Conserved Introns Reveal Novel Transcripts in *Drosophila melanogaster*

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  - ...identify novel mRNA-like ncRNAs

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- Capped, polyadenylated, often (alternatively) spliced (just like protein-coding genes), but lack discernible open reading frames

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- Gene regulators: Evf-2, Xist, roX1, Tsix, XistAS, roX2, H19, mei, LPW, KvDMR1, DGCR5, CMPD

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$\longrightarrow$ functionally important ncRNA class

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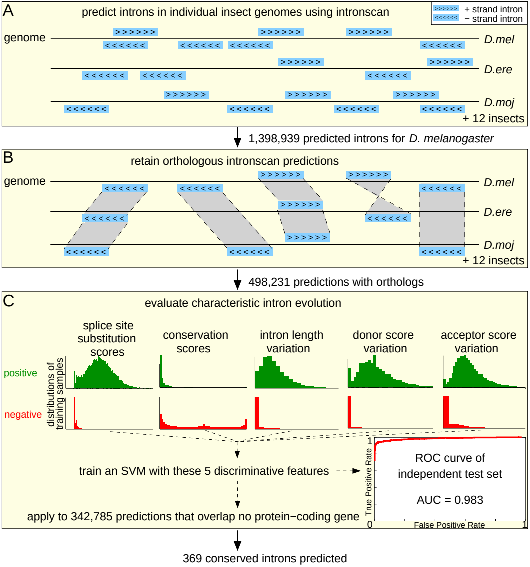
→ Find the intron → it guides you to your novel transcript

The data

12 drosophila genomes (fly)
+ *Anopheles gambiae* (mosquito)
+ *Tribolium castaneum* (beetle)
+ *Apis melifera* (honeybee)

The method



A predict introns in individual insect genomes using intronscan

1,398,939 predicted introns for *D. melanogaster*

B retain orthologous intronscan predictions

498,231 predictions with orthologs

C evaluate characteristic intron evolution

train an SVM with these 5 discriminative features

apply to 342,785 predictions that overlap no protein−coding gene

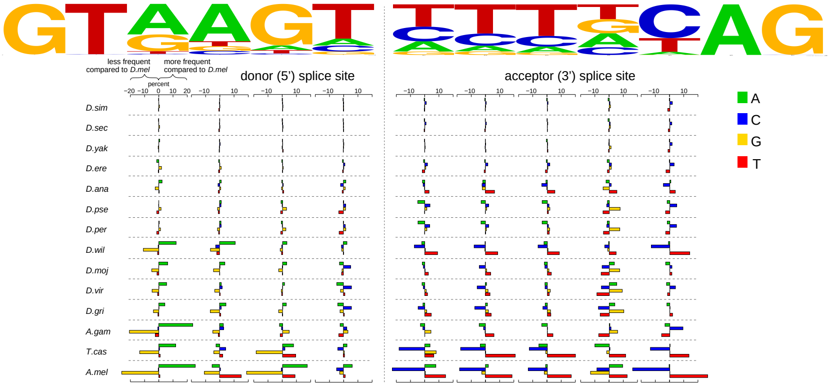
369 conserved introns predicted

intronscan

alignments

SVM

Nucleotide frequencies in SS positions differ



less frequent ← | → more frequent
(compared to Dmel)

e.g. Apis prefers A over G (donor +3) and T over C (accepor -3)

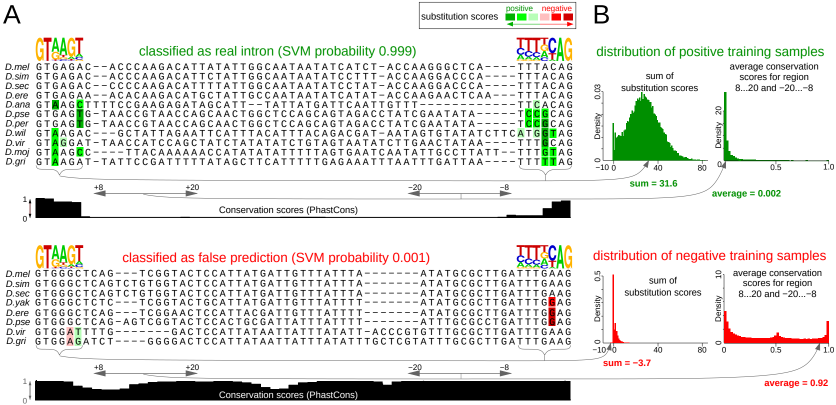
Learn log-odd substitution scores

$$log_2\left(\frac{freq_{\mathrm{pos}}(x,y)}{freq_{\mathrm{neg}}(x,y)}\right) \rightarrow \text{substitution matrix}$$

$$\forall x, y \in \{A, T, C, G\}$$

$$x \neq y$$

Evaluating intron evolution - an example



classified as real intron (SVM probability 0.999)

```
D.mel  GTGAGAC--ACCCAAGACATTATATTGGCAATAATATCATCT-ACCAAGGGCTCA----TTTACAG
D.sim  GTGAGAC--ACCCAAGACATTCTATTGGCAATAATATCCTTT-ACCAAGGACCCA----TTTACAG
D.sec  GTGAGAC--CCCCAAGACATTTTATTGGCAATAATATCCTAT-ACCAAGGACCCA----TTTACAG
D.ere  GTGAGAA--ACACAAGACATGCTATTGCCAATAATATCATAT-ACCAAGAACTCAA---TTTACAG
D.ana  GTAAGCTTTTCCGAAGAGATAGCATT--TATTATGATTCAATTGTTT------------TTCACAG
D.pse  GTGAGTG-TAACCGTAACCAGCAACTGGCTCCAGCAGTAGACCTATCGAATATA-----TCCGCAG
D.per  GTGAGTG-TAACCGTAACCAGCAACTGGCTCCAGCAGTAGACCTATCGAATATA-----TCCGCAG
D.wil  GTAAGAC---GCTATTAGAATTCATTTACATTTACAGACGAT-AATAGTGTATATCTTCATGGTAG
D.vir  GTAGGAT-TAACCATCCAGCTATCTATATATCTGTAGTAATATCTTGAACTATAA----TTTGCAG
D.moj  GTAAGCC----TTACAAAAAACCATATATATTTTTAGTGAATCAATATTGCCTTATT--TTTGTAG
D.gri  GTAAGAT-TATTCCGATTTTTATAGCTTCATTTTTGAGAAATTTAATTTGATTAA----TTTTTAG
```

Conservation scores (PhastCons)

sum = 31.6

average = 0.002

classified as false prediction (SVM probability 0.001)

```
D.mel  GTGGGCTCAG---TCGGTACTCCATTATGATTGTTTATTTA-------ATATGCGCTTGATTTGAAG
D.sim  GTGGGCTCAGTCTGTGGTACTCCATTATGATTGTTTATTTA-------ATATGCGCTTGATTTGAAG
D.sec  GTGGGCTCAGTCTGTGGTACTCCATTATGATTGTTTATTTA-------ATATGCGCTTGATTTGAAG
D.yak  GTGGGCTCTC---TCGGTACTGCATTATGATTGTTTATTTT-------ATATGCGCTTGATTTGGAG
D.ere  GTGGGCTCAG---TCGGAACTCCATTACGATTGTTTATTTT-------ATATGCGCTTGATTTGGAG
D.pse  GTGGGCTCAG-AGTCGGTACTCCACTGCGATTATTTATTTT-------ATTTGCGCCTGATTTGGAG
D.vir  GTGGATTTTG-------GACTCCATTATAATTATTTATATT-ACCCGTGTTTGCGCTTGATTTGAAG
D.gri  GTGGAGATCT----GGGGACTCCATTATAATTATTTATATTTGCTCGTATTTGCGCTTGATTTGAAG
```

Conservation scores (PhastCons)

distribution of positive training samples

distribution of negative training samples

sum = −3.7

average = 0.92

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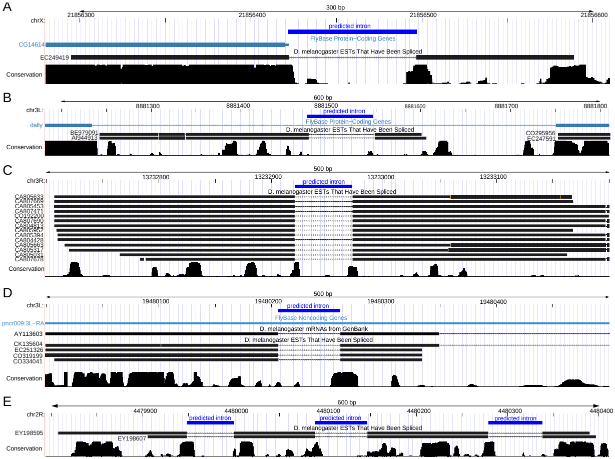
- p$>$0.99: 72% true positives at 0.07% false positives (4 FP, manual inspection: 3 are true introns → 1 FP)

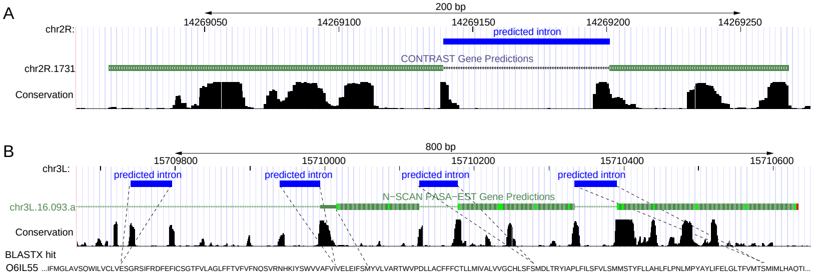
Novel spliced transcripts



369 predictions outside of protein-cod. genes ($p > 0.95$)

131 EST/FlyBase-transcript confirmed introns, 238 unconfirmed

Novel protein-coding genes



A) CONTRAST predicted coding gene, B) NSCAN coding gene

- 20/238 located within 100nt upstream of cod. genes
- 14/20 no annotated 5’UTR

  (in contrast to 77/218, Fischer’s exact test, p=0.005)
- 23 extend CDS, 30 belong to novel CDS

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- 129 *bona fide* mlncRNAs

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- Mostly unstructured (just 2 transcripts have RNAz hit)

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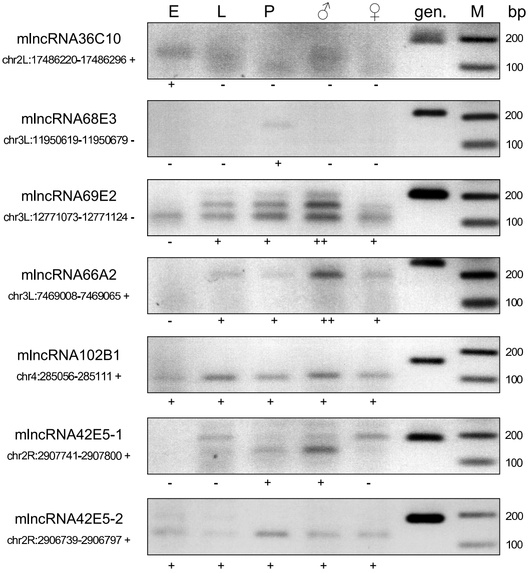
  mlncRNAs: 7/12
  introns in putative coding transcripts: 11/17

Experimental verification of mlncRNAs



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Limitations: Transcript start, transcript end?

Thank you

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