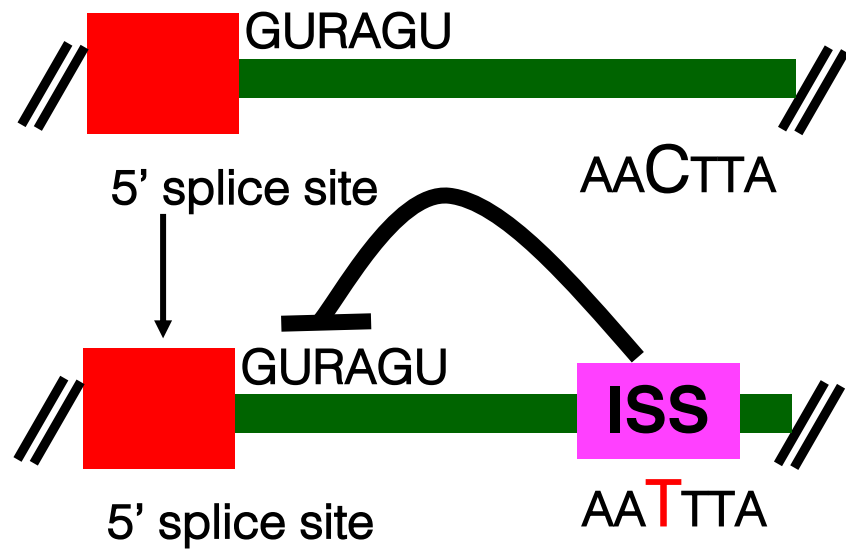
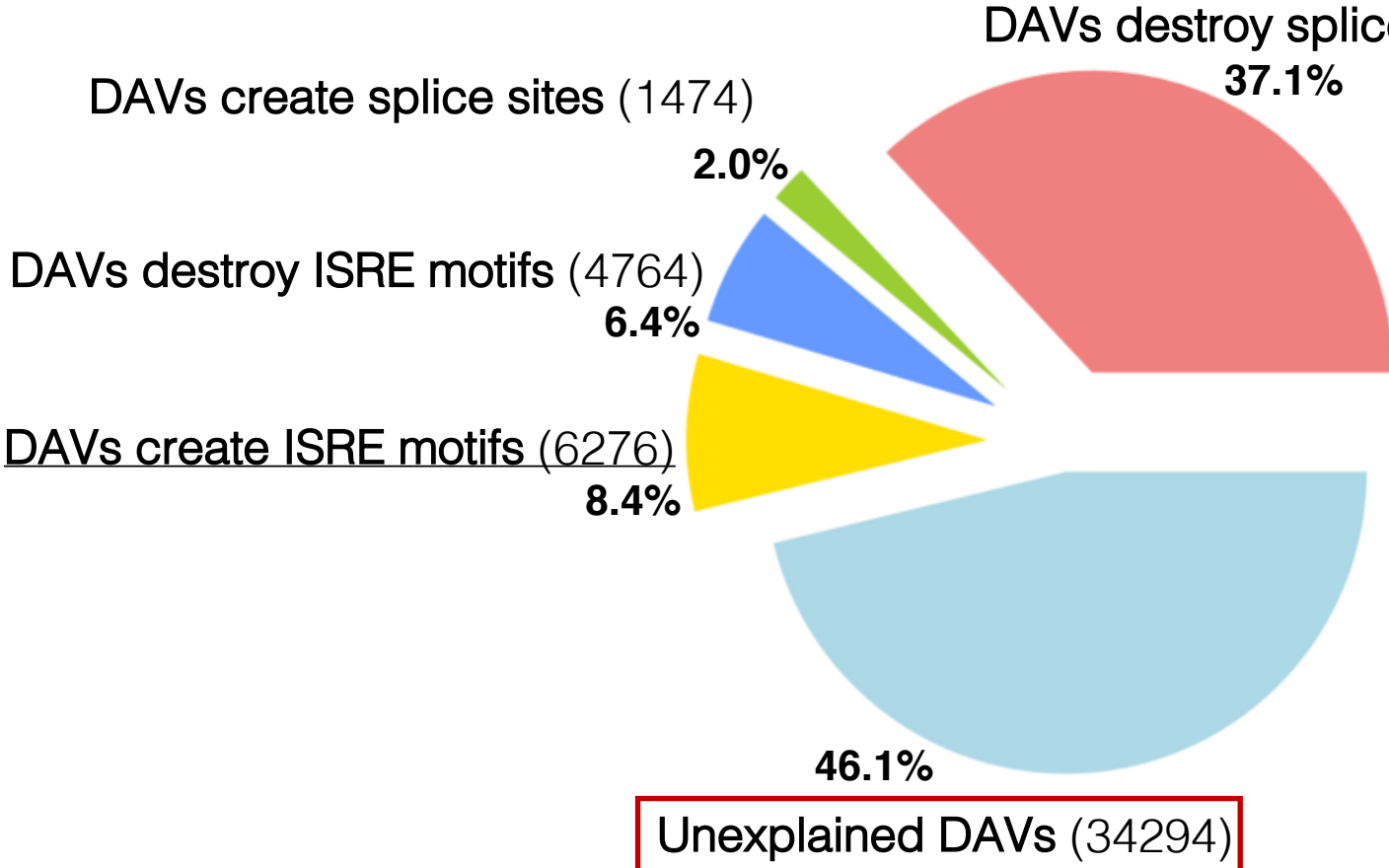
Splicing code 2.0: Intronic Splicing Regulatory Elements (ISREs)

- Wang et al., 2012 identified hexamers
- 87 intronic splicing enhancers
- 50 intronic splicing silencers

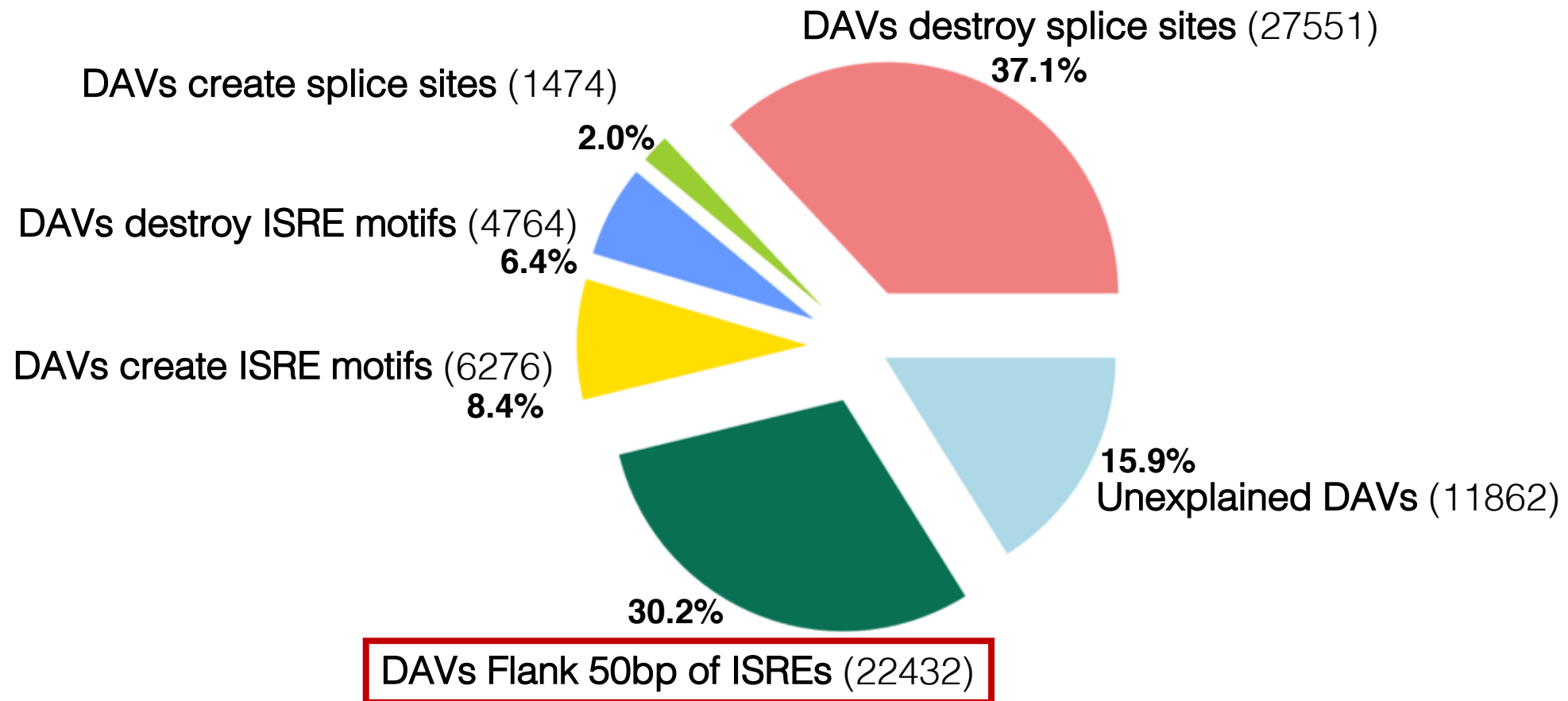
Impact of DAVs within introns



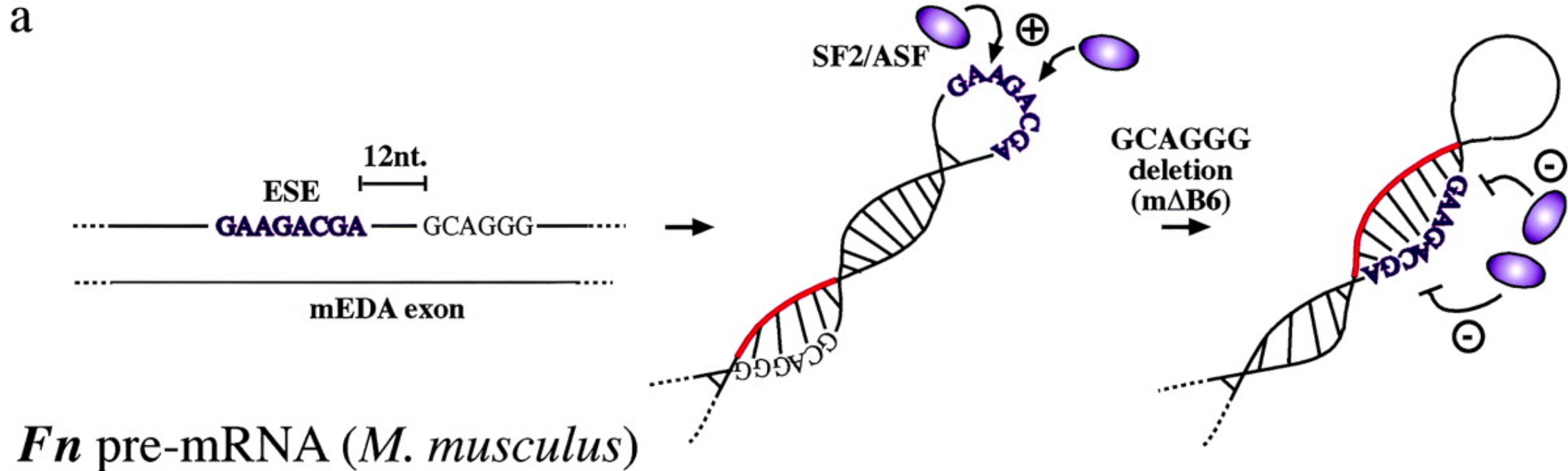
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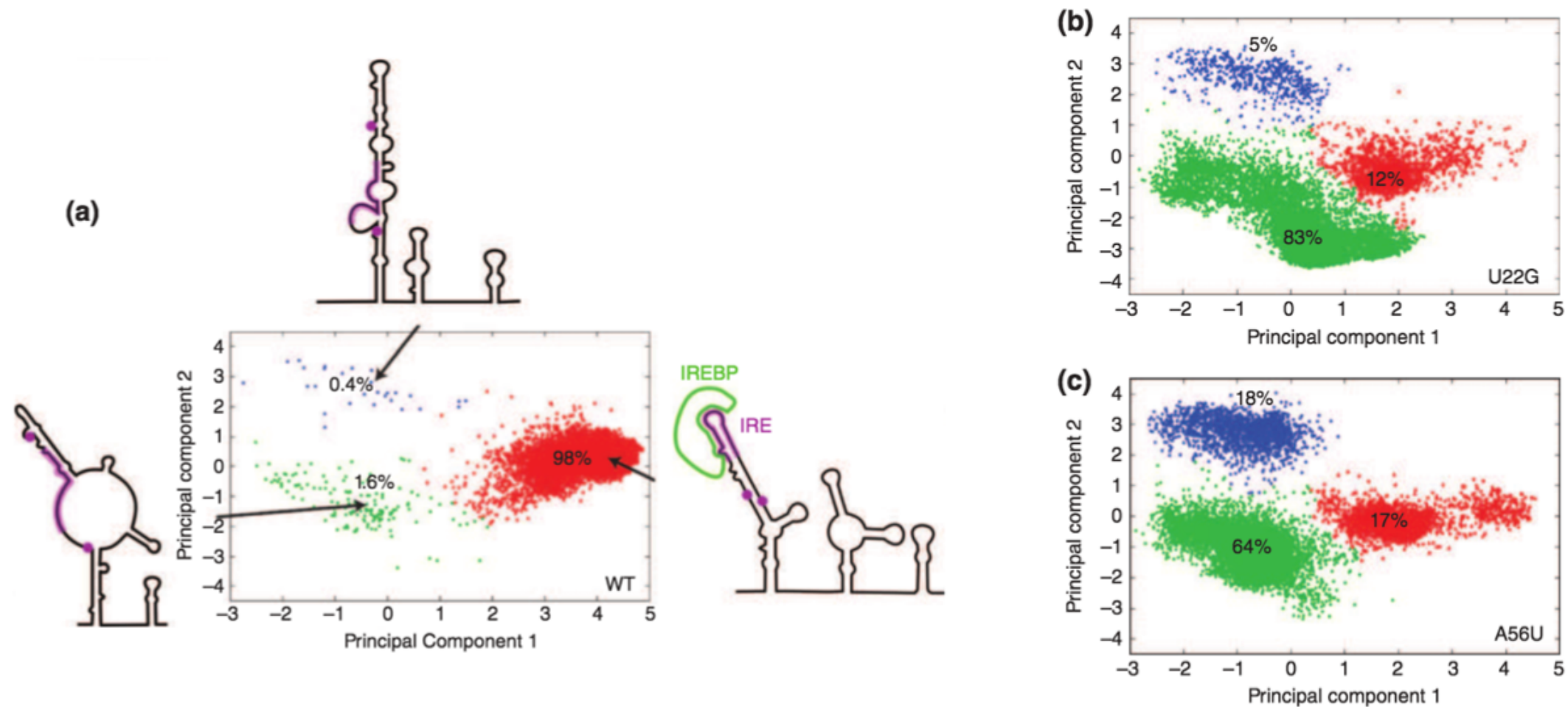
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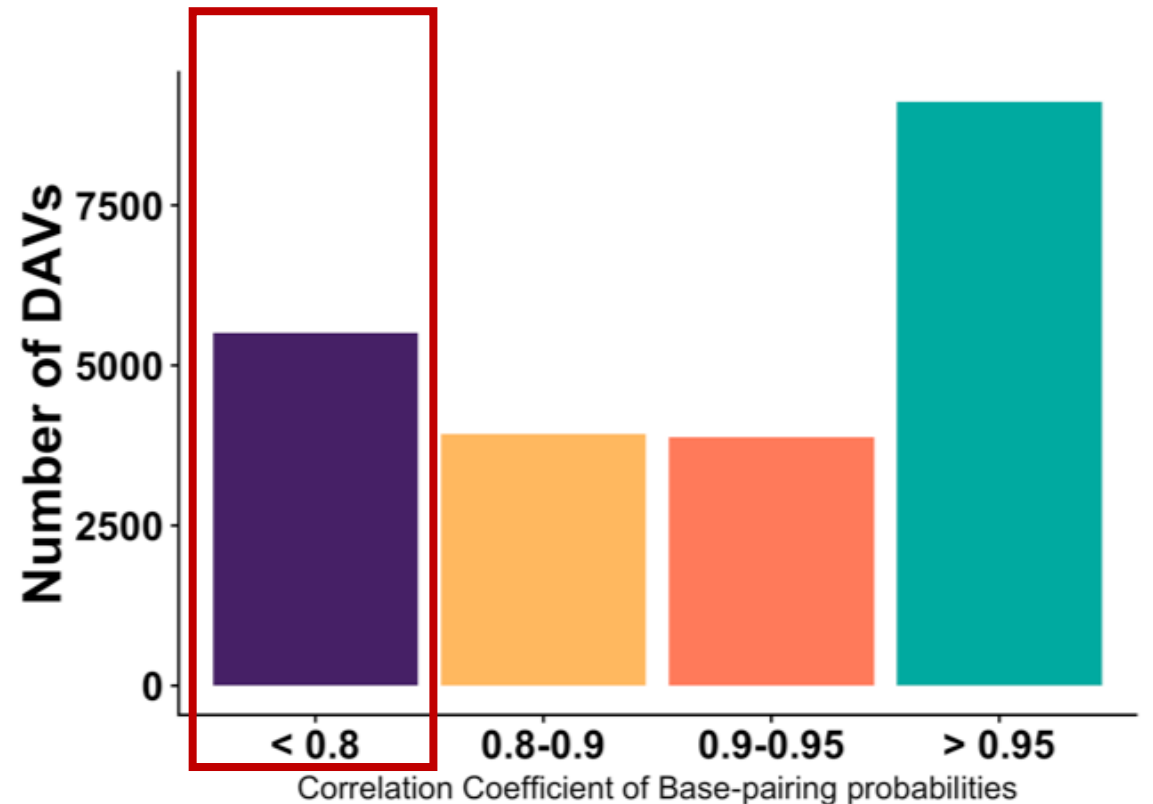
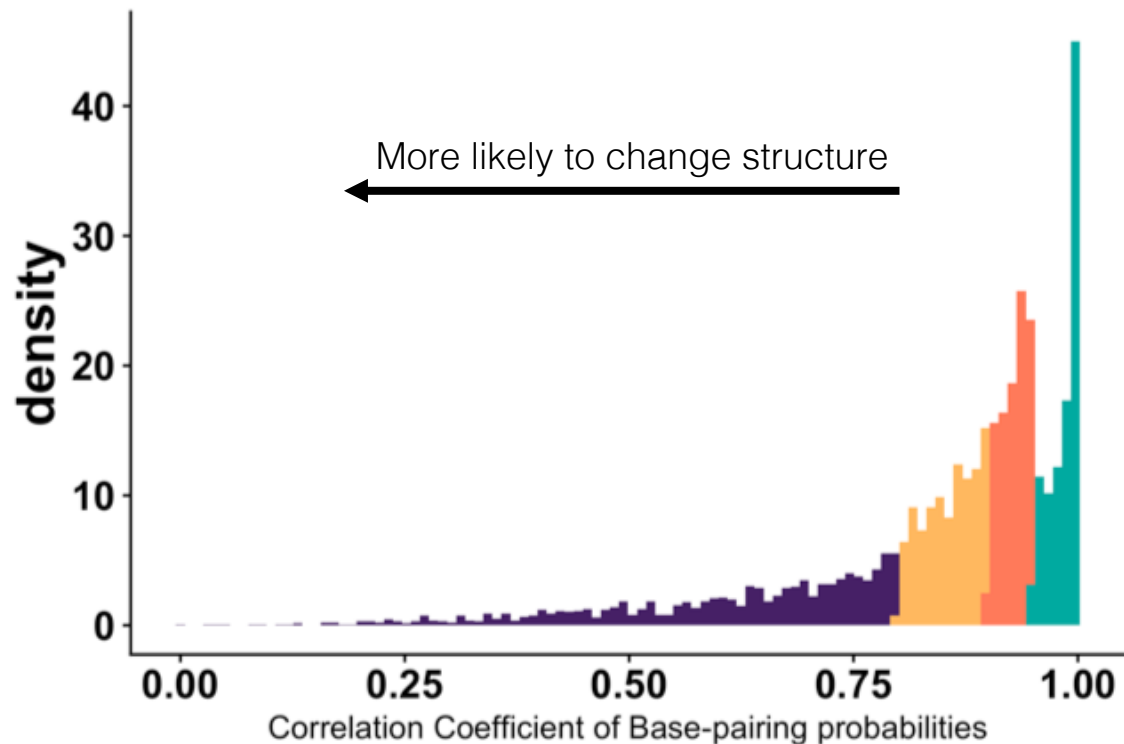
RNA Secondary Structures aiding splicing



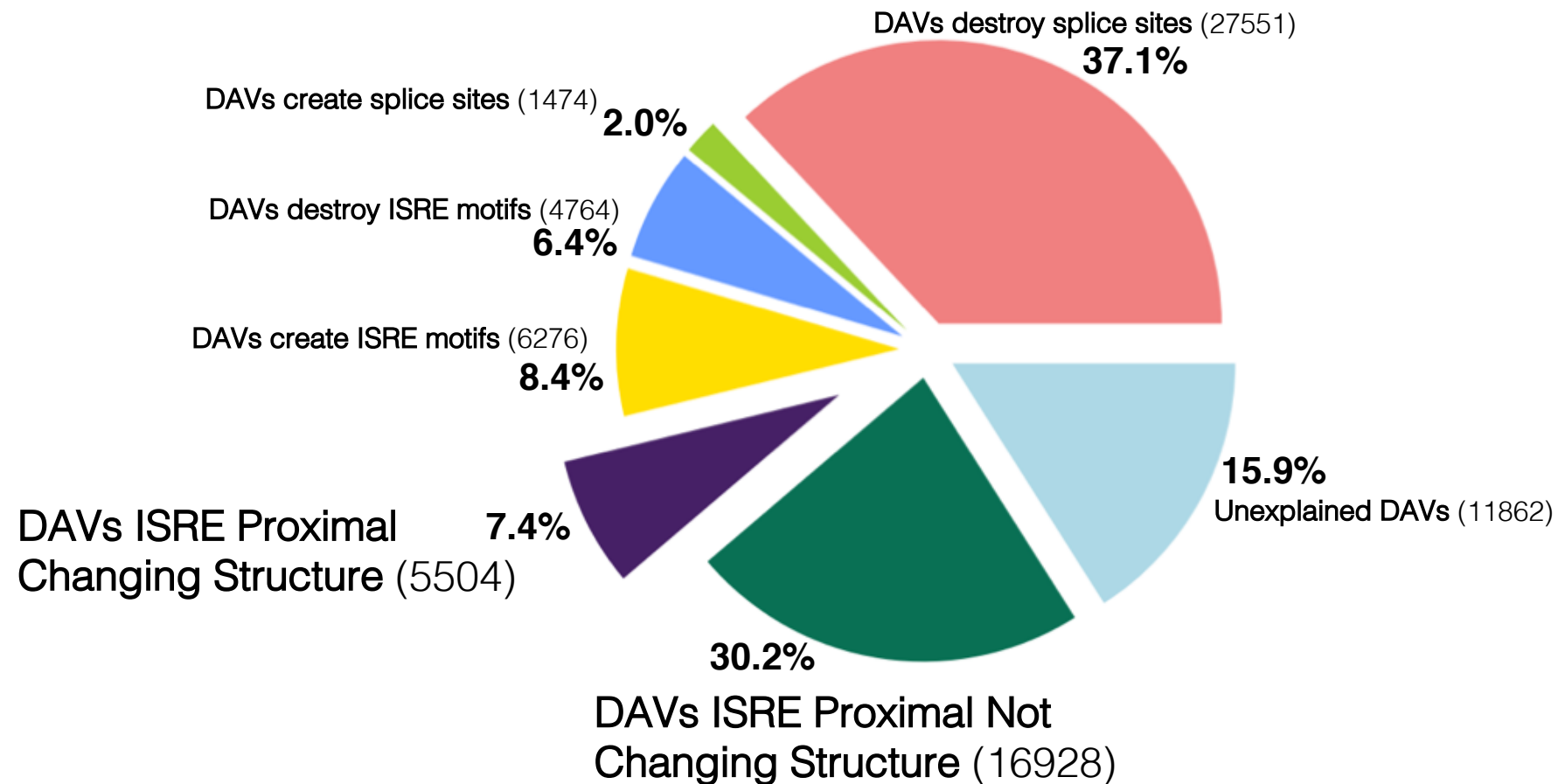
RiboSNitch in 5'UTR of Ferritin Light Chain (FTL) gene



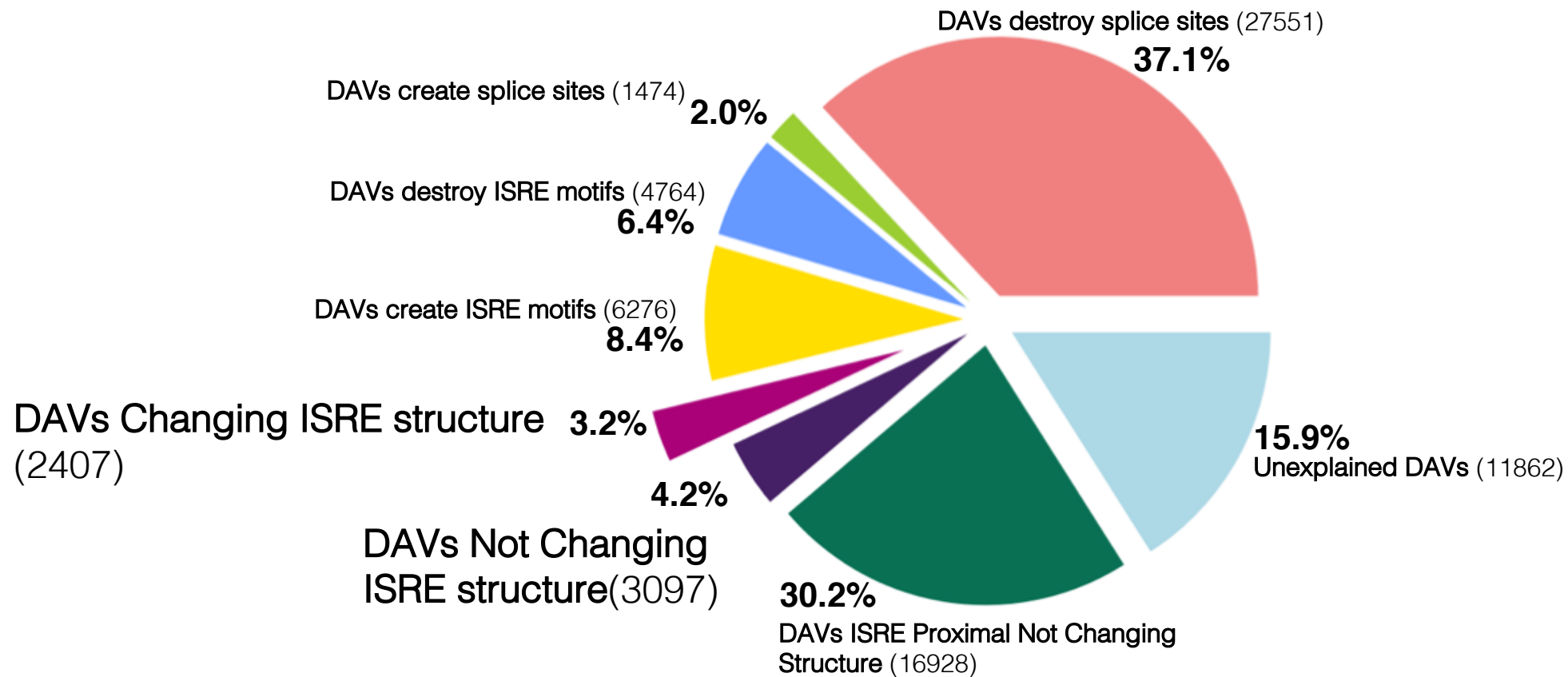
Changes in base-pairing probabilities for DAVs flanking ISREs



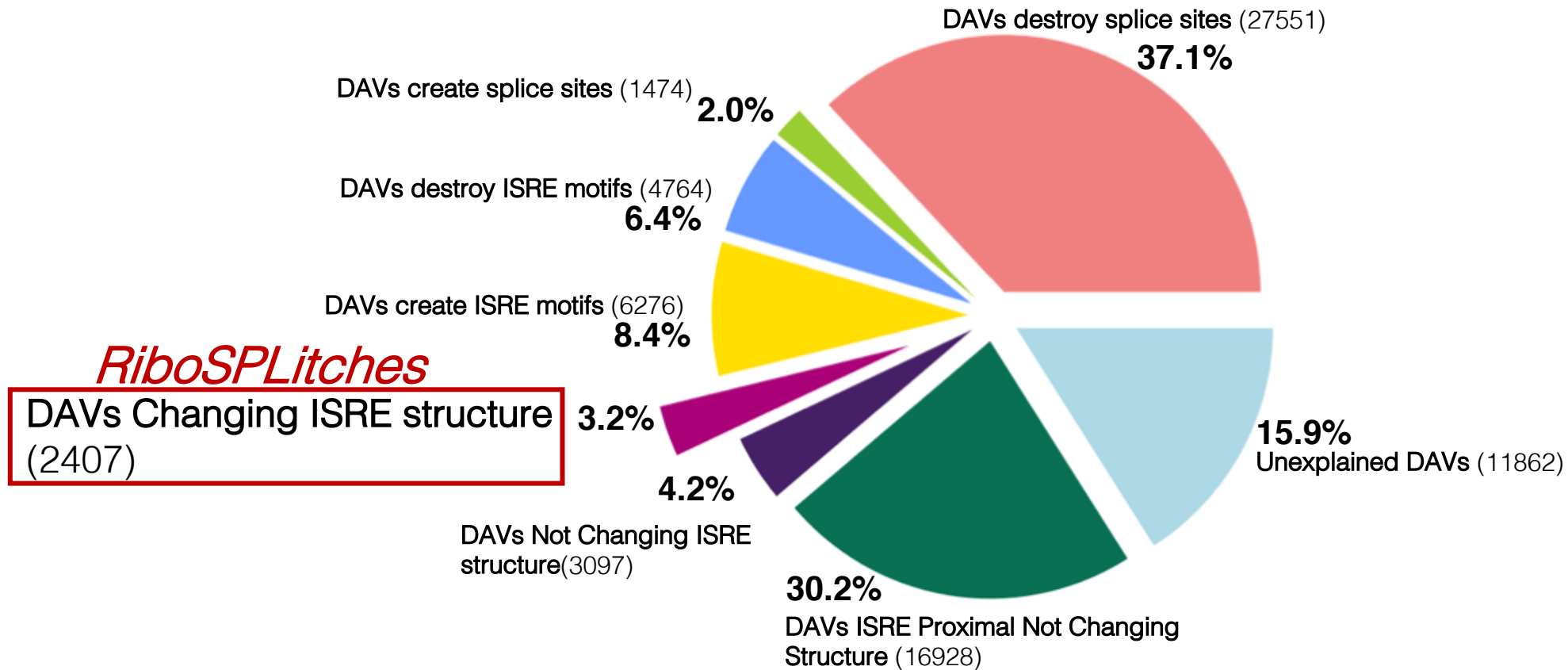
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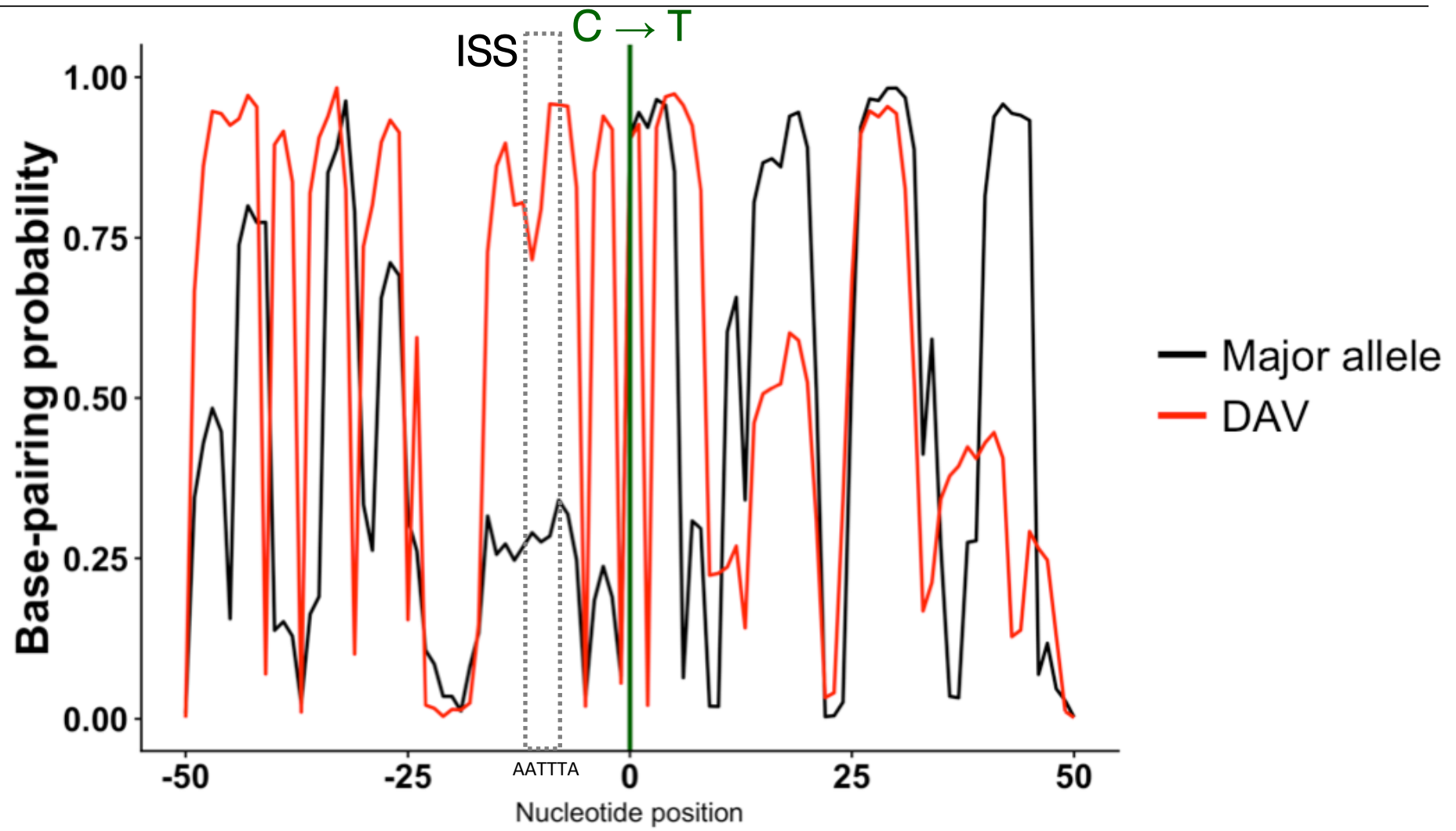
Impact of DAVs within introns



Impact of DAVs within introns



Predicted structural change for rs150393520



Conclusions

- Structure can be detected within introns
- RiboSPLitches: Intronic RNAs with DAVs that alter structure and impact splicing
- By filtering out DAVs that have obvious implications, we are able to predict RiboSPLitches

Acknowledgements

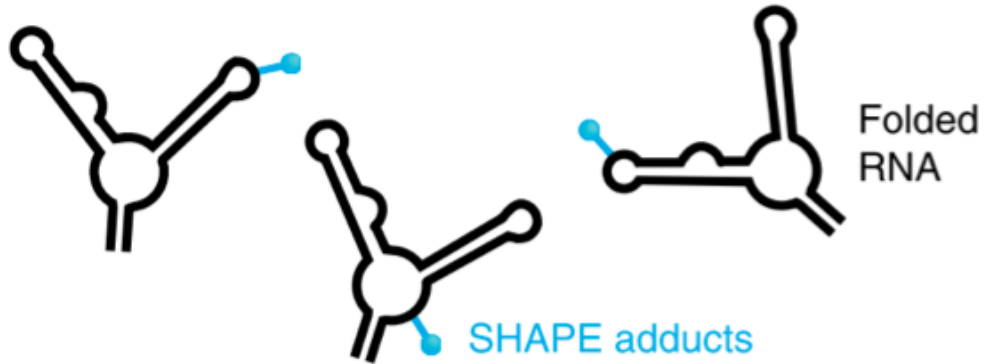
Laederach Lab at UNC Chapel Hill

- Alain Laederach
- Lela Lackey
- Aaztli Coria
- Lakshmi Ramasamy
- Anais Monroy-Eklund

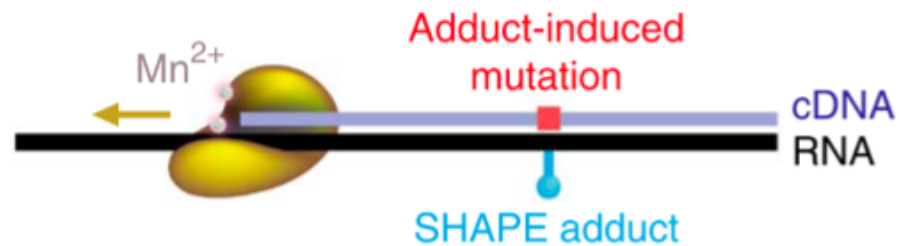


Structure probing: SHAPE-MaP

SHAPE modification



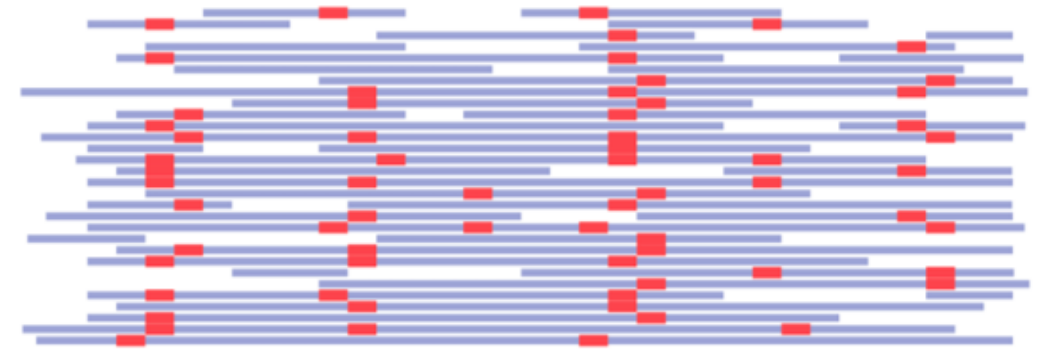
Mutational profiling



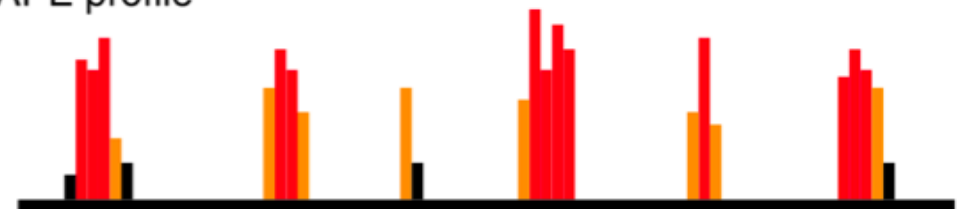
Library preparation and sequencing



Mutation counting



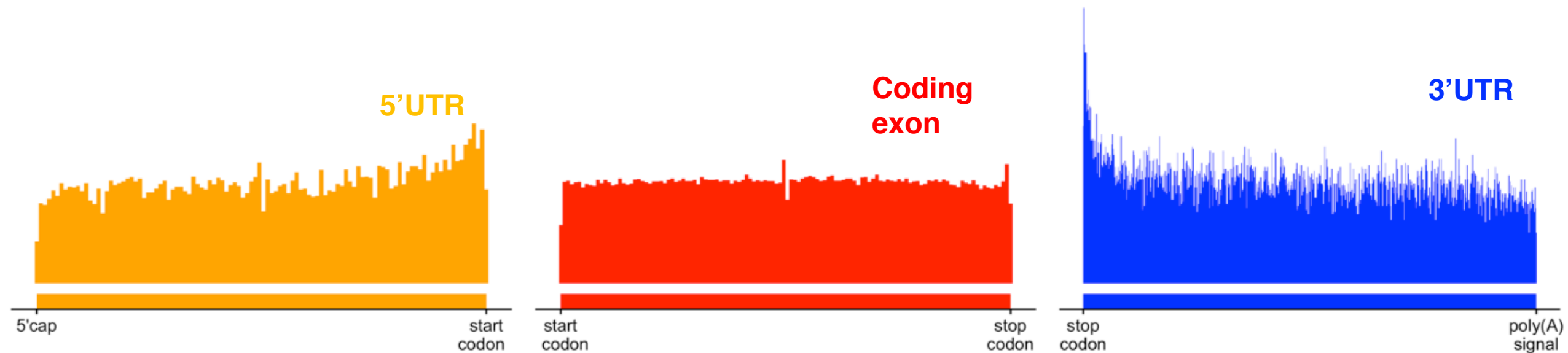
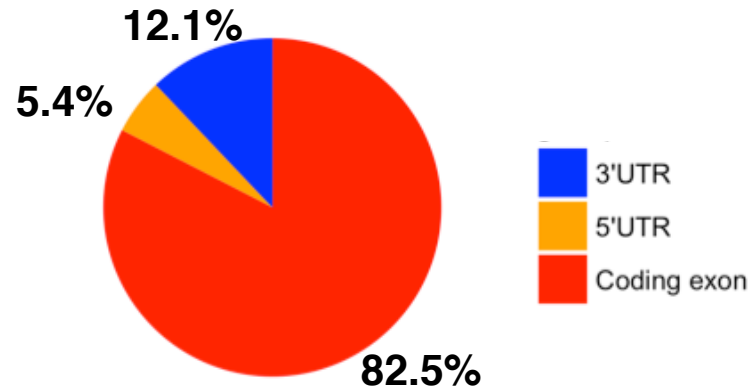
SHAPE profile



Future work

- Genome-wide analysis of RNA secondary structure in intronic regions
- Splicing assays
 - Verify the impact of DAVs on splicing
- Structure probing assays
 - Perform SHAPE on these specific regions to see if DAVs are altering base-pairing of nucleotides

DAV distribution: A typical mRNA



Splicing code

