

Supplementary Information for "Conserved Introns Reveal Novel Transcripts in *Drosophila melanogaster*"

Table S2: Experimentally tested introns.

This table lists the loci and sequences of the tested introns, the used primers and the results.

The first column indicates the experimental result: pos = verified; neg = no transcript; neg* = RT-PCR amplified a unspliced or too short transcript. We tested expression in the following stages: E=embryo; L=larva; P=pupa; M=male; F=female. The expression signal is indicated by + = expressed; - = not expressed; 0 = negative for all stages. As the expression intensity for one intron should be roughly comparable between different stages (same experimental condition, same primers), we indicate variation as + and ++. Note that the expression intensity is not comparable between different introns.

result	locus	fwd primer	rev primer	E	L	P	M	F
introns in putative mlncRNAs								
pos	chr2L:17486220-17486296:+	aatatggactgtgtgagctg	ttgtcgtataatcgcccaag	+	-	-	-	-
GTAATGAATTTGTAATGCTATCAAAACAGGGCTTTTAAATCATATTACTATTTTATTTTCATTTTGTGTTGTAAAG								
pos	chr3L:11950619-11950679:-	atTTTTTTTgcagtcgggc	tagagtggctcggttactatgga	-	-	+	-	-
GTAAATGTGCCGATCGGTTAACCAGTATAATGTCTAGGAAAATAATTTTACATTTCTAG								
pos	chr3L:12771073-12771124:-	gcctaagagatcaacagctggga	gctattggcttcaacaactgcga	+	+	+	++	+
GTGAGTGGGGCTTTCTAGCTTAATCCTTGTTTAAATGCGAATCATAAATAG								
pos	chr3L:7469008-7469065:+	acctagtgtcatgcttcattgc	aaagtggtgacgtcctctg	-	+	+	++	+
GTAGCTCCTTATAGGGTCTTCTGAATCAGTAATATGCATATTCTAAAATTATTTTCAG								
pos	chr4:285056-285111:+	tcgaacctccgatccacggaa	tgtcaggatcactatgatctcttcgtt	+	+	+	+	+
GTTTGTAATTTAATGTCTTTTATAATAATATTTTGATAAATAGTAAATTTTAAG								
pos	chr2R:2907741-2907800:+	tttttacctgctcggtgc	acgataccacagcaatcc	-	-	+	+	-
GTAAGTTTTTCAAATTTGGCTGCAAATGAACCAAACTAATCGATCATCTTATGATTAATAAG								
pos	chr2R:2906739-2906797:+	cacttgatcctgttcgacttcca	cctcagggttcgggtatagc	+	+	+	+	+
GTAGGTAAATATGTATGATTATTGCAATATTGATCGTTTATCTATTATTATAATTCAG								
neg	chr2R:10106353-10106417:-	cgaaagtcaggcactgcttcg	tcgcccggaagataatgccacc	0	0	0	0	0
GTGAGCACTTGAAGAGTGCTAATTGATTAAGGCAAAAGCAAAAGCAAGTGAACCGCGATTTGTAG								
neg	chr3R:5977483-5977539:-	tgttcagtgctgtgttgca	ggaattgaactgtacgacccgtg	0	0	0	0	0
GTTAGTAAATACCATATATCAAGAGTTTCGGATTAAAAAGTATAGTTATCTCCAAG								
neg	chr2R:10510037-10510091:+	gaaattgcagactcaacct	tcacggatatcaaacgggt	0	0	0	0	0
GTAATTTAAACTTAAATTACTATATAACACTAAATCAGTTTAATTGATGATAAG								
neg	chrX:4142467-4142529:+	ccctgaatagagctcagtttgc	tgttcgtcacactcagagaggac	0	0	0	0	0
GTAAGTTATTTTCATATTTCCCATGAGCTTAAATGGAACTTAAACCAACATTTGAAATAG								
neg	chr2R:2911578-2911644:+	gtttgggtgtgtcgaaacca	cgtaaacacacagtcagtggtgca	0	0	0	0	0
GTAAGAATAAACACGTTTTTATCTTTAGACTTAACAAATCATTTAAATCAAATTTGTTGTTTCAG								
introns belonging to putative protein-coding genes								
pos	chr2R:19040486-19040544:+	caccttgacttggtgatgctcag	gatagcagcgtgctctgctgc	-	+	+	++	+
GTGAGAGATACAATAAAAACGCGGTGTATTTCCAAATGAAAACCCATTTTCCCGGTCAG								
pos	chr2L:17486655-17486729:+	tcgttacaaaacgctgacaacacc	tcggagtctggtcatcccca	+	-	-	-	-
GTAAGTTTCGTAGTGACTCAATATTAACCTCAATGCCCCATGTATTACCTATTTTACCTATTTTGCCACCAG								
pos	chr2L:13119936-13119995:+	gcatacgcaacaacacctgtc	ggtcatatcggtgactgtcagtc	-	-	+	++	+
GTAAGTTTTAAATCTCACATAACGCCAAATTGTAATTTATATATATATACATTTTTTAAG								
pos	chr2R:12268491-12268550:+	caatctggagggcagaatgg	ttccgatcgattgagtggtgca	+	++	-	-	-
GTATTTTTCATATGGCTTTTCGCAAAGAATTACTCTGTTTATAGCATCTGTTTATTAG								
pos	chr2R:7751812-7751876:+	ctgcattaaattgcgagagcctg	cacttacgattaaagctccgccca	-	+	-	-	-
GTAAGTTTGGGGCTGGCAGATCCATTTAAATGATCACCCCTGGAATGCATCCACCTTCATCTAG								
pos	chr2R:7732172-7732229:+	atcaggtgtctctgaagcggta	ccggtaggttcttgagtgcga	-	+	+	+	-
GTTAACCCTGAAGCAAAATTAATTGAGGAAATATCTTAAGAATCCTGTGATTTTGCAG								
pos	chrX:7223271-7223350:+	ctgctacttcgcccgttgcca	gcatagccttttgaacgtgggca	+	+	++	++	+
GTAATATATATAGGTAGATATATATATATATAATATTTATGTAATTGCAATTCCACAATTTCTATTTAATGTTTGCAG								
pos	chr2R:20502310-20502367:-	ctgtgggtcatcttgcgggtc	cgcaacgacatcgagtacga	-	+	+	+	+
GTGAGTATCTCTCAGAGCCGTCTGTTTATCCCGCACTGCCAGTGTCTCTTCCAG								

result	locus	fwd primer	rev primer	E	L	P	M	F
pos	chr3L:5122667-5122722:+	ccgagcattaacaatgtggcctc	aaagtgtcaagccgcagtc	-	-	+	+	+
GTGAGTTATTTAAATAAACCATTAATTTTAATACATAAAGTACTACTTCTTAAGCAG								
pos	chr3L:4473080-4473137:+	gcttacagtcactggcactttgac	atgatgccgctgggtgcttg	-	++	+	-	-
GTAACGGATCAACGATAGGTTCTAGGTTCCGCATTTACCAATCCATTTTATTTTAG								
pos	chr2L:19966361-19966419:-	gtttcaaatcatggccagcagac	gagtgatgatgccgctgggtg	-	-	-	++	++
GTAAGATCCTTAGATTTGCTTATATGCTTTTGAATCAACAGAAACCTTTAACTTTCAG								
neg*	chr2R:14499932-14499987:-	ggcattgtccaagccctagc	ggacaagagcatggcccagc	+	+	+	+	+
GTAAGTGAATACTTTTATCCTTAAATTTGTAAAGATATCCAATCGCTGTTCTACAG								
neg*	chr3L:13129956-13130018:+	cttttgaggccatatgcgagg	gaacaatcccttcgtgatcca	-	-	+	+	-
GTGAATCATCTAATAATTCAATTTGTATTTTGAATATTTAAAGTTCATTTTTTATTCAG								
neg*	chrX:16207852-16207912:+	caccacttgaaatgcccca	tcaattcccatcgtgctcc	++	++	++	++	++
GTATGAAAACGCCGATATCAGCTATGGAATACTATTCTTATCTATATATTTTCTATTTCAG								
neg*	chr3R:18595958-18596014:-	aagtcagtgccagacagttc	tgatggggttttcgatgcca	++	++	++	+	+
GTAGGTTTCCAAGTAACTCGGATTGTGAAAAAGTGGTAATAAATTTATTCCATATAG								
neg	chr2R:14119561-14119613:+	tacttagcacttcgagagctg	ggaatatcttgccagccaaatgg	0	0	0	0	0
GTGTTTGTATTCTTTACAACCTTGGGACGTTTTTAATTGATTGATTCTAG								
neg	chr3L:19947195-19947245:-	cactgtgatacaaatgtctccga	ttcgaaccataagtgtgaagc	0	0	0	0	0
GTATGTATATAATATAATTAAAGATGGTTTTTACAAAATATATGTATTTAG								

Table 2:

Table S3: Experimentally tested orthologous introns.

This table lists the loci, the used primers and the results for experimentally tested orthologous introns. The given sequences are the spliced partial transcript (not the predicted intron). The first column gives the corresponding *D. melanogaster* mlncRNA. We tested expression in the following stages: L=larva; P=pupa; M=male; F=female. The expression signal is indicated by + = expressed; - = not expressed; 0 = negative for all tested stages. As the expression intensity for one intron should be roughly comparable between different stages (same experimental condition, same primers), we indicate variation as + and ++. Note that the expression intensity is not comparable between different introns.

n.o. = no ortholog, n.t. = not tested, n.d. = not detected in any tested stage.

mlncRNA	species	location	fwd primer	rev primer	E	L	P	M	F
36C10	<i>D. simulans</i>	chr2L:17190719-17190786:+	ACAACCAACAACATGGACTG	AATAATCGCCAAAGTACTCCTC	n.t.	+	-	+	+
	TACAACCACAACATGGACTGCTGTGAGCTGCCGGAATATTTTTGAGCTTCCTCAACGACCTCTTCTTCTCGGACATAACTGATGAGGAGTACTTTGGCGATTATTA								
	<i>D. erecta</i>	scaffold_4845:6762561-6762620:-	AATATGGACTTCTGTGAACTGC	AATAATCGCCAAAGTACTCCTC	n.t.	+	-	+	-
	TATGGACTTCTGTGAACTGCCGGAATATTTTTGGGCTTCCTCAACGACCTCTTCTTCTCAGACATAACCGATGAGGAGTACTTTGGCGATTA								
	<i>D. pseudoobscura</i>	chr4_group3:10952381-10952448:-	CAAAATCCCGCAAGTATCCAG	AATCACCAAAGTAATCCTCGTC	n.t.	+	+	+	-
	CAAAATCCCGCAAGTATCCAGATTATATCATACACCATGGATTGCTGTGAGCTTATTGAAATATTTCTGGCTTTCTCCTCAACGATTATTCTTCTCCGATATAACGGACGAGGATTACTTTGGG								
68E3	<i>D. simulans</i>	chr3L:11319476-11319536:-	TTTTTTTTGCAGTGCGGCAAGGATCTGTTCTTATCTACGTAGGATTGTTGGCTTTCATTGCAATTGTGCGATTGCCAGGAAAACAATAATCTCGTGTCCATACTAACCGA	TCGGTTAGTATGGACACGAG	n.t.	-	+	-	-
	<i>D. erecta</i>	scaffold_4784:11940187-11940247:-	CAGAACAAATTCGGAGGACAC	CAGAAGGCACATAATCCTGAAC	n.t.	-	+	-	-
	CAGAAGGCACATAATCCTGAACCTTAGGGCATTGACAAATAATTTTTTTTTGCAGTGCGGCAAGGATCTGCTGTTATCTGCCTAGGATTGTTGGCTTTCATTGCAGTTGTTGACTGCCAAGCAATCAATAATCTCGCGCCACACACTAACCGAACACTCTATACAATGCCACCGTTTCGACGATCCCTACCACAATGTGATGTTGCCATATCCCAACGACTGTCGCAAGTATTACGTATGCCAAAAAGGACGCGCCTTCGAGTACCAGTGTCTCCGAATTTGTTCTG								
	<i>D. pseudoobscura</i>	chrXR_group6:1941812-1941865:-	TGGAGGTGAGGCATATAATGAC	TGGAATGGCAGTAACACAAC	n.t.	-	+	-	-
	TGGAGGTGAGGCATATAATGACGTGGCCATCAATGCTGACATGTCTATTGGGATGGGACAATGCCACCCACGATGATCCCGGCCGCGTTGTGTACTGCCATTCCA								
69E2	<i>D. simulans</i>	chr3L:12157582-12157632:-	ACAGCCCTTAGATGACGATG	GCCTTGAAACAAC TGCGAAC	n.t.	+	+	++	+
	ACAGCCCTTAGATGACGATGGTATATGCATGAAGAATCTATTTGGTTTCGAGTTGTTTCAAGGCA								
	ACAGCCCTTAGATGACGATGGTATATGGTTTGAATGCAATGTGCCCTTTCGAGTAAATATTCCCTTTCCTATTAGCATGAAGAATCTATTTGGTTTCGAGTTGT TCAAGGCA								

mlncRNA	species	location	fwd primer	rev primer	E	L	P	M	F
69E2	<i>D. erecta</i>	scaffold_4784:12778685-12778735:-	TGAACAGCCCCCTTGATGAC	GCCTGGAAATAGCTGGGAAC	n.t.	+	+	++	-
	TTGAACAGCCCCCTTGATGGCGGATGGCATATGCATAAAGAATCTATTTGGTTCACAGCTATTTCCAGGC								
	TTGAACAGCCCCCTTGATGACAATGGCATATGGTTTGAATGCAATGTGCCCTTTTCGAGTAAATATTCCTTTTCCTATTAGCAGAAGAATCTATTGGTTCACAGCTATTTCCAGGC								
	<i>D. pseudoobscura</i>	chrXR_group6:11702713-11702778:+	ACGACAATCAAGTCAAATCCCCTCTAAAAACGACCTGCAAAGTTGGCGGTGGACAGTATTAATACTGATGGTGTGGTTCGAGTGCAATGTCA	GACATTGCACTCGAACCAAAC	n.t.	+	+	+	-
	ACGACAATCAAGTCAAATCCCCTCTAAAAACGACCTGCAAAGTTGGCGGTGGACAGTATTAATACTGATGGTGTGGTTCGAGTGCAATGTCA								
66A2	<i>D. simulans</i>	chr3L:6988970-6989027:+	GCACTTCTTTCTCTGGCATTG	ACCGATAAGCCCGTGTGAG	n.t.	-	-	+	-
	TGCACTTCTTTCTCTGGCATTGGAATTTATGGAACGATTATGAATACAATCTTCATAATTGCTGATGTTTCGTGTTGATAACATTCTGCTTATTCAAGA TAGTCATGTGGATAGTGTGTAAGAATCTGTGCGCAATGTCAGCGGAGGACGTCACCCACGTATGGTTCCAGCTCAACACGGGGCTTATCG								
	<i>D. erecta</i>	scaffold_4784:3866115-3866172:-	ATGCGAAAGACGAACACC	TGGGGACAGATTCTTACACAC	n.t.	-	+	+	-
	ATGCGAAAGACGAACACCATGAGATTGCCGCCAATTAACCTAATAGCCTGCTTCATTGCATTATAACATGATATTGGAATTTATGGAACGATTATGAAA ACACTCTTTGTAATTAGAATGCAGATGTTTCATACTGATATCATTCTGCTTAGTTAAGATAGTCATGTGGATAGTGTGTAAGAATCTGTCCCCA								
	<i>D. pseudoobscura</i>	chrXR_group6:1469444-1469507:+	CTTCGTCTTGAACCTCCTTCC	TCCAGAGCGACATCTTTCC	n.t.	-	-	+	-
CTTCGTCTTGAACCTCCTTCCCTATAGCCATCCTCTGTGCGGGCCTATATGCGGCGATACGCCAGAATCTCAAGCTTATGCCAAATGCTTATTTTCGCATTGGTAA GCTACTTCCTGGAAGATGTCGCTCTGGA									
102B1	<i>D. simulans</i>	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.
	<i>D. erecta</i>	scaffold_4512:1016083-1016139:-	TAGTGGACCCTCAACCAAGCAG	GGCTTTCCGCCAGATCACTATG	n.t.	+	+	+	+
	TAGTGGACCCTCAACCAAGCAGTTTATCAGATGTCGCTTTCGAACCTCCGATTCAAGAAATGCGGAATGACCATATCAATGATTTAAATAACTTTGATTTAAAA AATGGCTAGATAAGCAGGCGGGTCGTGGTATCGGCGGAAGGCC								
	<i>D. pseudoobscura</i>	Unknown_group_723:2863-2916:-	AATCATTGATGAGTCGTCAG	TGACTAACAGAGCCATACCC	n.t.	+	+	+	+
	AATCATTGATGAGTCGTCAGTAGTTCAAAACAGCGACGCAACAAGTTCTGCAGATGAGGTTTTCGAGTATCCGAGACAAACGAAAGGGTATGGCTCTGTTGG TCA								
42E5-1	<i>D. simulans</i>	chr2R:1708382-1708441:+	TTTTTACCTTGCCTGGTGC	CTGTGTAATTCGACGATACCC	n.t.	-	+	++	+
	TTTTTACCTTGCCTGGTGCCTGCTTCAAATATTATTCCTGGGAGAAATGTGAACCAAGTTAATTTTGAAAATATGTTATTTTGTGCGGCTTGGGTTCGGCGCCAGT GGATTGCTGTGGGTATCGTCGAATTTACACAG								
	<i>D. erecta</i>	n.d.	n.d.	n.d.	n.t.	0	0	0	0
	<i>D. pseudoobscura</i>	n.d.	n.d.	n.d.	n.t.	0	0	0	0
42E5-2	<i>D. simulans</i>	chr2R:1707301-1707356:+	TGCACTTCCAAGGAGAACAC	TTCGGGAATAGCTTGGCAG	n.t.	-	-	+	-
	TTCGACTTCCAAGGAGAACACTTGGGTACCAATGAAAAAGTTTCACTACTTTGGTATCGGGCTTATGTTGGTCTCCCTTCTAACAGTCGCAAGCTATTCCCG AA								
	<i>D. erecta</i>	n.d.	n.d.	n.d.	n.t.	0	0	0	0
	<i>D. pseudoobscura</i>	n.d.	n.d.	n.d.	n.t.	0	0	0	0

Table 3:

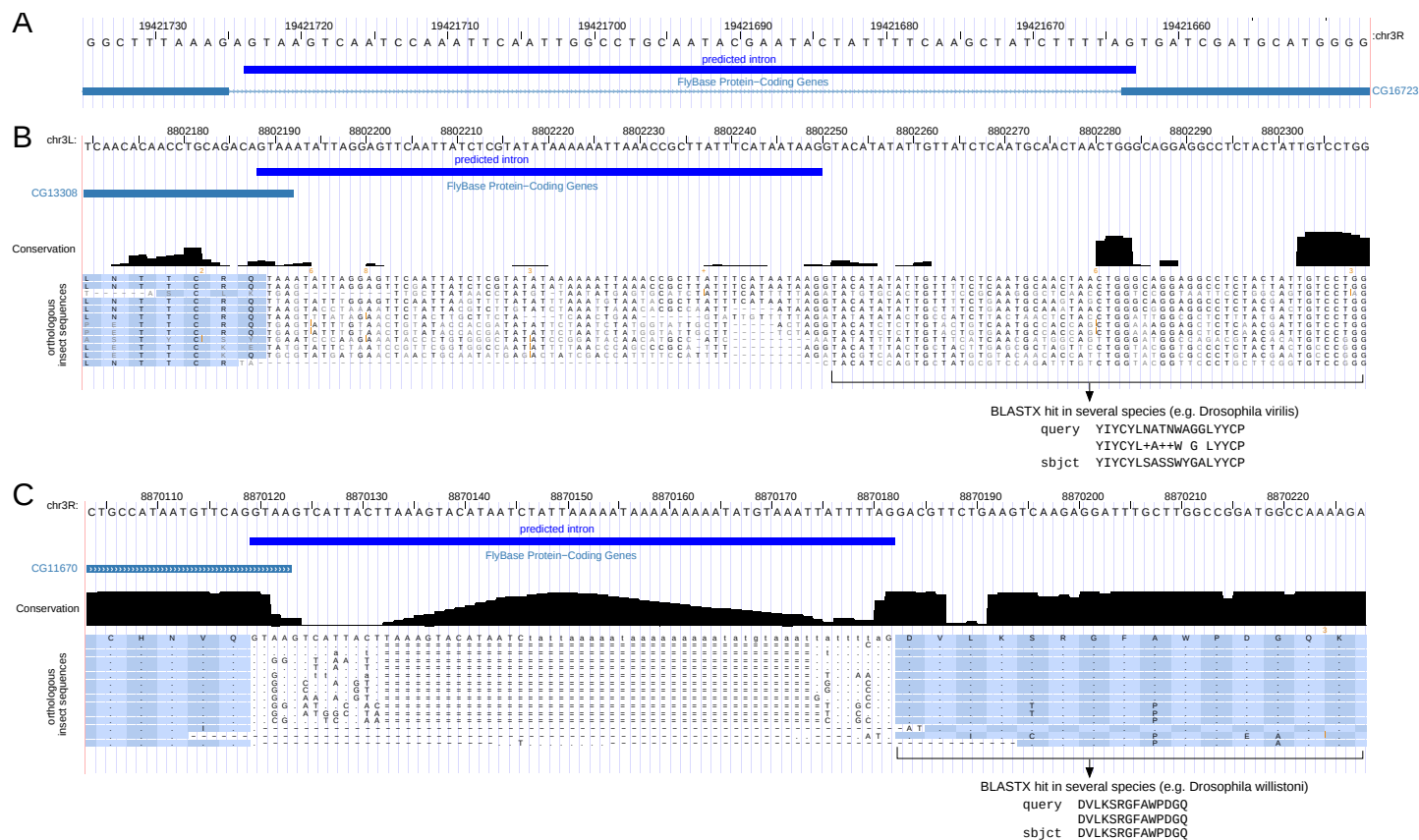


Fig. S 1. **Three predictions in our test set initially considered as false are likely to be real introns.**

(A) Potential misaligned intron: As shown in the UCSC genome browser screenshot, the RefSeq and FlyBase annotated intron is exactly shifted by one position upstream, leading to AG - TA intron boundaries. Our predicted intron (SVM probability 1.0) provides canonical GT - AG boundaries that are conserved and show characteristic splice site evolution. A single A/G SNP at the last position of the upstream exon would lead to a G in the transcript and a correct alignment.

(B) Potential missing part of the coding sequence: The upstream gene CG13308 has no annotated 3' UTR. A BLASTX run with the sequence downstream gives multiple hits in several *Drosophila* species, making it likely that this downstream part belongs to the gene with the incomplete 3' end. Our predicted intron (SVM probability 1.0) would bridge the upstream and downstream coding part without changing the reading frame.

(C) Potential missing part of the coding sequence: As in (B) the upstream gene (CG11670) has no annotated 3' UTR. The UCSC browser screenshot shows a conserved protein coding part downstream (that can also be found by a BLASTX run), which is not annotated in RefSeq or FlyBase. The upstream and downstream coding part is exactly split by our predicted intron (SVM probability 1.0).

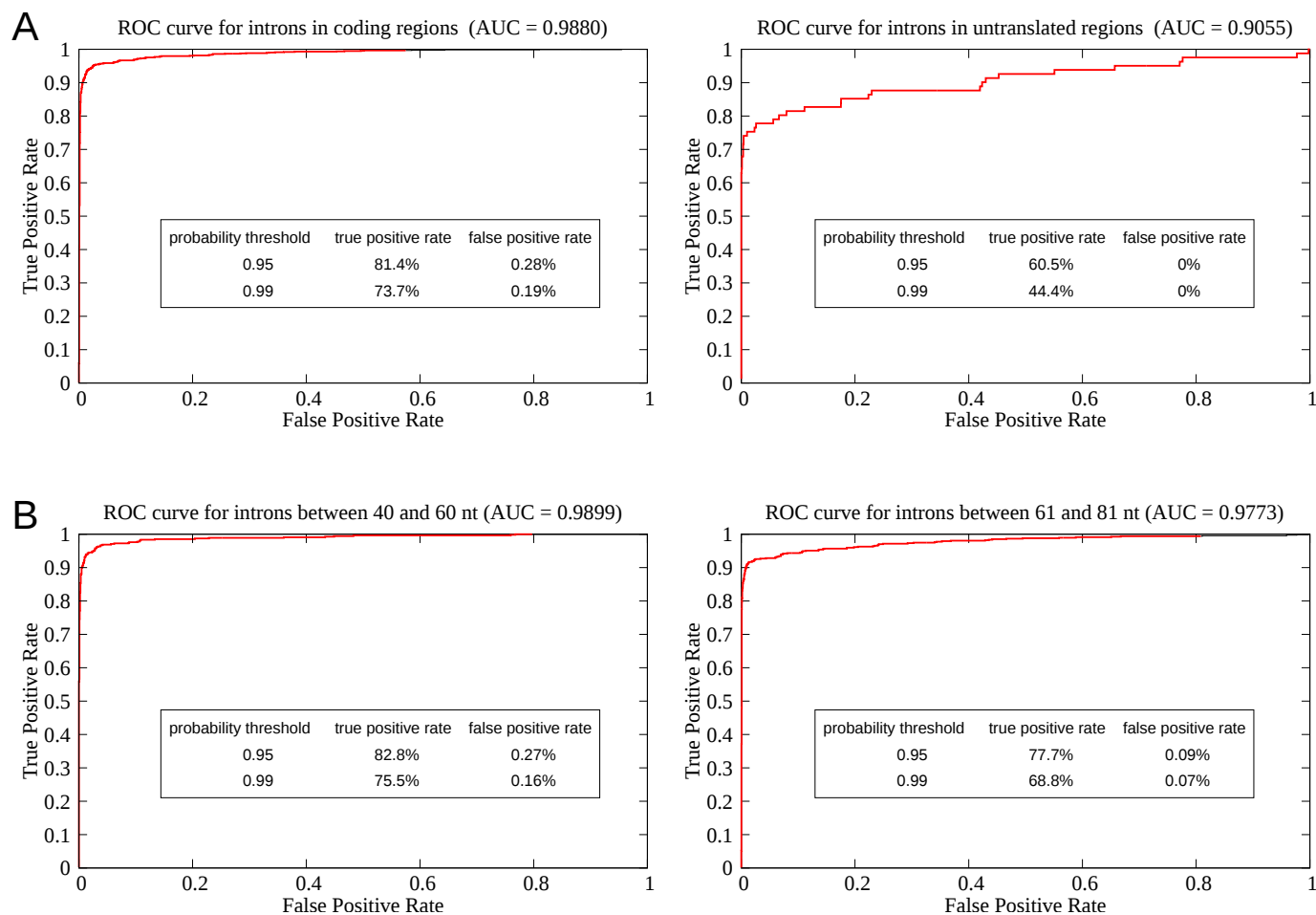


Fig. S 2. SVM performance for different intron classes.

We compared the performance of the SVM for introns that reside in UTR vs. CDS (A) and introns that are between 40 and 60 nt long vs. between 61 and 81 nt long (B). The performance for CDS introns is better than for UTR introns, as the true positive rates are lower for UTR introns. However, very low false positive rates indicate that a subset of UTR introns can still be predicted with high specificity. The performance for introns longer or shorter than 60 nt is similar, probably because our features are designed to be independent of the length. Longer introns have a lower true positive rate but also a slightly lower false positive rate.

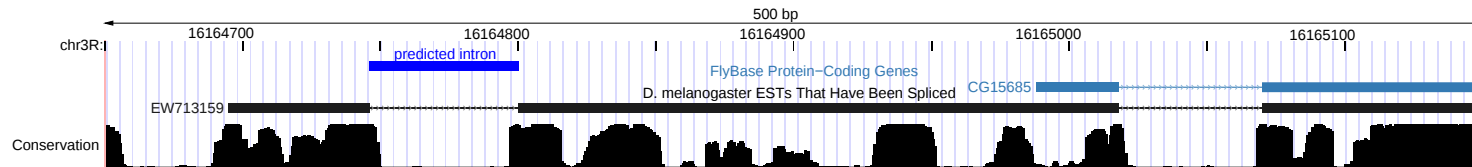


Fig. S 3. **Example of a confirmed intron prediction belonging to an incomplete CDS.**

A predicted intron (blue) is located upstream of the protein-coding gene CG15685, for which no 5'UTR is annotated. CONTRAST and NSCAN predictions (not shown) suggest that this intron belongs to the incomplete CDS. Our prediction is confirmed by one EST (EW713159), recently sequenced in the MODENCODE project.

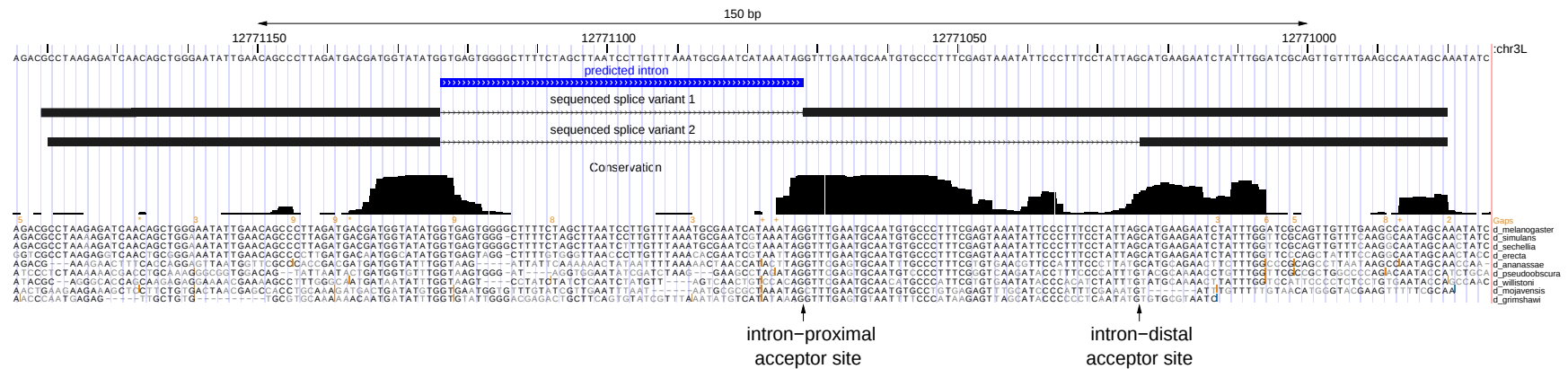


Fig. S 4. **MlnCrRNA69E2 is alternatively spliced.**

MlnCrRNA69E2 produces two transcripts differing by the use of the acceptor site, visible as two bands in Fig. 6. While the proximal acceptor that corresponds to the predicted intron is deeply conserved, the distal acceptor located 48 nt downstream is only conserved in the *melanogaster* subgroup. Conserved sequence parts that are excluded in the shorter splice variant might suggest that both transcript differ in their function.

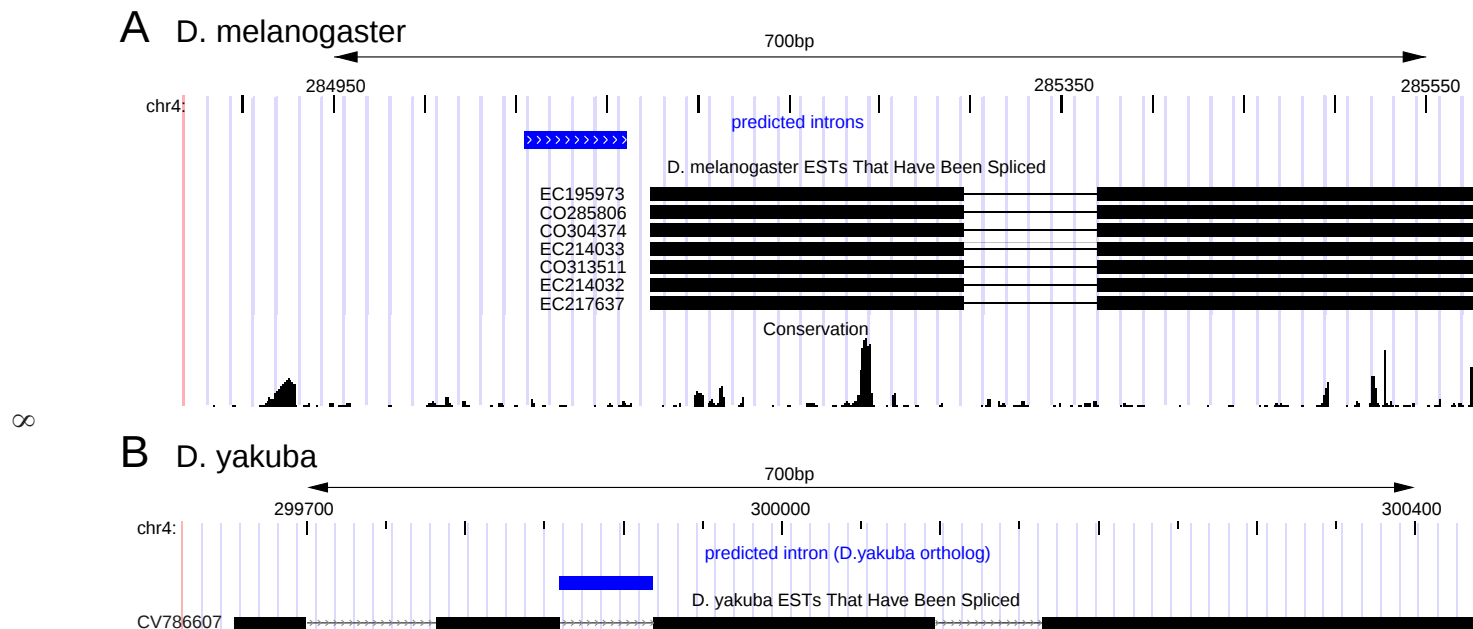


Fig. S 5. **Genomic location of intron mIncrNA102B1-1.**

(A) The experimentally verified intron mIncrNA102B1-1 in combination with spliced ESTs indicates the existence of a longer transcript in *D. melanogaster*.

(B) The orthologous intron in *D. yakuba* is confirmed by a *D. yakuba* EST.

A mlncRNA66A2

D.melanogaster	---CAACTTAAACCTAGTTGCATGCTTCATTGCACTTCTATCTCTGGCATTGGAATTTA	57
D.pseudoobscura	GGTGTGCTTCGTCTTGAACCTTCCTTATAGCCATCCTCTGTGCGG-----GCCTATA	54
	*** * * * * * * * * * * * * * * * * * * *	
D.melanogaster	TGGAACGATAATGAATACAATCTTCATAATTCGATTGCTGATGTTCTGTTGATTACATT	117
D.pseudoobscura	TGCGGCGATACGCCAGAATCTCAAGCTTATGCAAATGCTTATTTTCGCATTGGTAAGCTA	114
	** ***** * * * * * * * * * * * * * * * * *	
D.melanogaster	CTGCTTATTCAAGATAGTCATGTGGATAGTGATAAGAATCTGTCGCCAATGTCAGCAGA	177
D.pseudoobscura	CTTCCTGGGAAAGATGTCGCTCTGGATCGTTGCTGGGAACCAGGGAGGAGTCGTCGAGCT	174
	** * * ***** * * * * * * * * * * * * * *	
D.melanogaster	GGACGTCACCCA	189
D.pseudoobscura	GGTGGTCAC---	183
	** *****	

B mlncRNA69E2

D.melanogaster	CAATCCTAAACGTAGAGAAGAGAACGTAAAAAGACGCCTAAGAGATCAACAGCTGGG--AAT	58
D.pseudoobscura	CAAGCCGAAGCGTACGACAATCAAGTCAAATCCCTCTAAAAAACGACCTGCAAAGTTGGC	60
	*** ** * * * * * * * * * * * * * * * * * *	
D.melanogaster	ATTGAACAGCCCTTAGATGACGATGGTATATGGTTTGAATGCAATGTGCCCTTTTCGAGTA	118
D.pseudoobscura	GGTGGACAGTATT--AATACTGATGGTGTTTGGTTTCGAGTGCAATGTCCCCTTTTCGGGTC	118
	** ***** * * * * * * * * * * * * * * * * *	
D.melanogaster	AATATTCCTTTTCCTATTAGCATGAAGAATCTATTTGGATCGCAGTTGTTTGAAGCCAAT	178
D.pseudoobscura	AAGATACCTTTCCCCATTTGTACGCAAAACCTGTTTGGGAATTCGCTACCCGCTGGCCCC	178
	** ** * * * * * * * * * * * * * * * * * * *	

Fig. S 6. **Conserved expression of transcripts with low sequence conservation.**

A ClustalW alignment of the sequence up- and downstream of the conserved intron mlncRNA66A2 (A) and mlncRNA69E2 (B) shows little sequence similarity between *D. melanogaster* and *D. pseudoobscura*. Still, both introns belong to a transcript that is expressed in both species. The sequences do not contain the intron, thus represent the spliced transcript.

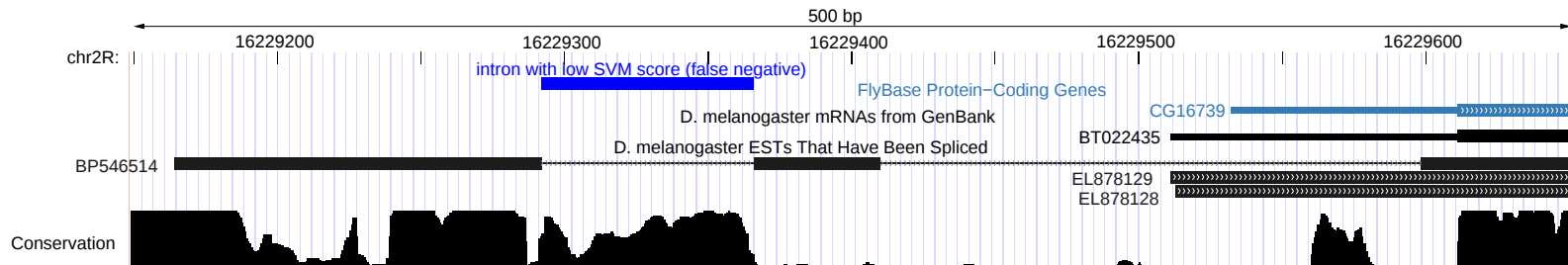


Fig. S 7. **Example of a false negative prediction.**

This intron gets a low SVM probability (0.003) for being a real intron, likely because the entire intron resides in a region of high sequence conservation (indicated by high PhastCons scores). The downstream gene CG16739 probably has two transcription start sites (downstream start supported by two ESTs and one mRNA, upstream start supported by one EST) and the transcript derived from the upstream start site contains our intron prediction (blue bar). It is tempting to speculate that the high intronic sequence conservation is due to overlap with promoter elements crucial for the downstream transcription start site. Despite the fact that the great majority of real introns has a low sequence conservation in the middle of the intron, some introns overlap regions having another function, leading to this unusual high conservation. Such cases are falsely classified by our approach.