

RNA Structures Regulating Alternative Splicing in Paralogs

Dmitri Pervouchine¹

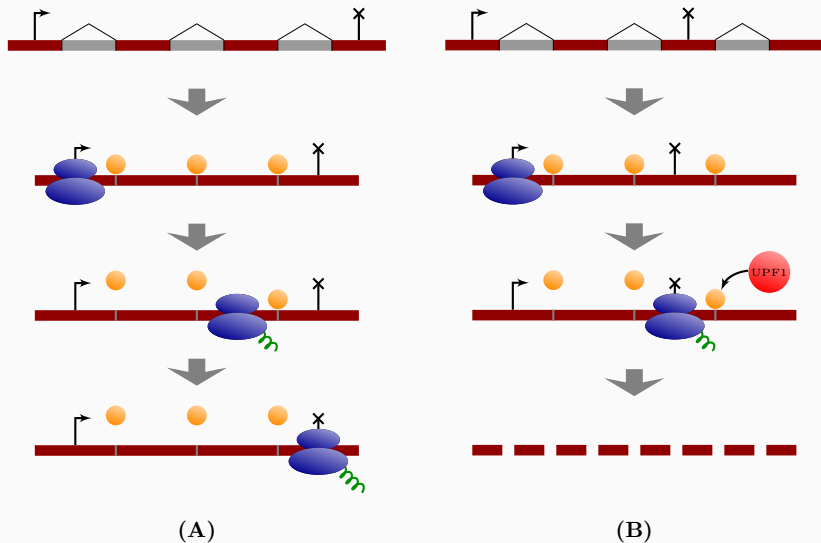
Benasque, July 9, 2026

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RNA structures regulating alternative splicing

Org.	Gene	AS event	IRBIS	PREPH	RIC-seq	Publ.
D. mel	CG33298	Alt. 5'ss	+	+		2009
	Gug	Alt. 3'ss	+	+		2009
	Nmnat	Alt. term. exon	+	+		2009
H. sap.	SF1	Intron retention	+	+		2012
	PHF20L1	Cassette exon	+	+	+	2023
	CASK	Cassette exon	+	+	+	2023
	ATE1	Mut. excl. exon	+	+	+/-	2021
	MARK2	Cassette exon	+	+	+	2023
	BRD2	Poison exon	+	+	+	2024
	BRD3	Poison exon	+	-	-	2024
	SCN9A	Mut. excl. exon	+	+	+	2026

Nonsense-mediated mRNA decay (NMD) pathway



NMD degrades mRNAs with premature termination codons (PTCs)

Poison and essential exons, unproductive splicing

- Poison exons generate PTCs when included



Poison and essential exons, unproductive splicing

- Poison exons generate PTCs when included



- Essential exons generates PTCs when skipped



Poison and essential exons, unproductive splicing

- Poison exons generate PTCs when included



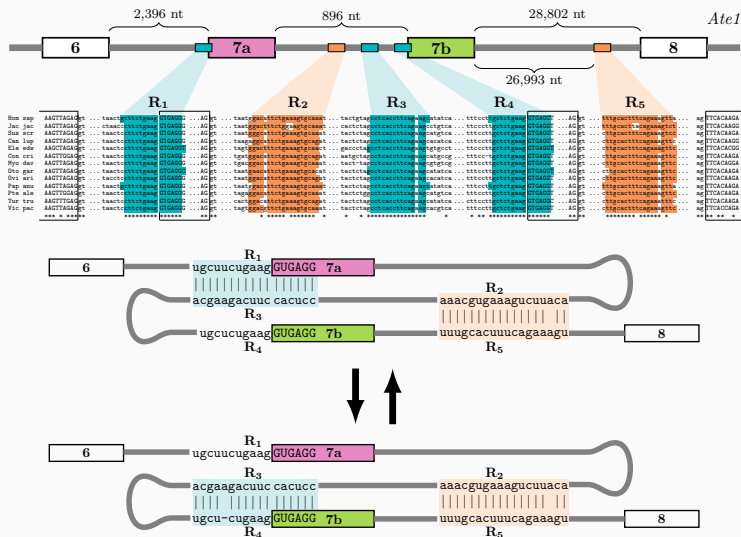
- Essential exons generate PTCs when skipped



- Pairs of mutually exclusive exons can generate PTCs

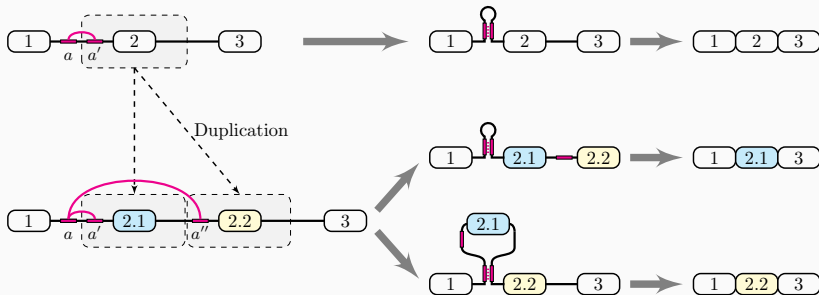


Competing RNA structures regulate mutually exclusive exons (MXE)



*Kalinina et al, Nucleic Acids Res. 49(1):479-490 (2021)

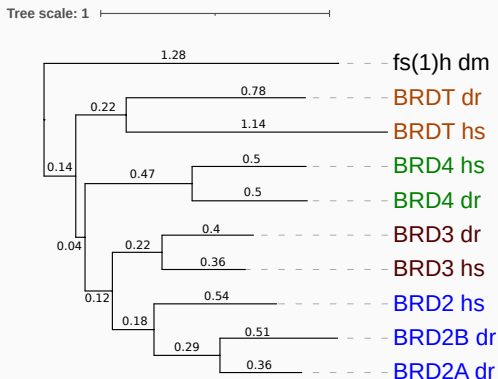
Tandem duplications generate competing RNA structures



*Ivanov & Pervouchine, Genes (Basel) 9(7):356 (2018).

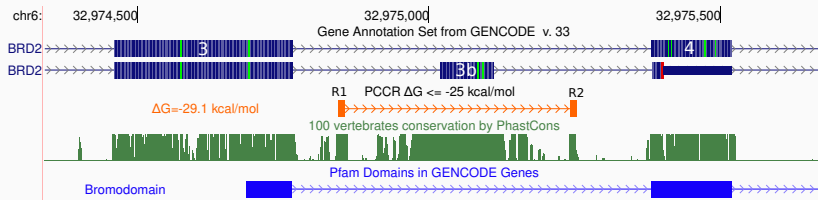
The Bromodomain and Extraterminal (BET) gene family

The BET gene family consists of four epigenetic readers regulating transcription widely implicated in cancer and inflammatory diseases

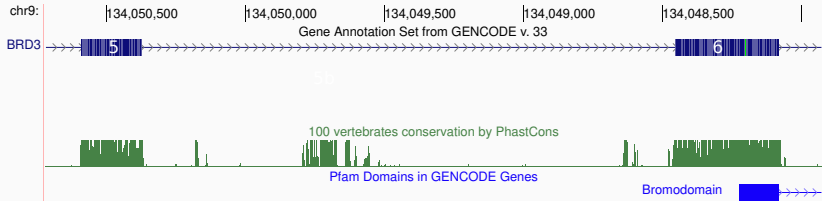
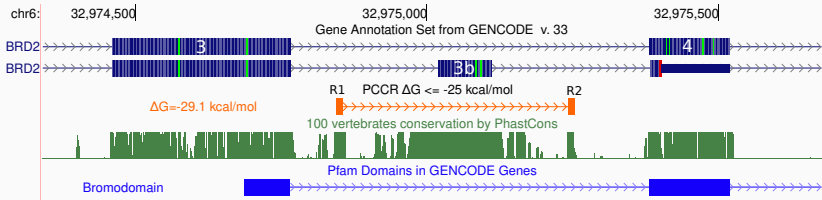


*Petrova et al NAR Genom Bioinform. 2024 Jan 15;6(1):lqad113

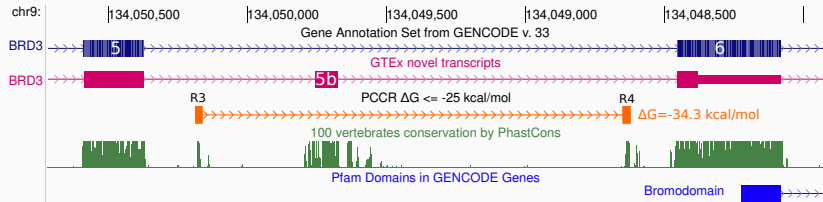
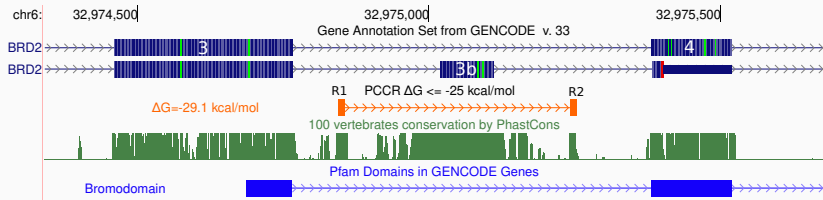
Poison exons in the BET gene family



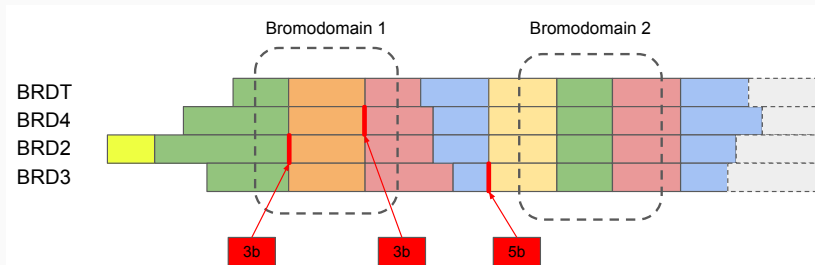
Poison exons in the BET gene family



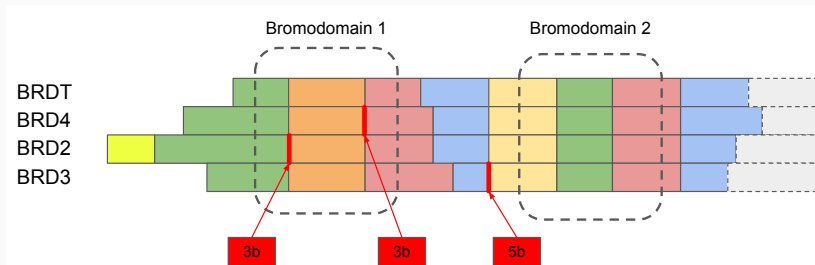
Poison exons in the BET gene family



Poison exons located in non-homologous introns

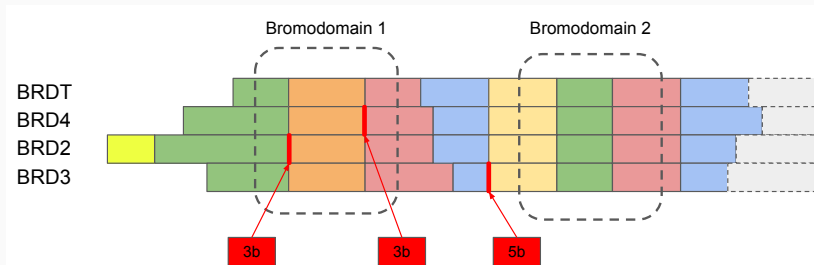


Poison exons located in non-homologous introns



Poison exons and regulatory RNA structures around them were acquired independently in convergent evolution.

Poison exons located in non-homologous introns

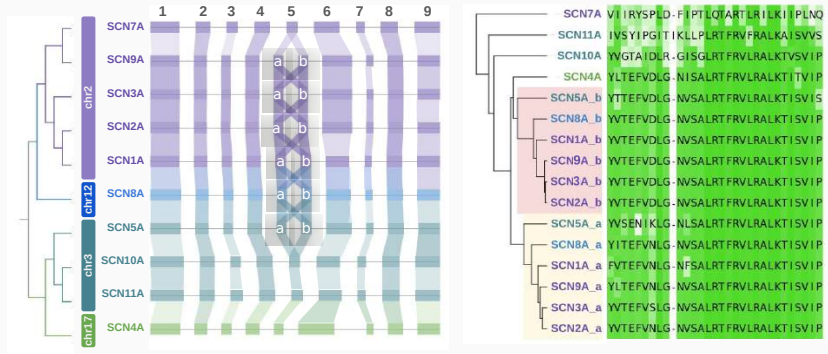


Poison exons and regulatory RNA structures around them were acquired independently in convergent evolution.

Possibly to enable tissue-specific regulation through RNAPII elongation rate

The SCN_{α} (Na^{+} voltage-gated channel α subunit) gene family

SCN_{α} genes encode the principal pore-forming proteins of voltage-gated sodium channels.

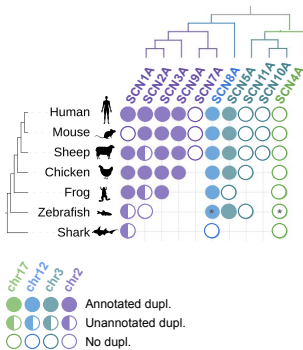


Exon 5 duplication happened in the common ancestor of (some) SCN_{α} genes, followed by gene duplication

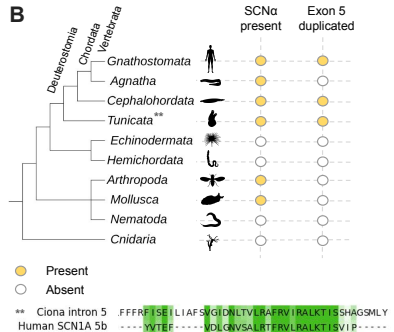
*Chernyavskaya et al, RNA 18;32(6):827-842 (2026).

Exon 5 duplication was present in Deuterostomia

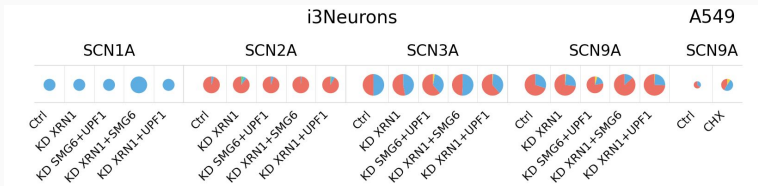
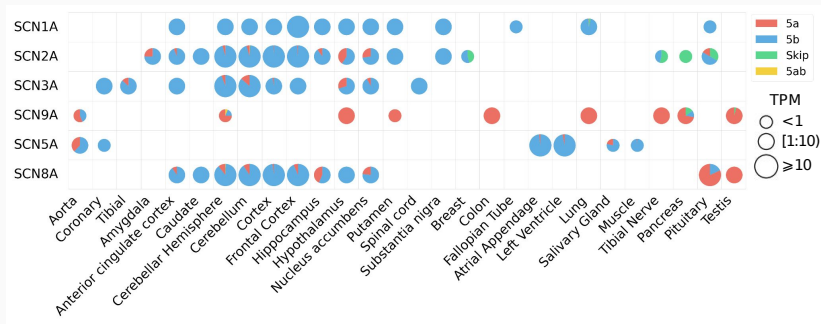
A



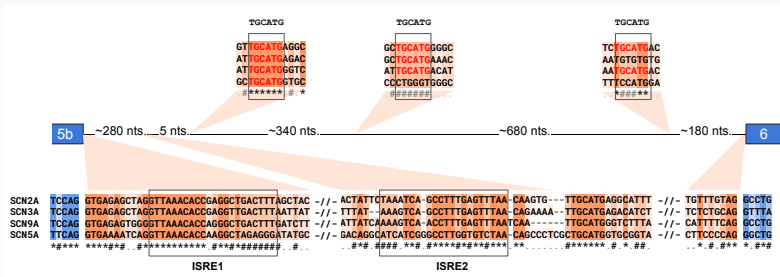
B



Exons 5a and 5b are mutually exclusive not due to NMD

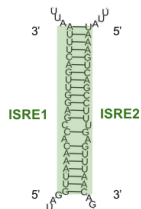
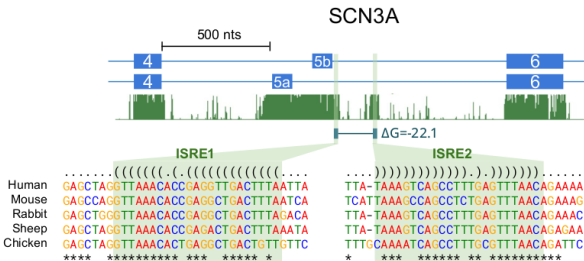
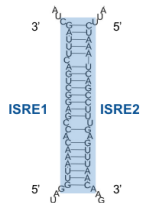
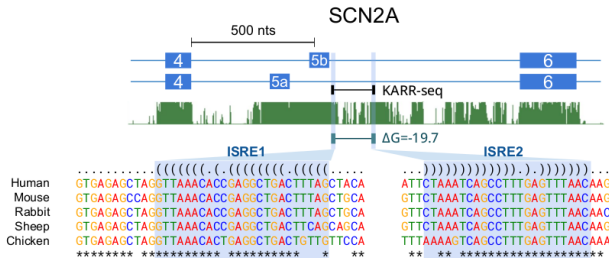


SCN α genes share intronic splicing regulatory elements (ISRE)

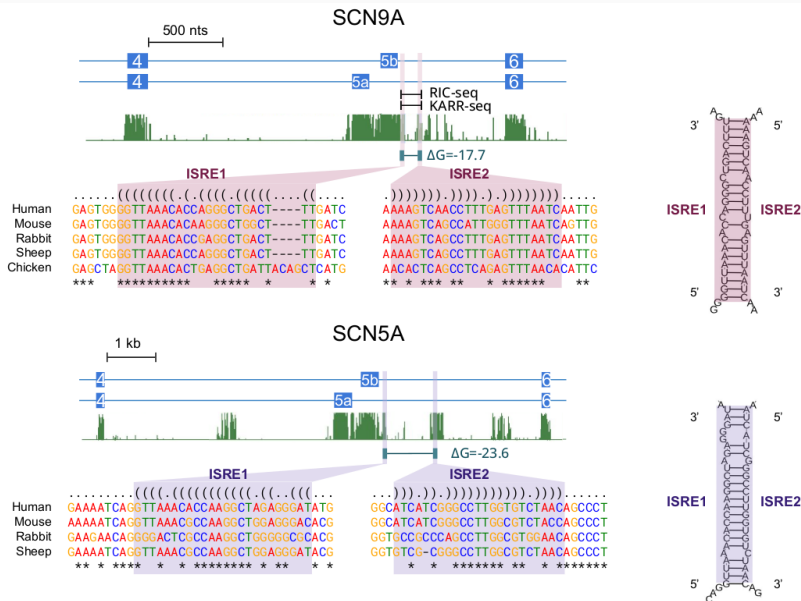


- ISRE1 and ISRE2 can form a long dsRNA helix
- Base pairing is supported by KARR-seq and RIC-seq
- TGCATG is a binding site of RBFOX2
- ISRE are very conserved in orthologs (no variation)
- ISRE are homologous between paralogs
- As ancient as the common ancestor!

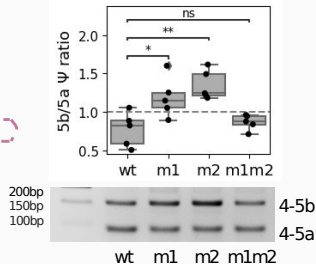
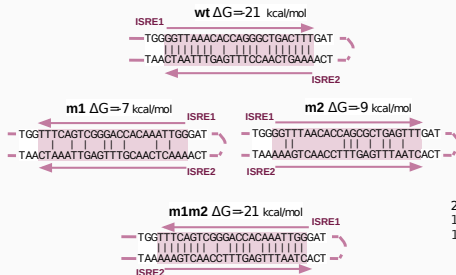
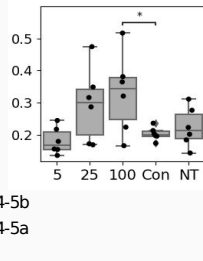
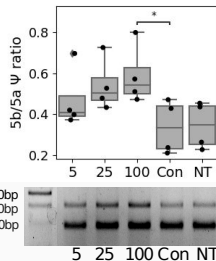
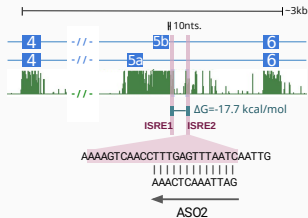
ISRE1 and ISRE2 are complementary



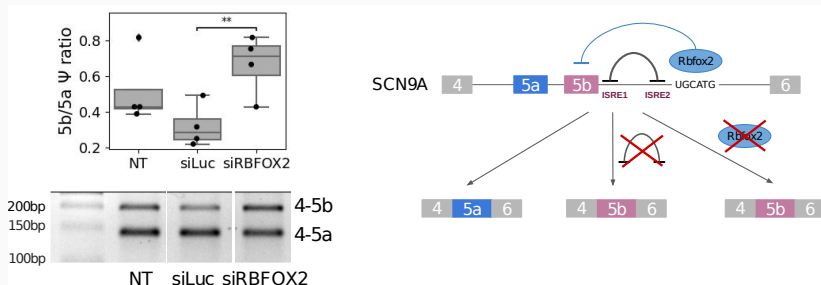
ISRE1 and ISRE2 are complementary



Disruption of RNA structure promotes exon 5b inclusion

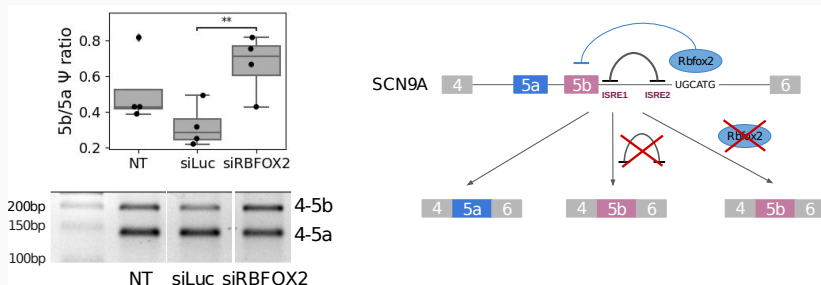


RBFOX2 is suppressing exon 5b through an RNA bridge



- RBFOX2 usually acts as splicing activator, not repressor

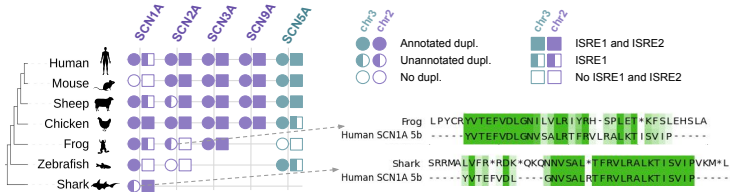
RBFOX2 is suppressing exon 5b through an RNA bridge



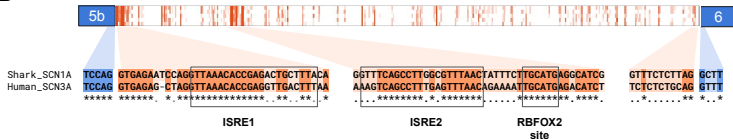
- RBFOX2 usually acts as splicing activator, not repressor
- The experiment was done in the *huh7* cell line, where the direction of regulation may be different

ISRE1 and ISRE2 date back to elephant shark

A



B



- In order to discover functional RNA structures through covariation, one needs variation
- Most known functional RNA structures in vertebrates lack sufficient level of sequence variation
- Comparing homologous non-coding sequences in paralogs (e.g., introns) helps discover functional regulatory elements including but not limited to RNA structures

Acknowledgments



Maria
Vlasenok



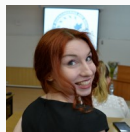
Dmitry
Skvortsov



Lev
Zavileiskiy



Rita
Vorobeva



Marina
Kalinina



Sergei
Margasyuk



Yulia
Uglova



Antonina
Kuznetsova



Ekaterina
Chernyavskaya



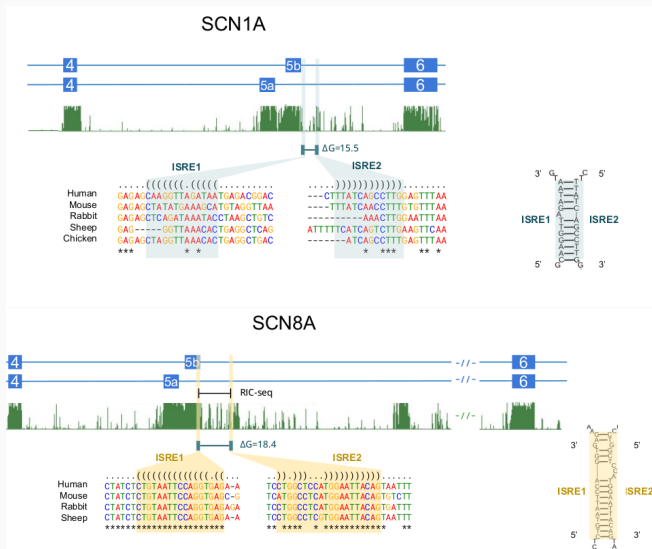
Olga
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RNA Structures in SCN5 α and SCN8 α



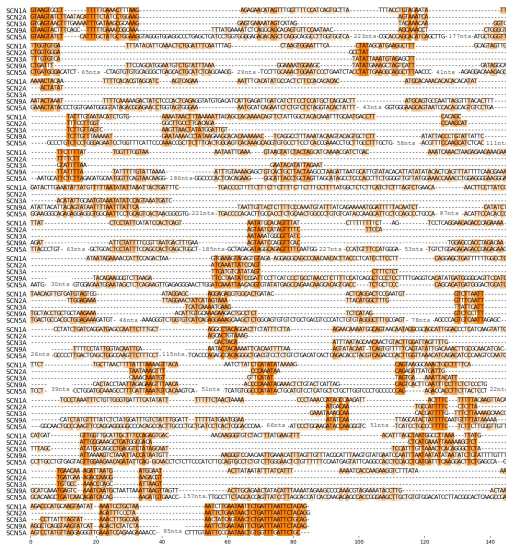
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SCN1A	ATGTTT	TAGCATTCTCTTT	AGCTT	TGAGTTAAGCA	TTCTG	TATGTCATATCTGATTTT	GGGTCGAGGAC	CTG
SCN2A	TTCTTTAAACATATCTAA	CTG	AGCTT	TGAGTTAAGCA	TTCTG	TATGTCATATCTGATTTT	ACAGGATCTACAGCT	CTG
SCN3A	ATTACAGTGATTTATAAATC	CTG	AGCTT	TGAGTTAAGCA	TTCTG	TATGTCATATCTGATTTT	AAATCTGATCGAGATCTTGAAA	CTG
SCN9A	ATTATACAAA	CTG	AGCTT	TGAGTTAAGCA	TTCTG	TATGTCATATCTGATTTT	ATGCTATCTTTGGGCTC	CTG
SCN5A	GTG-22nts	AGGCCATTTCTGGCAACAGCATCTCTGGACGGGATCATGGGCTGGTGTCTAGAGCTCTGATCTGCTGAGTGTAGGACATGATCGATGAGCT-27nts	TGGCATCT	CTG	AGCTT	TGAGTTAAGCA	TTCTG	CTG
SCN1A	CAGCT	ACAAATATGAGGTTTCTTTT	TTT	TTCAGCATCTGTTTATGGAAATTTGG	CTG	TTT	AAATGTGGTCTG	CTG
SCN2A	CATCT	TATATAGATGAGCTTTCTG	CTG	CATGAGAGCTACATGAG-AGAT	CTG	CTG	CAGCTGTA	CTG
SCN3A	GGCTC	CTCTCTCTCTTTTCA	CTG	CTACCATGGCTTTAC	CTG	CTG	ACAGCA	CTG
SCN9A	CATCT	CAATTTGATATCTCTG	CTG	CTGCTCTTGAGGTTTCTG	CTG	CTG	TTTTCATCTTTCTATACAG	CTG
SCN5A	CATCT	GGAAATCTAGGCCGATAGATCTCTTGAAGACATCTCATC-60nts	GTGCTGAGCTCTGCTGCTGAGAGAGATCTATCTCTAGTTTGTATTTCTCTCTCCAGATCTGAGGCT-33nts	GTAGCT	CTG	AGCTT	TGAGTTAAGCA	TTCTG
SCN1A	-TCTTGAT	TCTTTATGAGCTGATTATGATCATGTGATAGATCTCTTGATTTT	TGCTAT	TAATTAAGAG	CTG	TGAT	TAGTATGATATGAGATCTTGAGCT	CTG
SCN2A	-TCTGAT	TCTTTATGAGCTGATTATGATCATGTGATAGATCTCTTGATTTT	TGCTAT	TAATTAAGAG	CTG	TGAT	TAGTATGATATGAGATCTTGAGCT	CTG
SCN3A	-TCTTGAT	TCTTTATGAGCTGATTATGATCATGTGATAGATCTCTTGATTTT	TGCTAT	TAATTAAGAG	CTG	TGAT	TAGTATGATATGAGATCTTGAGCT	CTG
SCN9A	-TCTTGAT	TCTTTATGAGCTGATTATGATCATGTGATAGATCTCTTGATTTT	TGCTAT	TAATTAAGAG	CTG	TGAT	TAGTATGATATGAGATCTTGAGCT	CTG
SCN5A	AGCTTGTGATC-TTGACAGCTGATGATCTCTGAGACAGCACTGATCTGACCATCCGACCGAGAGG	CTG	TGCTAT	TAATTAAGAG	CTG	TGAT	TAGTATGATATGAGATCTTGAGCT	CTG

[illegible]

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 SCN1B TTCTCA TGAATATG TTTATCTATGCAACACATTAATCATATCTTGCAATG TTTGCAAGGATCATTTTAT TTTGCAAGGATCATTTG
 SCN3A AGATG TTTGCAAGGATCATTTTAT TTTGCAAGGATCATTTTAT TTTGCAAGGATCATTTTAT TTTGCAAGGATCATTTTAT
 SCN9A TTTT TTTTGAATGAGTCTG TTAGCTGATCATTTGATATTA TGAATGCTG TTTGCAAGGATCATTTTAT TTTGCAAGGATCATTTTAT
 SCN5A CCTCAGAGGATGCTCTATTCTCTCCATCTCAGATCAAGCTGAGGCTGCTCATTTGCTCCTCTCTCTCATGATGATGATTAAGG 52nta TGTGTGTCCTGAGAAAGACAGCTTTCAGACCATCCG

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