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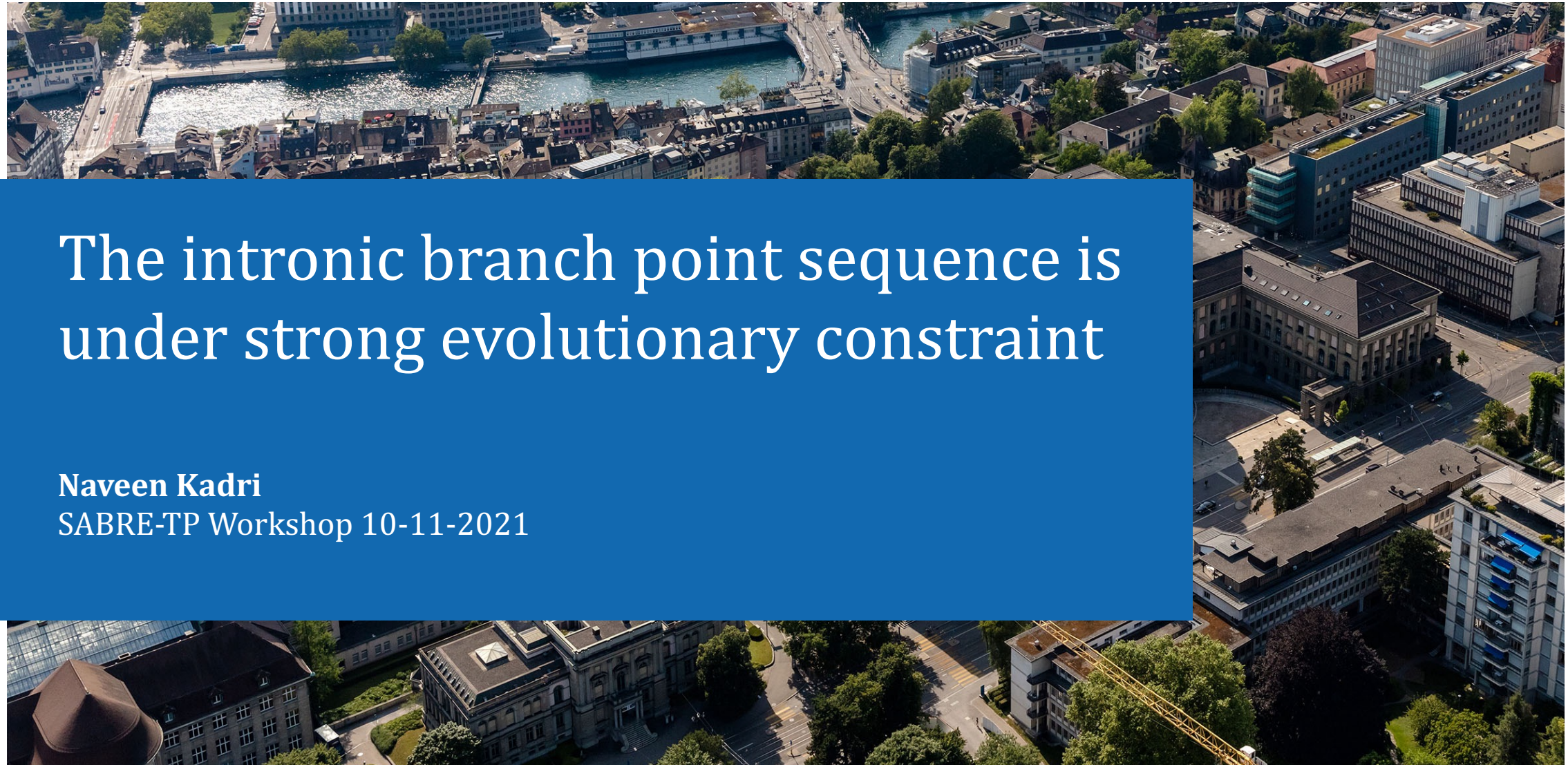


The intronic branch point sequence is under strong evolutionary constraint

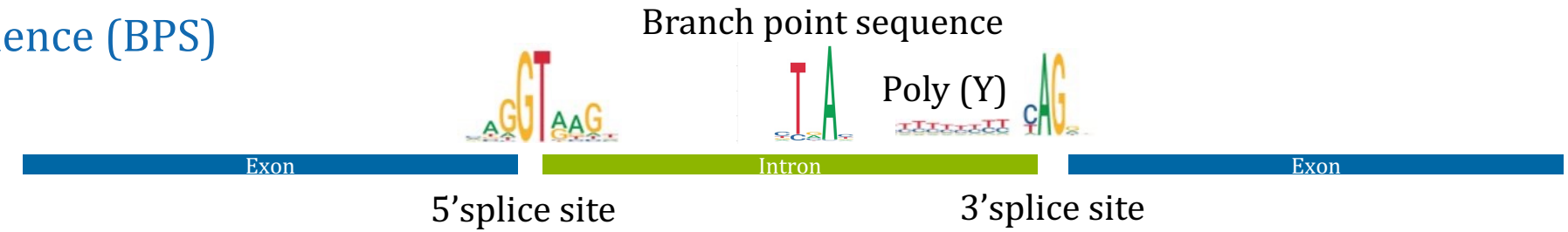
Naveen Kadri

SABRE-TP Workshop 10-11-2021

Introduction

- Noncoding sequences are an important source of genetic variation
  - Regulatory elements → gene expression & alternative splicing
  - QTL / significant contribution to h^2

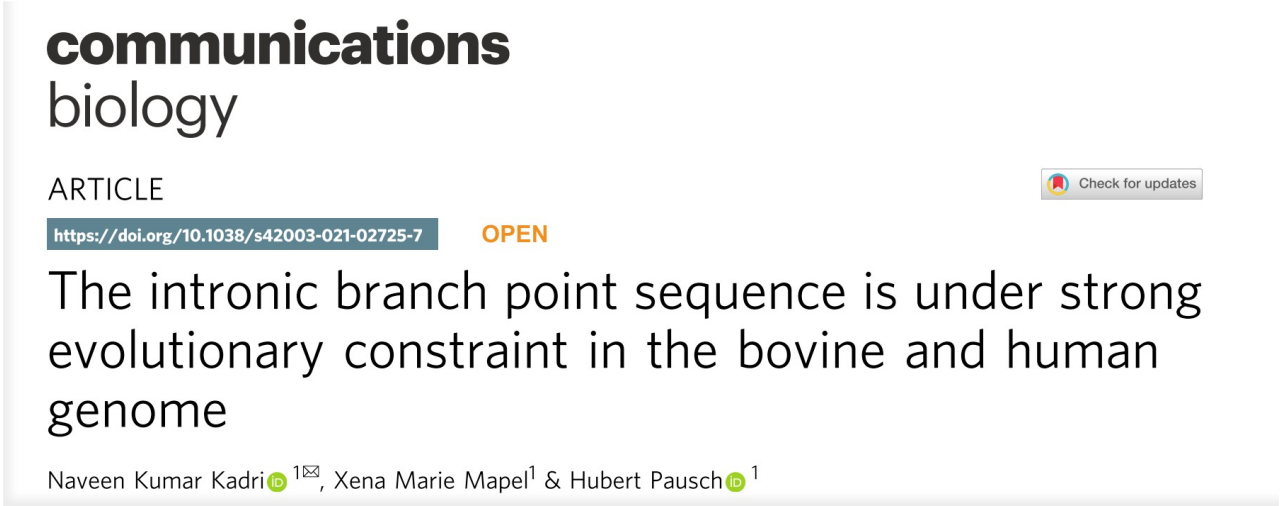
- m-RNA splicing Removal of (noncoding) introns and ligation of (coding) exons]
  - Spliceosome → evolutionarily conserved across eukaryotes
  - Requires motifs contained in intronic and exonic sequences (cis-acting)
    - Splice acceptor and donors
    - Polypyrimidine tract
    - Branch point sequence (BPS)



Introduction

- This study → focus on BPS
  - So far neglected feature
  - Associated with human diseases
  - Programs predicting functional consequences (e.g., VEP) are blind

  - Characterize bovine BPS
  - Strong evolutionary constraint
  - Splicing QTL overlapping bovine BPS
  - Confirmation in human BPS



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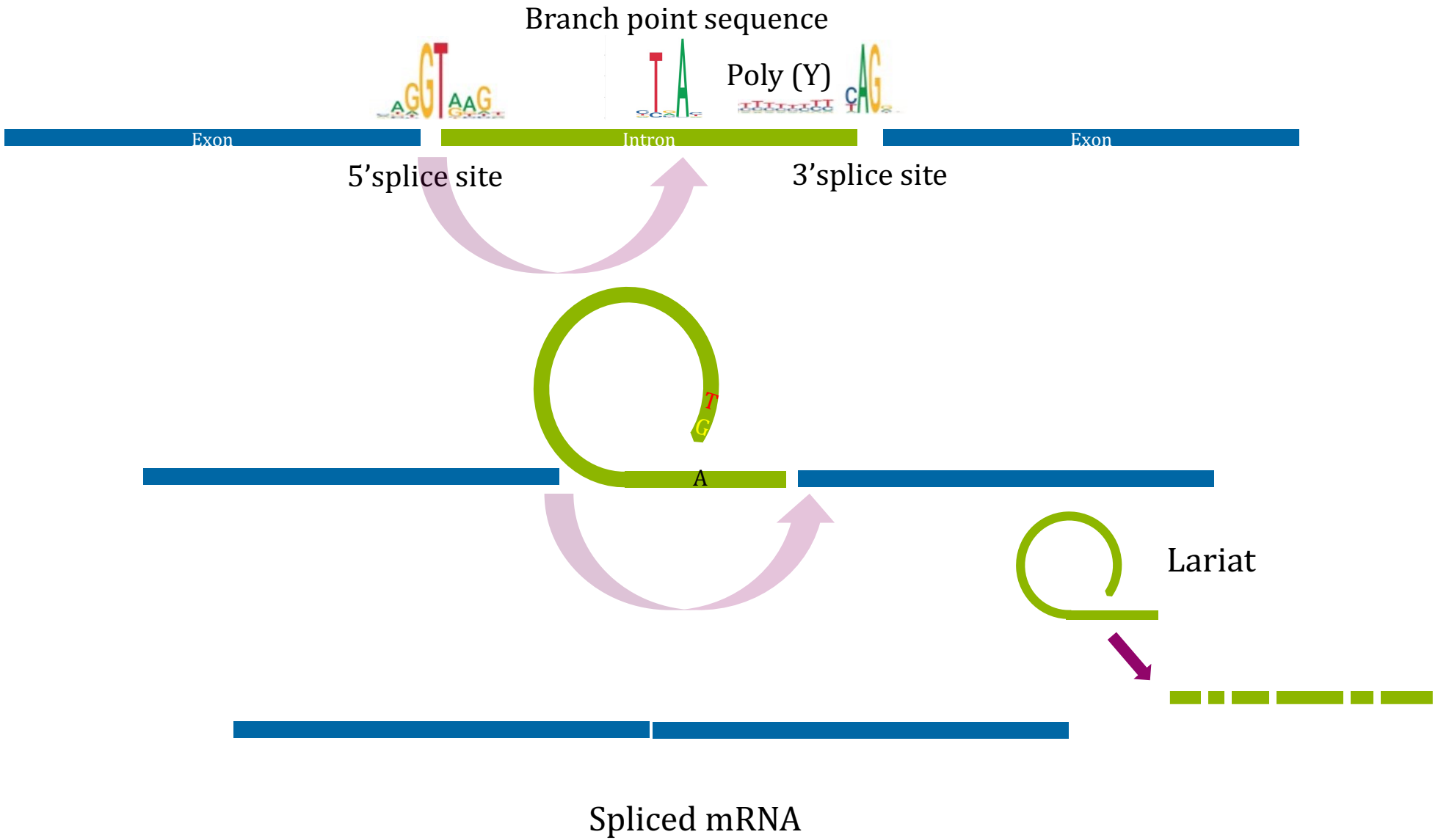
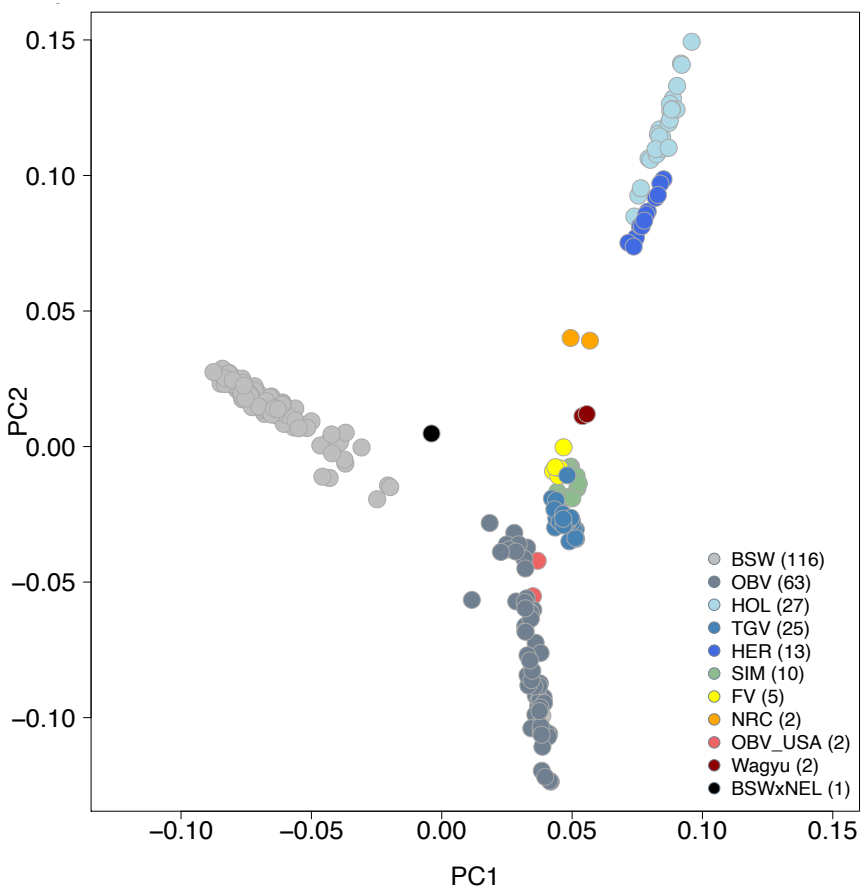


Data

- 266 cattle from 11 breeds
  - Sequenced to an average depth of 16.28
- 29,227,950 (bi-allelic) and 190,200 (multi-allelic)
- One variant site every 85 bp

- Functional features
  - Ensemble (release 104) annotation of ARS-UCD1.2



Constraint on annotated features

- 35,848 transcripts from 20,785 genes

- Variation expressed as variants /100 bp

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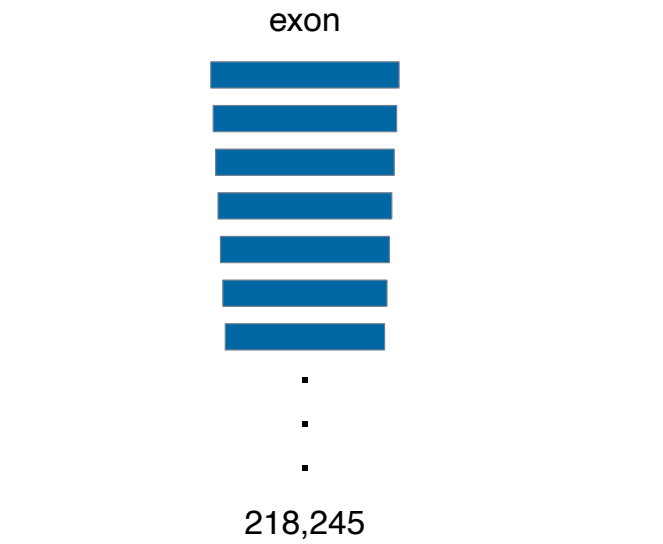
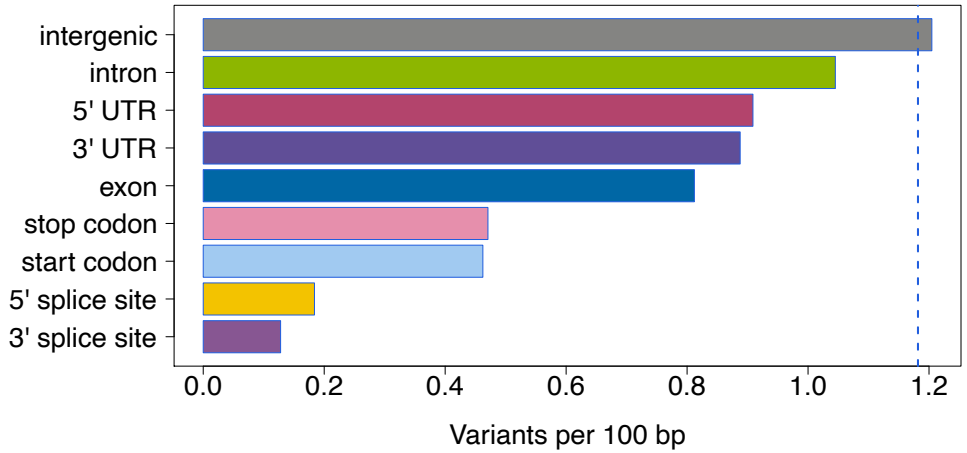


267,564 / 32,947,475
0.81 variants per 100 bp

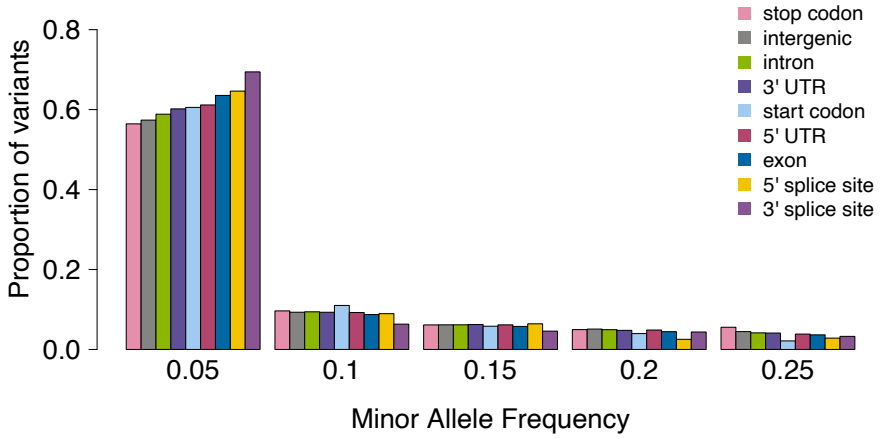
- Variation expressed as variants /100 bp

Constraint on annotated features

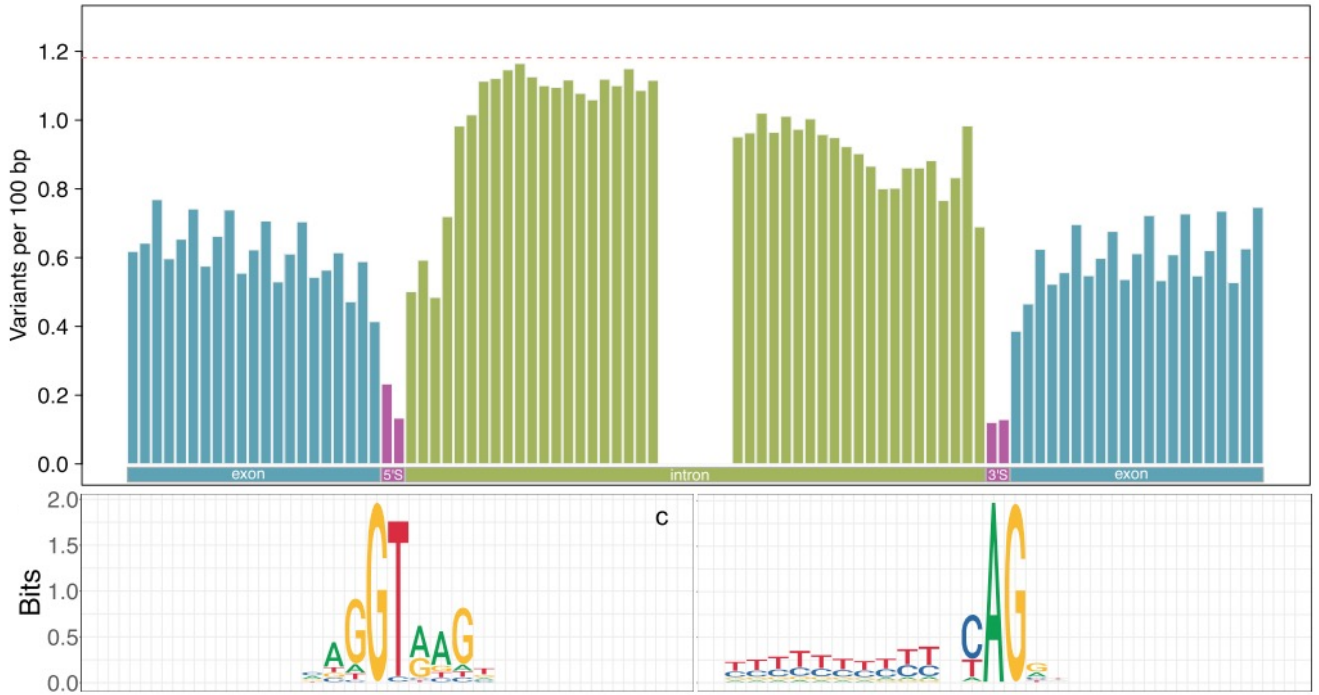
- Intergenic region more variable
- Genic features less variable
- Splice sites least variable



- Enriched for low-frequency/rare variants



Local variation around the Splice sites



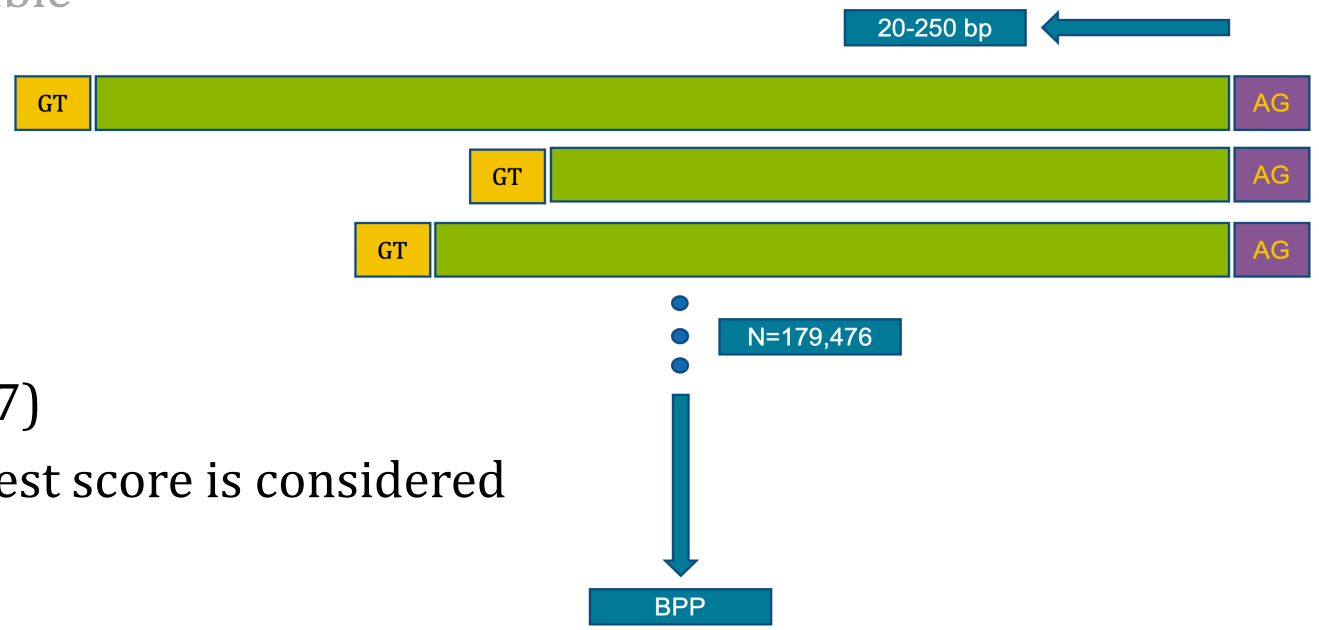
- Spice sites indeed less variable than surrounding intronic & exonic sequences
- Known patterns retrieved
  - Wobble at the third position of triplet codon

Prediction of Bovine branch point sequence

- Hitherto neglected → not available from GTF files
  - Computational prediction is possible

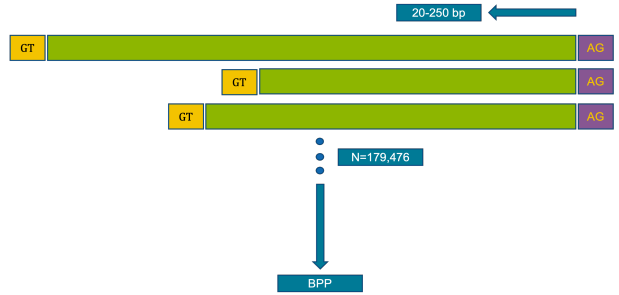
- 179,476 bovine introns >20 bp long
  - Up to 250 bp from the 3'SS

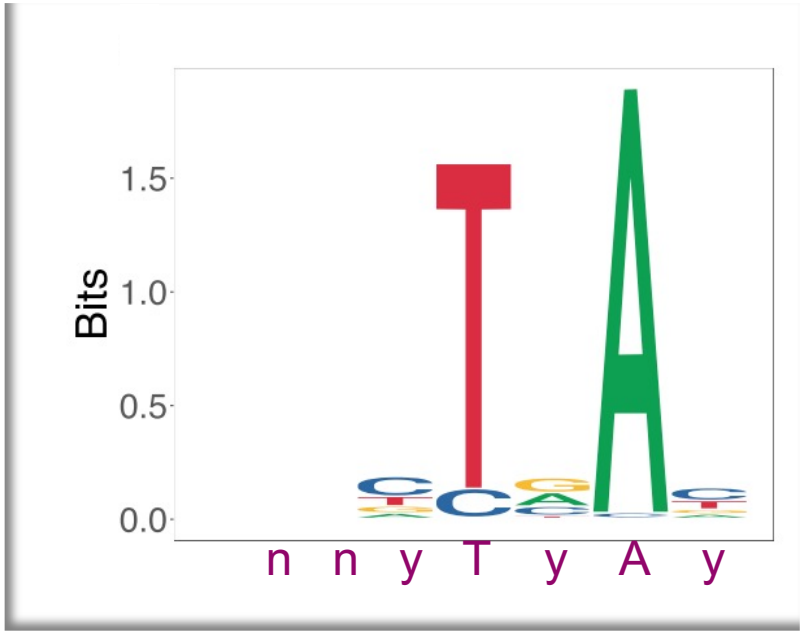
- Prediction using BPP (Zhang et al., 2017)
  - Only one BP/intron with the highest score is considered



Bovine branch point sequence

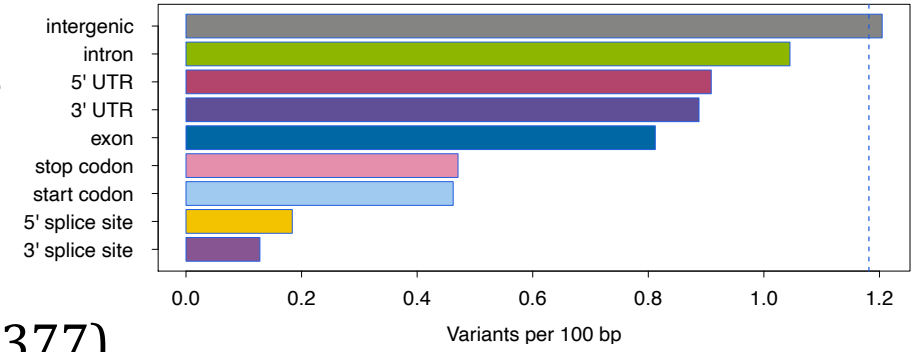
- Location 14-145 bp upstream of 3'ss (mean = 28.8 ± 11.2)
  - 82% between 18-37 bp
- Predicted branch point was predominantly Adenine (98.64%)
  - Cytosine (1.28%) or Thymine (0.08%)
- Degenerate heptamer → nnyTyAy
  - Highly conserved Thymine at position 4 (91.56%)
- >90% BPS were "TNA" at position 4 to 6 (canonical)
  - "CNA" → ~8%

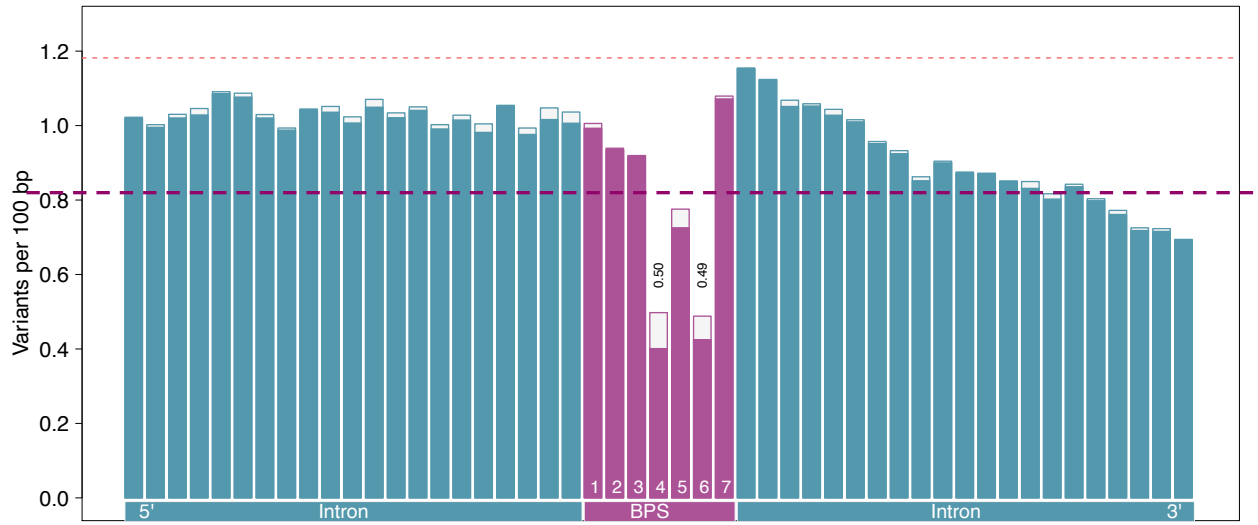




Constraint on bovine Branch point sequence

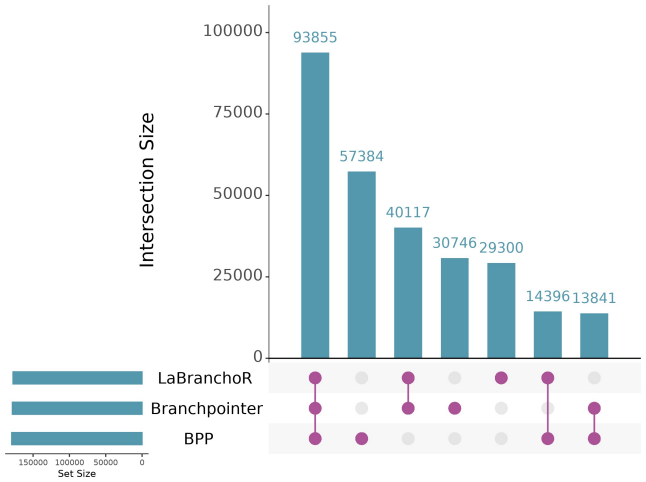
- Less variable than the surrounding intronic sequence
  - Highly conserved 4(T) and 6 (A)
- Lower than coding sequence
- Stronger constraint in canonical ("TNA") BPS (n=162,377)

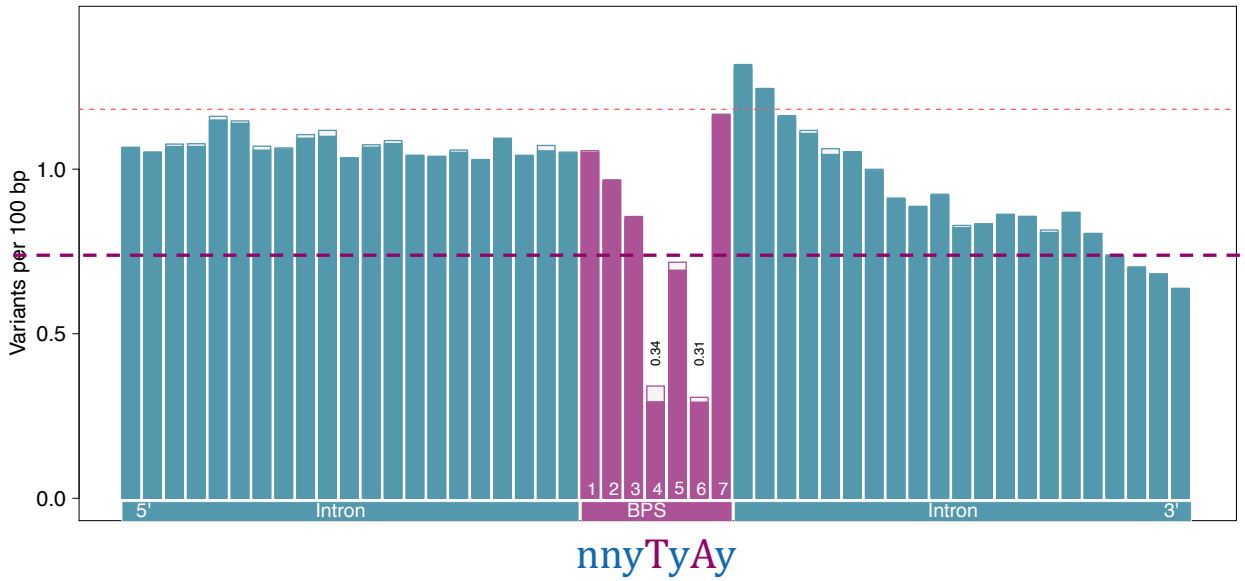




Prediction tools

- Prediction from two other tools → branchpointer[1] and LaBranchOR[2]
- Matching ~53% (n=93,855)
- Even higher constraint on these 93,855 BPS





[1]Paggi et al., RNA 24, 1647–1658 (2018). [2]Signal et al., Bioinformatics 34, 920–927 (2018).

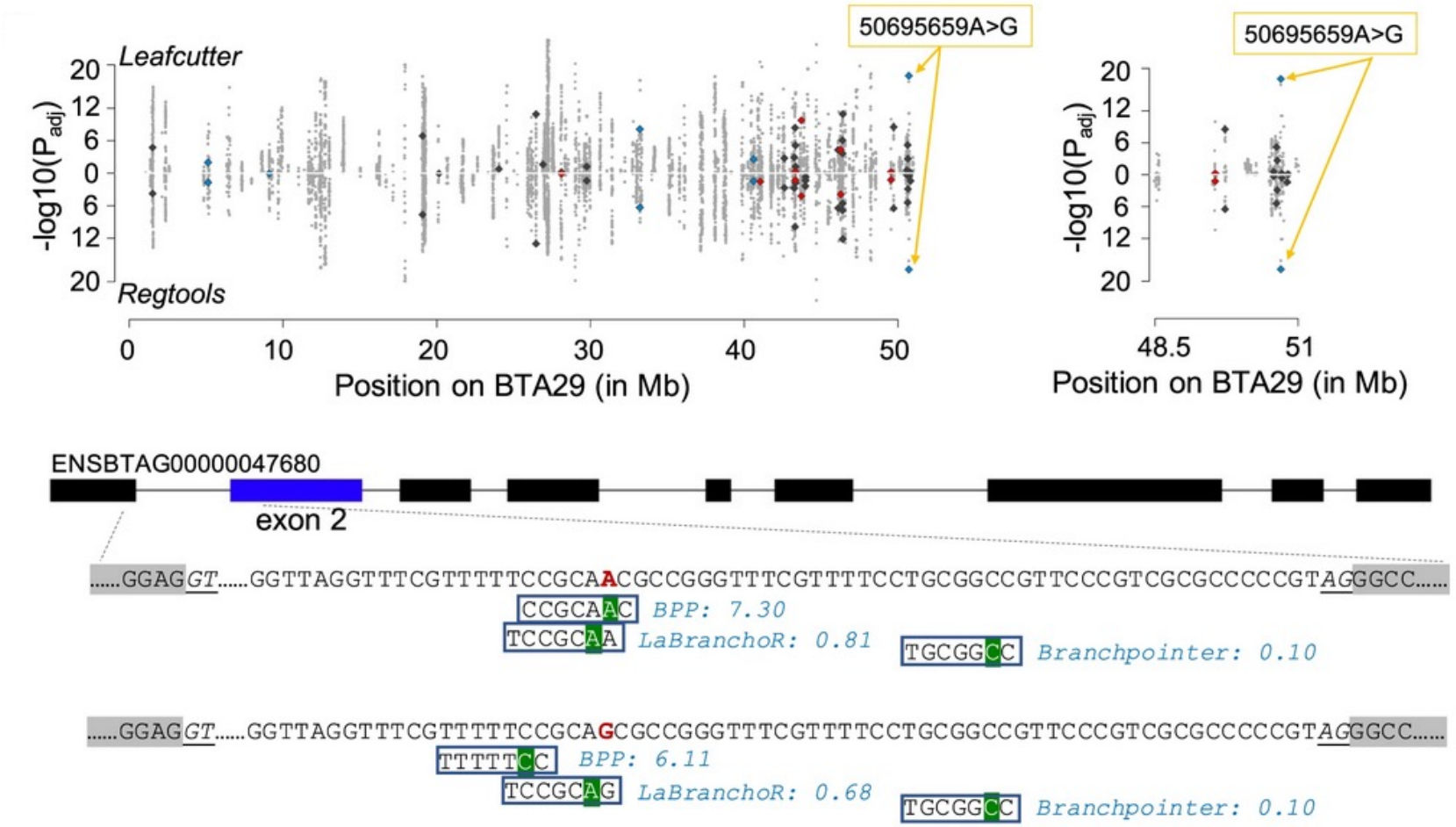
Splicing QTL

- sQTL cohort
  - Testis tissue sampled from 76 mature bulls
  - DNA sequencing to an average of 12.6-fold depth
  - Deep total paired-end RNA sequencing (283.6 million reads per sample)

- Splicing events characterized using *Leafcutter*[1] and *Regtools*[2]
- cis-sQTL detection using *FastQTL*[3]

[1]Li et al., ***Nat. Genet***. 50, 151–158 (2018), [2]Cotto et al., ***bioRxiv*** https://doi.org/10.1101/436634 (2021), [3]Ongen et al., ***Bioinformatics*** 32, 1479–1485 (2016)

A branch point mutation leads to utilization of alternative 3’ splice acceptor sites in *IRF7*



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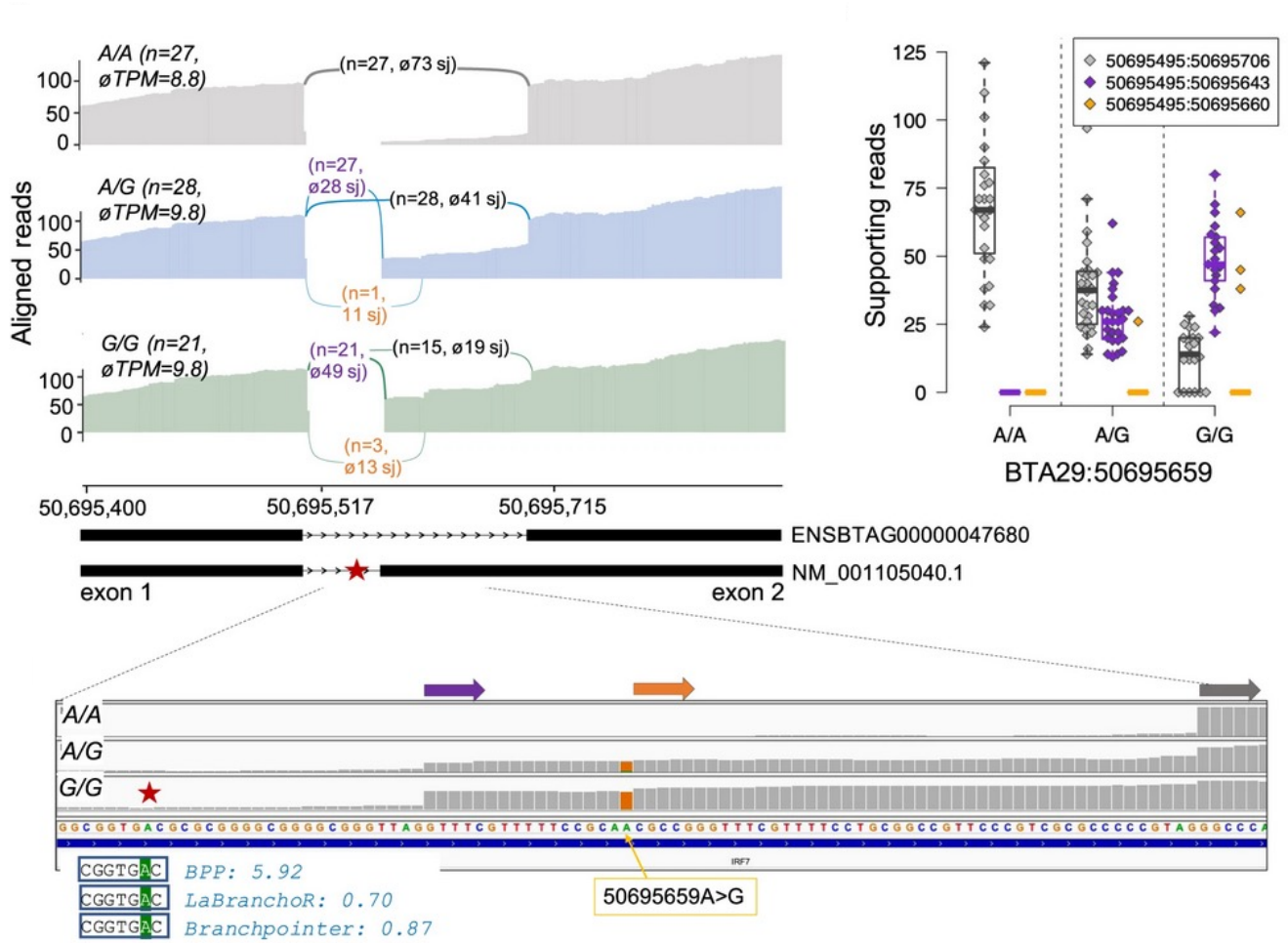
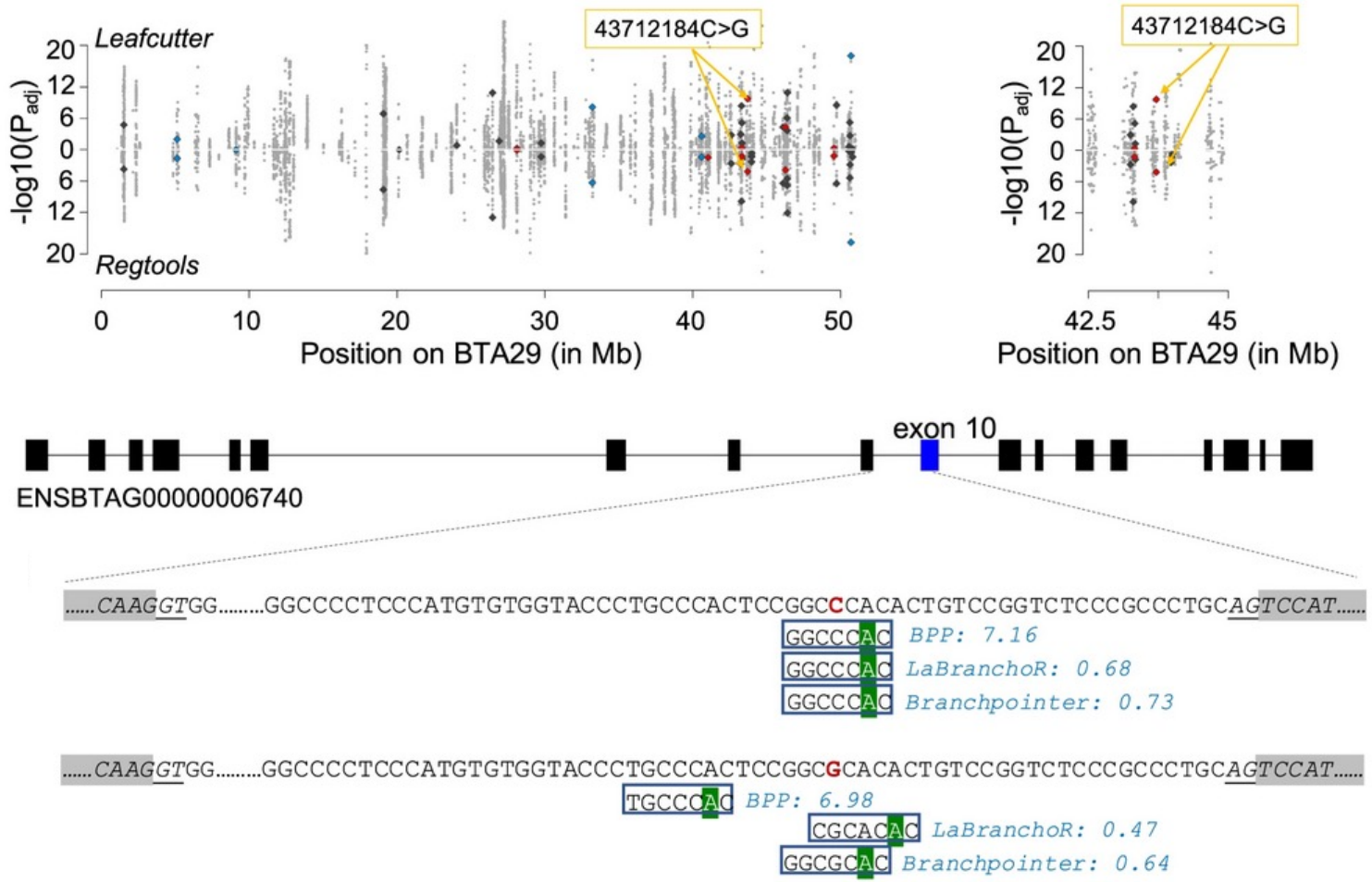


A branch point sequence mutation leads to alternative 3’ splicing of *SCYL1* exon 10



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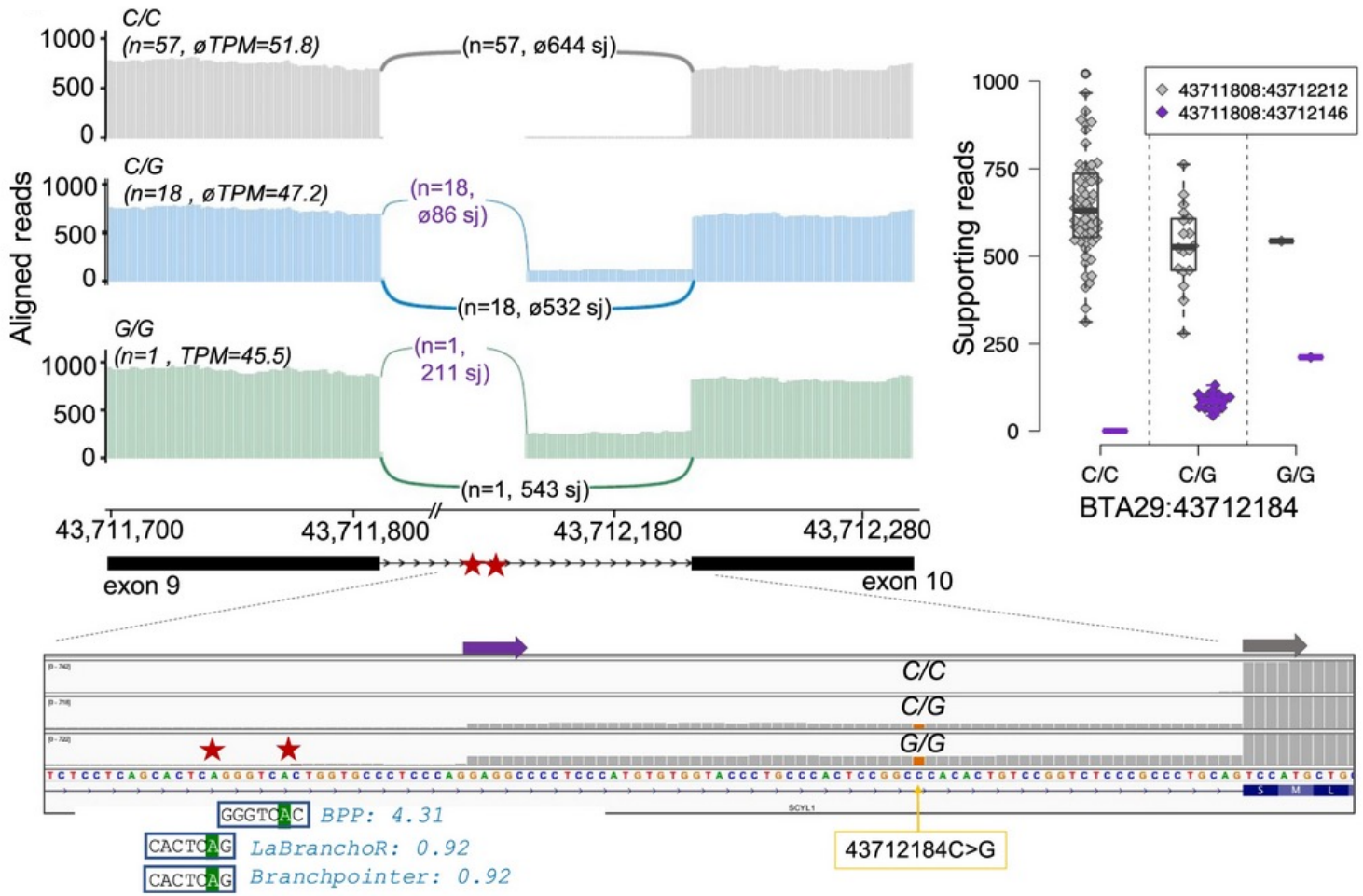
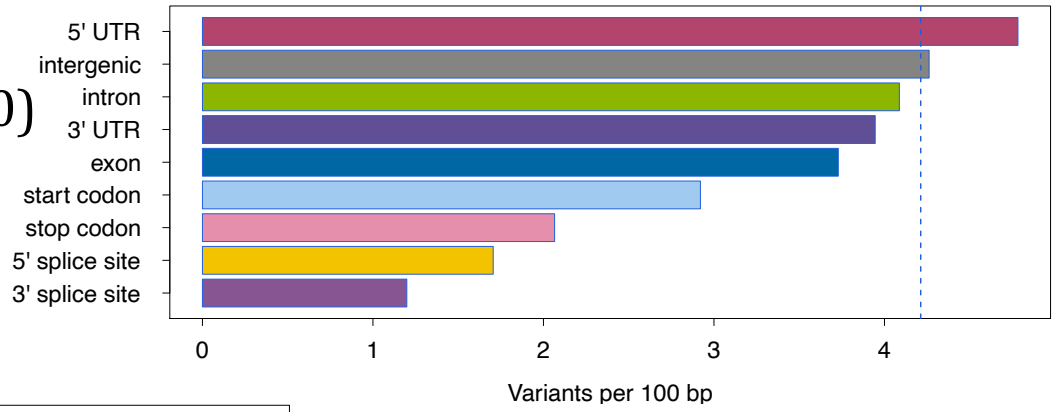
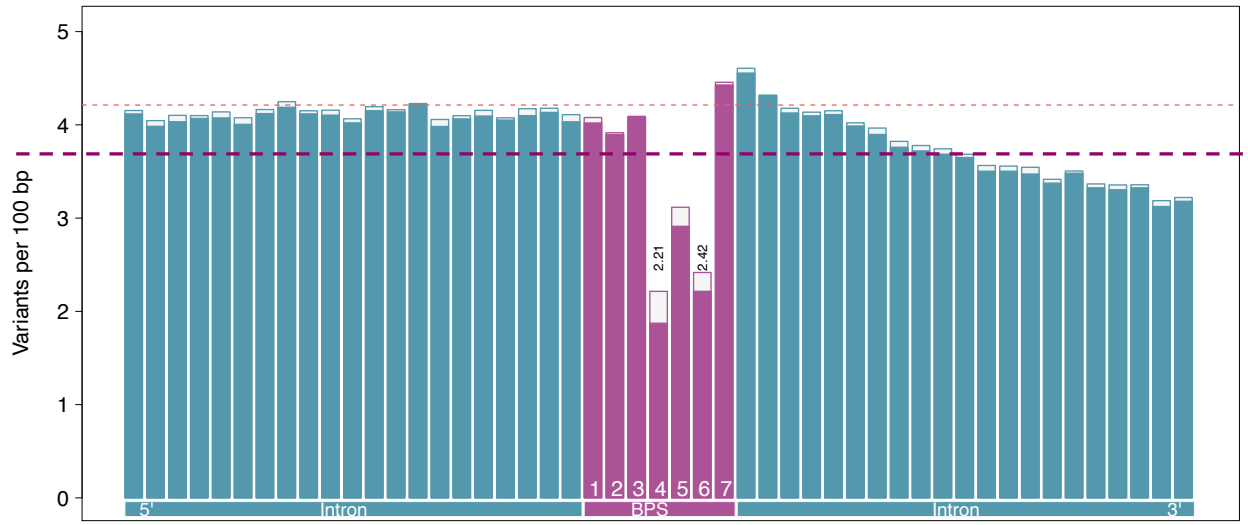


Confirmation in Human functional features

- Variant catalogue of 115,640,370
  - 3942 human genomes (HGDP + 1KG; gnomAD v3.0)
  - 4.21 variants/ 100 bp
- BPS predicted for 201,832 introns → nnyTyAy
- Higher constraint on the Thymine at position 4





Genome-wide Exonic

nnyTyAy

Conclusions

- First description of Bovine BPS
  - Features very similar to other species

- Strong constraint → stronger than coding sequence
  - Possible false positives, true constraint might be even stronger

- Variants associated with alternative mRNA splicing

- Ignored as candidate casual variants
  - We provide annotations
  - Genotyping BPS variants in large cohorts with customized SNP chip

Acknowledgements

Thank you …

BovReg
Understanding cattle genomes

European Commission
Horizon 2020
European Union funding
for Research & Innovation

AgroVet Strickhof
Eine Kooperation in Bildung und Forschung:
strickhof ETH zürich University of Zurich

ETH zürich
University of Zurich
Functional Genomics Center Zurich

FNSNF
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