

Ejemplo comparación de resultados predictores in silico

Cambio de estudio UEVLD c.821-1G>C (chr 11:18544797 G/C, COSV57873687 o NM_001040697.4: c.821-1G>C)

Exón 8 e intrones adyacentes:

[illegible]

El cambio se encuentra en posición justo antes del exón 8 (la **g** en color naranja).

Se va a obtener los resultados que produce analizar esta variable con los diferente predictores y ver cuál de ellos es más preciso.

NetGene2

Donor splice sites, direct strand

pos	5'→3'	phase	strand	confidence	5'	exon	intron	3'
475		1	+	1.00	GAAGACAAAG	^	GTAAGAAGTA	H
482		2	+	0.90	AAGGTAAGAA	^	GTACGTTTTT	H

Donor splice sites, complement strand

pos	3'→5'	pos	5'→3'	phase	strand	confidence	5'	exon	intron	3'
282		493		1	-	0.47	TGCATACAAG	^	GTAATAAATG	

Acceptor splice sites, direct strand

pos	5'→3'	phase	strand	confidence	5'	intron	exon	3'
300		2	+	0.18	CAATATTTAG	^	TGGAAATCAT	
419		0	+	0.17	TGTTTTGAAG	^	GCACAGACTT	
425		0	+	0.18	GAAGGCACAG	^	ACTTCAGGCA	
432		1	+	0.18	CAGACTTCAG	^	GCAAAGAAGT	
438		1	+	0.07	TCAGGCAAAG	^	AAGTATGGGT	

Acceptor splice sites, complement strand

pos	3'→5'	pos	5'→3'	phase	strand	confidence	5'	intron	exon	3'
375		400		1	-	0.23	GTGAATCCAG	^	ATTACATCCA	

Donor splice sites, direct strand

pos	5'→3'	phase	strand	confidence	5'	exon	intron	3'
475		1	+	1.00	GAAGACAAAG	^	GTAAGAAGTA	H
482		2	+	0.90	AAGGTAAGAA	^	GTACGTTTTT	H

Donor splice sites, complement strand

pos	3'→5'	pos	5'→3'	phase	strand	confidence	5'	exon	intron	3'
282		493		1	-	0.46	TGCATACAAG	^	GTAATAAATG	

Acceptor splice sites, direct strand

pos	5'→3'	phase	strand	confidence	5'	intron	exon	3'
419		0	+	0.17	TGTTTTGAAG	^	GCACAGACTT	
425		0	+	0.18	GAAGGCACAG	^	ACTTCAGGCA	
432		1	+	0.18	CAGACTTCAG	^	GCAAAGAAGT	
438		1	+	0.07	TCAGGCAAAG	^	AAGTATGGGT	

Acceptor splice sites, complement strand

pos	3'→5'	pos	5'→3'	phase	strand	confidence	5'	intron	exon	3'
375		400		1	-	0.17	GTGAATCCAG	^	ATTACATCCA	

Se pierde un sitio *acceptor* (en rojo) en la secuencia mutante. Este sitio es el sitio *acceptor* para el exón 8, por lo que es muy probable que esté afectando al *splicing*, bien teniendo que utilizar el sitio *acceptor* del exón siguiente y produciéndose *skipping*, o bien se emplea el sitio *acceptor* siguiente (TGTTTTGAAG^GCACAGACTT), lo que llevaría a que se perdieran 119 nt del exón.

Splice Site Prediction by Neural Network (NNSplice)

Donor site predictions for 85.53.15.54.13512.0 :

Start	End	Score	Exon	Intron
468	482	1.00	gacaaag	gt aagaag

Donor site predictions for 85.53.15.54.13548.0 :

Start	End	Score	Exon	Intron
468	482	1.00	gacaaag	gt aagaag

Acceptor site predictions for 85.53.15.54.13512.0 :

Start	End	Score	Intron	Exon
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Acceptor site predictions for 85.53.15.54.13548.0 :


Start	End	Score	Intron	Exon
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No hay cambios entre ambas predicciones por lo que la mutación no alterará el *splicing*.

Spliceman

Point mutation	Wildtype (wt)	Mutation (mt)	L1 distance	Ranking (L1)
attta(g/c)tgga	gtggaa	ctggaa	26847	54%

Human Splicing Finder

 Broken WT Acceptor Site	Alteration of the WT Acceptor site, most probably affecting splicing		
Algorithm/Matix	position	sequences	variation
HSF Acceptor site (matrix AG)	chr11:18544808	- REF : TGCAATATTTAGTG - ALT : TGCAATATTTACTG	73.93 > 46.06 => -37.7%











SVM-BPfinder

seq_id	agez	ss_dist	bp_seq	bp_scr	y_cont	ppt_off	ppt_len	ppt_scr	svm_scr
wt	17	478	gcctcagtg		0.584713373252	0.450317124736	20	8	13
wt	17	469	cattgagga		-1.41158331758	0.450431034483	11	8	13
wt	17	461	acctaatat		0.676060530093	0.451754385965	3	8	13
wt	17	453	tacttatta		-2.07085582872	0.448660714286	14	7	11
wt	17	450	ttattaaaa		-3.80619613029	0.447191011236	11	7	11
wt	17	449	tattaaaa		-0.837525156699	0.448198198198	10	7	11
wt	17	444	aaataataa		-1.03280410638	0.451025056948	5	7	11
wt	17	441	taataaata		-1.27787709617	0.451834862385	2	7	11
wt	17	431	ttgtgagct		-0.223675079758	0.450704225352	18	31	53
wt	17	405	tttttaacc		-3.40490496754	0.4475 2	21	38	-0.55946855
wt	17	404	ttttaacct		1.5934919779	0.448621553885	1	21	38
wt	17	398	cccttacat		-1.94302375298	0.442748091603	1	15	27
wt	17	389	tttttacct		-1.4022471159	0.434895833333	56	10	16
wt	17	371	tatttagtg		-3.15274944295	0.426229508197	38	10	16
wt	17	361	aatcatgta		-1.90179999332	0.429775280899	28	10	16
wt	17	358	tcatgacct		1.44656785974	0.43059490085	25	10	16
wt	17	349	atgtaacat		0.810705042143	0.427325581395	16	10	16
wt	17	337	aactgagta		-0.212087509166	0.430722891566	4	10	16
wt	17	313	gagtgatcg		0.64422040507	0.425324675325	53	6	13
wt	17	297	atgtaatct		0.213449085422	0.428082191781	37	6	13
wt	17	287	gattcacag		0.180208682911	0.421985815603	27	6	13
wt	17	278	agattacag		-1.91409118524	0.424908424908	18	6	13
wt	17	268	atattatta		-4.03088613247	0.422053231939	8	6	13
wt	17	265	ttattacaa		-1.98121444058	0.419230769231	5	6	13
wt	17	253	ttttgaagg		-1.42059083227	0.415322580645	68	7	15
wt	17	239	accttcaggc		-2.49276363167	0.41452991453	54	7	15
wt	17	220	gggttattg		-2.84843019216	0.427906976744	35	7	15
wt	17	192	aggttaagaa		-1.949418832	0.459893048128	7	7	15
wt	17	178	tttttaggc		-4.39554662582	0.456647398844	16	11	14
wt	17	159	ggctcatgc		1.63283634736	0.461038961039	86	12	19
wt	17	150	ctgtaattc		0.608926339302	0.455172413793	77	12	19
wt	17	113	cgctgaagc		-0.839604118353	0.453703703704	40	12	19
wt	17	100	acttcaaga		-3.21441114553	0.452631578947	27	12	19
wt	17	52	agataaaaa		-2.65405085156	0.489361702128	47	0	0
wt	17	45	aaattagcc		-2.14552835715	0.525 40	0	0	-2.8006711

seq_id	agez	ss_dist	bp_seq	bp_scr	y_cont	ppt_off	ppt_len	ppt_scr	svm_scr
mut	17	478	gcctcagtg		0.584713373252	0.450317124736	20	8	13
mut	17	469	cattgagga		-1.41158331758	0.450431034483	11	8	13
mut	17	461	acctaatat		0.676060530093	0.451754385965	3	8	13
mut	17	453	tacttatta		-2.07085582872	0.448660714286	14	7	11
mut	17	450	ttattaaaa		-3.80619613029	0.447191011236	11	7	11
mut	17	449	tattaaaa		-0.837525156699	0.448198198198	10	7	11
mut	17	444	aaataataa		-1.03280410638	0.451025056948	5	7	11
mut	17	441	taataaata		-1.27787709617	0.451834862385	2	7	11
mut	17	431	ttgtgagct		-0.223675079758	0.450704225352	18	31	53
mut	17	405	tttttaacc		-3.40490496754	0.4475 2	21	38	-0.55946855
mut	17	404	ttttaacct		1.5934919779	0.448621553885	1	21	38
mut	17	398	cccttacat		-1.94302375298	0.442748091603	1	15	27
mut	17	389	tttttacct		-1.4022471159	0.434895833333	56	10	16
mut	17	371	tatttaatg		-3.20745702824	0.426229508197	38	10	16
mut	17	370	attttaatgg		-0.213283091637	0.427397260274	37	10	16
mut	17	361	aatcatgta		-1.90179999332	0.429775280899	28	10	16
mut	17	358	tcatgacct		1.44656785974	0.43059490085	25	10	16
mut	17	349	atgtaacat		0.810705042143	0.427325581395	16	10	16
mut	17	337	aactgagta		-0.212087509166	0.430722891566	4	10	16
mut	17	313	gagtgatcg		0.64422040507	0.425324675325	53	6	13
mut	17	297	atgtaatct		0.213449085422	0.428082191781	37	6	13
mut	17	287	gattcacag		0.180208682911	0.421985815603	27	6	13
mut	17	278	agattacag		-1.91409118524	0.424908424908	18	6	13
mut	17	268	atattatta		-4.03088613247	0.422053231939	8	6	13
mut	17	265	ttattacaa		-1.98121444058	0.419230769231	5	6	13
mut	17	253	ttttgaagg		-1.42059083227	0.415322580645	68	7	15
mut	17	239	accttcaggc		-2.49276363167	0.41452991453	54	7	15
mut	17	220	gggttattg		-2.84843019216	0.427906976744	35	7	15
mut	17	192	aggttaagaa		-1.949418832	0.459893048128	7	7	15
mut	17	178	tttttaggc		-4.39554662582	0.456647398844	16	11	14
mut	17	159	ggctcatgc		1.63283634736	0.461038961039	86	12	19
mut	17	150	ctgtaattc		0.608926339302	0.455172413793	77	12	19
mut	17	113	cgctgaagc		-0.839604118353	0.453703703704	40	12	19
mut	17	100	acttcaaga		-3.21441114553	0.452631578947	27	12	19
mut	17	52	agataaaaa		-2.65405085156	0.489361702128	47	0	0
mut	17	45	aaattagcc		-2.14552835715	0.525 40	0	0	-2.8006711

Entre las secuencias, la primera diferencia se da por la presencia del cambio (el resultado en 371 para ambas es el mismo cambiando la g por la a en la antepenúltima posición). El segundo cambio es que aparece un nuevo BP en la secuencia mutante (en 370). Sin embargo, este tiene una puntuación negativa, por lo que no se tendrá en cuenta.

Variant Effect Predictor tool

ENST00000320750.10:c.821-1G>C	11:18544797-18544797	G	 splice_acceptor_variant	UEVLD	ENSG00000151116	Transcript	ENST00000320750.10	protein_coding	-	-	COSV57873687
ENST00000320750.10:c.821-1G>C	11:18544797-18544797	G	 splice_acceptor_variant	UEVLD	ENSG00000151116	Transcript	ENST00000379387.8	protein_coding	-	-	COSV57873687
ENST00000320750.10:c.821-1G>C	11:18544797-18544797	G	 splice_acceptor_variant,  NMD_transcript_variant	UEVLD	ENSG00000151116	Transcript	ENST00000396196.6	nonsense_mediated_decay	-	-	COSV57873687
ENST00000320750.10:c.821-1G>C	11:18544797-18544797	G	 splice_acceptor_variant	UEVLD	ENSG00000151116	Transcript	ENST00000396197.8	protein_coding	-	-	COSV57873687
ENST00000320750.10:c.821-1G>C	11:18544797-18544797	G	 splice_acceptor_variant	UEVLD	ENSG00000151116	Transcript	ENST00000535484.5	protein_coding	-	-	COSV57873687
ENST00000320750.10:c.821-1G>C	11:18544797-18544797	G	 splice_acceptor_variant,  non_coding_transcript_variant	UEVLD	ENSG00000151116	Transcript	ENST00000540666.5	processed_transcript	-	-	COSV57873687
ENST00000320750.10:c.821-1G>C	11:18544797-18544797	G	 splice_acceptor_variant	UEVLD	ENSG00000151116	Transcript	ENST00000541984.5	protein_coding	-	-	COSV57873687
ENST00000320750.10:c.821-1G>C	11:18544797-18544797	G	 splice_acceptor_variant	UEVLD	ENSG00000151116	Transcript	ENST00000543987.5	protein_coding	-	-	COSV57873687

Se trata de una mutación que afecta al *splicing*, concretamente al *acceptor* del exón.

ESEfinder

Solo se obtiene un resultado positivo con la posición de interés en las matrices 5'SS:

271 (-504)	tacattttttacottgtatgcaatatttag	0.78320	271 (-504)	tacattttttacottgtatgcaatatttag	-16.40960	271 (-504)	tacattttttacottgtatgcaatatttag	0.32730	271 (-504)	tacattttttacottgtatgcaatatttag	-18.44020
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Sin embargo, estas puntuaciones en la secuencia mutante son prácticamente iguales:

271 (-504)	tacattttttacottgtatgcaatatttac	0.80520	271 (-504)	tacattttttacottgtatgcaatatttac	-16.44340	271 (-504)	tacattttttacottgtatgcaatatttac	0.34890	271 (-504)	tacattttttacottgtatgcaatatttac	-18.47080
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Por lo tanto, la mutación no afecta al *splicing*.