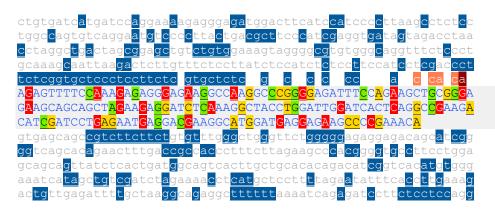
Ejemplo comparación de resultados predictores in sillico

Cambio de estudio CACNA1C c.1218-2A>G (chr12:2512810 A/G, COSV59770317 o NM_000719.7: c.1218-2A>G)

Exón 9 e intrones adyacentes:



El cambio se encuentra en la última posición del intrón 8 (la última **a** antes del exón en color granate).

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	t strand	d				_		_		
	pos 5'->3'	nhace	- strand	confidence	5' exon intron 3'	Donor splice s	sites, direct	t strand	d		
	105	0	+	0.70	TCCCATCGAG^GTGATAGTAG		pos 5'->3'	nhace	strand	confidence	5' exon intron 3'
	474	1	+	0.80	CCCGAAACA^GTGAGCAGCC		105	0	+	0.70	TCCCATCGAG^GTGATAGTAG
	., .	_		0.00			474	1	+	0.80	CCCCGAAACA^GTGAGCAGCC
Donor splice	sites, comple	ement st	trand								
						Donor splice s	sites, comple	ement si	trand		
pos 3'->5'	pos 5'->3'	phase	strand	confidence	5' exon intron 3'						
394	380	0	-	0.90	TCCAATCCAG^GTAGCCTTTG H	pos 3'->5'	pos 5'->3'	phase	strand	confidence	5' exon intron 3'
297	477	0	-	0.81	AAACTCTCTG^GTGAGAGTGA	394	380	0	-	0.90	TCCAATCCAG^GTAGCCTTTG H
						297	477	0	-	0.83	AAACTCTCCG^GTGAGAGTGA
Acceptor spli	ce sites, di	rect sti	rand								
						Acceptor splic	ce sites, di	rect sti	rand		
	pos 5'->3'		strand	confidence							
	300	2	+	1.00	CTCTCACCAG^AGAGTTTTCC H		pos 5'->3'		strand	confidence	
	302	1	+	0.85	CTCACCAGAG^AGTTTTCCAA		302	1	+	0.76	CTCACCGGAG^AGTTTTCCAA
	304	0	+	0.44	CACCAGAGAG^TTTTCCAAAG		304	0	+	0.42	CACCGGAGAG^TTTTCCAAAG
	314	1	+	0.31	TTTTCCAAAG^AGAGGGAGAA		314	1	+	0.20	TTTTCCAAAG^AGAGGGAGAA
	316	0	+	0.20	TTCCAAAGAG^AGGGAGAAGG		316	0	+	0.19	TTCCAAAGAG^AGGGAGAAGG
	318	2	+	0.19	CCAAAGAGAG^GGAGAAGGCC		318	2	+	0.19	CCAAAGAGAG^GGAGAAGGCC
	325	0	+	0.18	GAGGGAGAAG^GCCAAGGCCC		325	0	+	0.17	GAGGGAGAAG^GCCAAGGCCC
	569	1	+	0.27	CCTTTCTTAG^AAGCCCACGG		569	1	+	0.27	CCTTTCTTAG^AAGCCCACGG
Acceptor spli	ce sites, cor	mplement	t strand	I		Acceptor splic	ce sites, com	mplement	t strand	I	
pos 3'->5'	•	phase	strand	confidence		pos 3'->5'	pos 5'->3'	phase	strand	confidence	5' intron exon 3'
671	103	0	-	0.20	GGTTTTCTAG^ATCGACAGCT	671	103	0	-	0.20	GGTTTTCTAG^ATCGACAGCT
663	111	2	-	0.32	AGATCGACAG^CTATGATTTC	663	111	2	-	0.32	AGATCGACAG^CTATGATTTC
510	264	1	-	0.82	TCTCCCCCAG^AACCCAGCCC	510	264	1	-	0.82	TCTCCCCCAG^AACCCAGCCC
503	271	2	-	0.23	CAGAACCCAG^CCCAAACACA	503	271	2	-	0.23	CAGAACCCAG^CCCAAACACA
486	288	1	-	0.07	ACAGAAGAAG^ACGGCTGCTC	486	288	1	-	0.07	ACAGAAGAAG^ACGGCTGCTC
429	345	2	-	0.86	TCATTCTCAG^GATCGATGTC	429	345	2	-	0.86	TCATTCTCAG^GATCGATGTC
179	595	1	-	0.33	TGCTTTGCAG^GGAGAAACCT	179	595	1	-	0.33	TGCTTTGCAG^GGAGAAACCT
144	630	0	-	0.41	CTTTCCACAG^ACAGCTCCGC	144	630	0	-	0.41	CTTTCCACAG^ACAGCTCCGC
140	634	1	-	0.14	CCACAGACAG^CTCCGCTAGT	140	634	1	-	0.14	CCACAGACAG^CTCCGCTAGT
131	643	1	-	0.07	GCTCCGCTAG^TCAGCCTAGG	131	643	1	-	0.07	GCTCCGCTAG^TCAGCCTAGG
127	647	2	-	0.07	CGCTAGTCAG^CCTAGGTTAG	127	647	2	-	0.07	CGCTAGTCAG^CCTAGGTTAG

Desaparece uno de los sitios *acceptor* (en rojo) en la secuencia mutante. Esta región se encuentra entre el intrón 8 y el exón 9, pero el sitio aceptor que se utiliza es el que está dos pares de bases antes (el de *confidence* 1). Por lo tanto, es probable que no intervenga en el *splicing* y que la mutación no tenga efecto en él.

Splice Site Prediction by Neural Network (NNSplice)

Score

Donor site predictions for 89.130.114.18.16137.0:

467	481	0.51	cgaaaca gt gagcag
528	542	0.55	catcggg gt cagcac

Exon Intron

Donor site predictions for 89.130.114.18.16149.0:

on Intron	Exon	Score	End	Start
aaaca gt gagcag	cgaaa	0.51	481	467
tcggg gt cagcac	catcg	0.55	542	528

Acceptor site predictions for 89.130.114.18.16137.0:

Acceptor site predictions for 89.130.114.18.16149.0:

Start	Ena	Score	Intron Exon					
280	320	0.91	ccctcctctcactctcaccagagagttttccaaagagaggg	Start	End	Score	Intron	Exon
549	589	0.79	ttgaccgccaccctttctt ag aagcccacggggtgccttcc	549	589	0.79	ttgaccgccaccctttctt	agaagcccacggggtgccttcc
675	715	0.84	aaaacctcatgctcctttt ${f ag}$ aatatttcaccttgaaagac	675	715	0.84	aaaacctcatgctcctttt	ag aatatttcaccttgaaagac

Desaparece un sitio *acceptor* (en rojo) en la secuencia mutante. Este es el sitio *acceptor* para el exón 9 por lo que tiene es muy probable que esté implicado en el *splicing*.

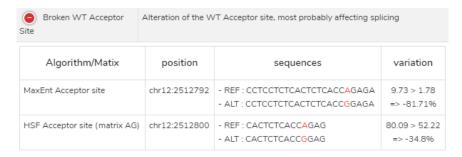
Spliceman

Start End

>sec

Point mutation	Wildtype (wt)	Mutation (mt)	L1 distance	Ranking (L1)	
tcacc(a/g)gagag	cagaga	cggaga	30560	78%	

Human Splicing Finder



SVM-BPfinder

con id	2007	ee die	t bo soa bo sen	v cont not off	nnt lon r	ont con	51/m 551				seg id	2007	ee die	t bo soo bo see	v cont not off	nnt lon nnt s	n sum ss			
seq_id		_	t bp_seq bp_scr								·-	agez		st bp_seq bp_scr						
wt	32	469	aggtgatag	-0.42974329402	0.4978448	327586	64	11	23	-3.4425258	mut	32	469	aggtgatag	-0.42974329402	0.49784482758	64	11	23	-3.4425258
wt	32	457	acctaacct	2.90622146329	0.5	52	11	23	-1.376	062	mut	32	457	acctaacct	2.90622146329	0.5 52	11	23	-1.3760	962
wt	32	447	ggctgacta	2.19207143891	0.5	12	11	23	-1.022	7023	mut	32	447	ggctgacta	2.19207143891	0.5 42	11	23	-1.0227	7023
wt	32	385	caattaaga	-2.77315332586	0.5105263	315789	4	49	95	0.11265428	mut	32	385	caattaaga	-2.77315332586	0.510526315789	9 4	49	95	0.11265428
wt	32	384	aattaagac	-1.15391170898	0.5118733	350923	3	49	95	0.81039728	mut	32	384	aattaagac	-1.15391170898	0.51187335092	3	49	95	0.81039728
wt	32	366	tccttatct	-1.00887583195	0.4986149	958449	1	33	62	0.68207737	mut	32	366	tccttatct	-1.00887583195	0.498614958449	9 1	33	62	0.68207737
wt	32	287	ctctcactc	2.20502740258	0.4007092	219858	192	18	34	-10.441971	mut	32	287	ctctcactc	2.20502740258	0.40070921985	3 192	18	34	-10.441971
wt	32	281	ctctcacca	2.29685319972	0.3913043	347826	186	18	34	-10.029265	mut	32	281	ctctcaccg	2.97993647541	0.39130434782	186	18	34	-9.7618054
wt	32	191	atctcaaag	-0.565957410292	0.4247311	182796	96	18	34	-5.4425456	mut	32	191	atctcaaag	-0.565957410292	0.42473118279	96	18	34	-5.4425456
wt	32	170	ggatcactc	-1.07616253186	0.4242424	124242	75	18	34	-4.3132081	mut	32	170	ggatcactc	-1.07616253186	0.42424242424	2 75	18	34	-4.3132081
wt	32	166	cactcaggc	0.396098241401	0.4161496	968323	71	18	34	-3.4861691	mut	32	166	cactcaggc	0.396098241401	0.41614906832	3 71	18	34	-3.4861691
wt	32	145	tcctgagaa	-0.418537077052	0.4142857	714286	50	18	34	-2.4764744	mut	32	145	tcctgagaa	-0.418537077052	0.41428571428	5 50	18	34	-2.4764744
wt	32	139	gaatgagga	-2.08492710738	0.4253731	134328	44	18	34	-2.745574	mut	32	139	gaatgagga	-2.08492710738	0.42537313432	3 44	18	34	-2.745574
wt	32	121	ggatgagga	-2.92643525945	0.4568965	51724	26	18	34	-1.9255133	mut	32	121	ggatgagga	-2.92643525945	0.45689655172	1 26	18	34	-1.9255133
wt	32	100	cagtgagca	-0.197461328582	0.4947368	342105	5	18	34	0.48449505	mut	32	100	cagtgagca	-0.197461328582	0.49473684210	5 5	18	34	0.48449505
wt	32	39	gggtcagca	-1.62948310594	0.5588235	529412	15	15	27	-0.75370517	mut	32	39	gggtcagca	-1.62948310594	0.55882352941	15	15	27	-0.75370517
wt	32	25	ctttgaccg	2.16975681196	0.65 1	L	15	27	1.6495	007	mut	32	25	ctttgaccg	2.16975681196	0.65 1	15	27	1.64956	907

Se trata de una variante que está afectando al sitio de splicing, concretamente en el sitio acceptor (todos menos 1 Varadiferrecip predactor posición (en amarillo). El BP tiene puntuación menos negativa en la resultado lo indican). Esto puede provocar o exón skipping o inclusión de un exón criptico. secuencia mutante, pero siguen siendo números muy bajos, por lo que es probable que no esté afectando al splicing.

ENST 1995	ENST00000402845.7:c.1218- 2A>G	12:2512810- 2512810	G	splice_acceptor_variant	CACNA1C	ENSG00000151067 Transcript	ENST00000327702.12	protein_coding	-	-	COSV59770317
ENST00000402845 7:c 1218- 225 2310		12:2512810- 2512810	G	splice_acceptor_variant	CACNA1C	ENSG00000151067 Transcript	ENST00000335762.9	protein_coding	-	-	COSV59770317
22-123-13 Splice_acceptor_variant CACNA1C ENSCIOND0402845.7c.1218- 22-12310 Splice_acceptor_variant CACNA1C		12:2512810- 2512810	G	splice_acceptor_variant	CACNA1C	ENSG00000151067 Transcript	ENST00000344100.7	protein_coding	-	-	COSV59770317
2512810 Spilice acceptor, variant CACNA1C ENSCO0000151067 Transcript ENST0000039996.5 protein_coding COSV59770317 2512810 Spilice acceptor, variant CACNA1C ENSCO0000151067 Transcript ENST0000039996.5 protein_coding COSV59770317 2512810 Spilice acceptor, variant CACNA1C ENSCO0000151067 Transcript ENST0000039997.5 protein_coding COSV59770317 2512810 Spilice acceptor, variant CACNA1C ENSCO0000151067 Transcript ENST0000039997.5 protein_coding COSV59770317 2512810 Spilice acceptor, variant CACNA1C ENSCO0000151067 Transcript ENST0000039997.5 protein_coding COSV59770317 2512810 Spilice acceptor, variant CACNA1C ENSCO0000151067 Transcript ENST000039990.5 protein_coding COSV59770317 2512810 Spilice acceptor, variant CACNA1C ENSC00000151067 Transcript ENST000039990.5 protein_coding COSV59770317 2512810 Spilice acceptor, variant CACNA1C ENSC00000151067 Transcript ENST000039990.5 protein_coding COSV59770317 2512810 Spilice acceptor, variant CACNA1C ENSC00000151067 Transcript ENST000039990.5 protein_coding COSV59770317 2512810 Spilice acceptor, variant CACNA1C ENSC00000151067 Transcript ENST0000039990.5 protein_coding COSV59770317 2512810 Spilice acceptor, variant CACNA1C ENSC00000151067 Transcript ENST0000039990.5 protein_coding COSV59770317 2512810 Spilice acceptor, variant CACNA1C ENSC00000151067 Transcript ENST0000039993.5 protein_coding COSV59770317 2512810 Spilice acceptor, variant CACNA1C ENSC00000151067 Transcript ENST0000039993.5 protein_coding COSV59770317 2512810 Spilice acceptor, variant CACNA1C ENSC00000151067 Transcript ENST0000039993.5 protein_coding COSV59770317 2512810 Spilice acceptor, variant CACNA1C ENSC00000151067 Transcript ENST0000039993.5 protein_coding COSV59770317 2512810 Spilice acceptor, variant CACNA1C ENSC00000151067 Transcript ENST0000039993.5 prot		12:2512810-		splice_acceptor_variant	CACNA1C	ENSG00000151067 Transcript	ENST00000347598.9	protein_coding	-	-	COSV59770317
2A-G		12:2512810- 2512810	G	splice_acceptor_variant	CACNA1C	ENSG00000151067 Transcript	ENST00000399591.5	protein_coding	-	-	COSV59770317
2A-G		12:2512810- 2512810	G	splice_acceptor_variant	CACNA1C	ENSG00000151067 Transcript	ENST00000399595.5	protein_coding	-	-	COSV59770317
24-96 2518310		12:2512810- 2512810	G	splice_acceptor_variant	CACNA1C	ENSG00000151067 Transcript	ENST00000399597.5	protein_coding	-	-	COSV59770317
2A-G		12:2512810- 2512810	G	splice_acceptor_variant	CACNA1C	ENSG00000151067 Transcript	ENST00000399601.5	protein_coding	-	-	COSV59770317
2A-G SET 2810 SINST00000402845.7c.1218- 12.2512810 G Isplice_acceptor_variant			G	splice_acceptor_variant	CACNA1C	ENSG00000151067 Transcript	ENST00000399603.6	protein_coding	-	-	COSV59770317
2A-G		12:2512810- 2512810	G	splice_acceptor_variant	CACNA1C	ENSG00000151067 Transcript	ENST00000399606.5	protein_coding	-	-	COSV59770317
ENST00000402845.7:c.1218- 12-2512810- G		12:2512810- 2512810	G	splice_acceptor_variant	CACNA1C	ENSG00000151067 Transcript	ENST00000399617.5	protein_coding	-	-	COSV59770317
2A-G 2512810 Splice acceptor variant CACNA1C			G	splice_acceptor_variant	CACNA1C	ENSG00000151067 Transcript	ENST00000399621.5	protein_coding	-	-	COSV59770317
2A-G 2512810			G	splice_acceptor_variant	CACNA1C	ENSG00000151067 Transcript	ENST00000399629.5	protein_coding	-	-	COSV59770317
ENST00000402845.7:c.1218- 12.2512810 C Splice acceptor_variant CACNA1C ENSG00000151067 Transcript ENST00000399638.5 protein_coding - COSV59770317			G	splice_acceptor_variant	CACNA1C	ENSG00000151067 Transcript	ENST00000399634.5	protein_coding	-	-	COSV59770317
2A>G			G	splice_acceptor_variant	CACNA1C	ENSG00000151067 Transcript	ENST00000399637.5	protein_coding	-	-	COSV59770317
2512810 ENST00000402845.7:c.1218-			G	splice_acceptor_variant	CACNA1C	ENSG00000151067 Transcript	ENST00000399638.5	protein_coding	-	-	COSV59770317
2A>G ENST00000402845.7:c.1218- 2A>G ENST00000402845.7:c.1218- 2A>G ENST00000402845.7:c.1218- 2A>G ENST00000402845.7:c.1218- 2A>G ENST00000402845.7:c.1218- 2512810 G Splice acceptor variant NMD transcript variant non coding transcript variant no			G	splice_acceptor_variant	CACNA1C	ENSG00000151067 Transcript	ENST00000399641.6	protein_coding	-	-	COSV59770317
25-128-10 ENST00000402845.7:c.1218-		12:2512810- 2512810	G	splice_acceptor_variant	CACNA1C	ENSG00000151067 Transcript	ENST00000399644.5	protein_coding	-	-	COSV59770317
ENST00000402845.7:c.1218- 2A>G ENST00000402845.7:c.1218- 2A>G ENST00000402845.7:c.1218- 2A>G ENST00000402845.7:c.1218- 2A>G ENST00000402845.7:c.1218- 2512810 G Splice acceptor variant, NMD transcript variant non-coding ranscript variant ENST00000480911.6 nonsense mediated decay COSV59770317 ENST00000402845.7:c.1218- 2512810 ENST00000402845.7:c.1218- 12:2512810- 3512810 G Splice acceptor variant, CACNA1C ENSG0000151067 Transcript ENST00000672806.1 nonsense mediated decay COSV59770317		12:2512810- 2512810	G	splice_acceptor_variant	CACNA1C	ENSG00000151067 Transcript	ENST00000399649.5	protein_coding	-	-	COSV59770317
ENST00000402845.7:c.1218- 2512810 ENST00000402845.7:c.1218- 2512810 ENST00000402845.7:c.1218- 2512810 G		12:2512810- 2512810	G	splice_acceptor_variant	CACNA1C	ENSG00000151067 Transcript	ENST00000399655.6	protein_coding	-	-	COSV59770317
2A>G		12:2512810- 2512810	G	splice_acceptor_variant	CACNA1C	ENSG00000151067 Transcript	ENST00000402845.7	protein_coding	-	-	COSV59770317
2A>G		12:2512810- 2512810	G	splice_acceptor_variant	CACNA1C	ENSG00000151067 Transcript	ENST00000406454.7	protein_coding	-	-	COSV59770317
2A>G			G		CACNA1C	ENSG00000151067 Transcript	ENST00000480911.6	nonsense_mediated_decay	-	-	COSV59770317
		12:2512810- 2512810	G		CACNA1C	ENSG00000151067 Transcript	ENST00000491104.1	processed_transcript	-	-	COSV59770317
			G	splice_acceptor_variant, NMD_transcript_variant	CACNA1C	ENSG00000151067 Transcript	ENST00000672806.1	nonsense_mediated_decay	-	-	COSV59770317

ESEfinder

Solo se obtiene un resultado positivo donde se encuentre la posición de estudio (a antes de las mayúsculas del exón) para las matrices 3'SS:

286 C-488 teteacteteaccagAGAGTTTTCCAAAGA -21.10300 286 C-488 CeteacteteaccagAGAGTTTTCCAAAGA -19.78690 286 C-488 CeteacteteaccagAGAGTTTTCCAAAGA -19.78690 C-488 C-488 CeteacteteaccagAGAGTTTTCCAAAGA -19.78690 C-488 C-488					
i/_400\i	200	286		1676TTTTCC777867 -10 78600 286	totoactotoaccagAGAGTTTTCCAAAGA 6 82410
			11/_488\	(-488)	0.02110

Si buscamos el resultado equivalente para la secuencia mutante, se observa que las puntuaciones son negativas para esas matrices:

	205
400	400
tctcactctcaccggAGAGTTTTCCAAAGA -23.40850 tctcactctcaccggAGAGTTTTCCAAAGA -9.57530	tctcactctcaccggAGAGTTTTCCAAAGA -22.14950 tctcactctcaccggAGAGTTTTCCAAAGA -7.71320
	88) (-488)

Se puede concluir que se está perdiendo un sitio 3'SS (acceptor) en la secuencia mutante.