

Ejemplo comparación de resultados predictores in silico

Cambio de estudio COG6 c.789-1074C>T (chr13:39686429 C/T, COSV62997589 o NM_001145079.2: c.789-1074C>T)

Exón 9 e intrones adyacentes:

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gaactttcaattgagggatggatgtcaggaatgggtggtgataatggaaaacaaggcttgag
tcaagtcaaaagaaatgggcactccaagagagctccaagccatttttataaattgaaatccca
aattgtcctattcccaggttaaaagctttccttccctaccagttccttcaacctagaaaag
cctttccatgctttttcaattataaatagggtgacttctaatacatgttttttaaaaagggt
atttgcttgatatgtgtttgtcagtaaatttaaggaaacatctgttatgtctaattattg
gagtcaaaataagcttttaagatgggtagcaatttttggagcaaatgttgatagat
tgtgtagtagtgtgttaaagaagacttttttaattgtagaagagaaaaatgtcaattttggg
attgcagaattccttttattttttctgagacaggggtcttgctctgtggctcaggctggagt
atagtgagcagtcactgttaactgcagcctgaacctcacgggctcaagtgatcccccta
cctcagccttccgagtagctgggactataggcatgcaaccacatgcctggctattttttt
tttttaataatgagaaaagggtttcattattttgcccaggctggtctcaaaactcctgggct
ccagtgaccaccttctcggcctcctaaagtgtctaggattgcaggtgtgaaccacgcac
ccagccttggatttgcagagttctaattataatcagtttttgagaaatgttatggcttta
cgtaaaacagtagagcatcatgttttagactgcaaatatgtataactgaaatataccgaaa
cctagtatagtgaatatactgaaactataaaaagtttaagtcttccacaccttggcttgattt
tcttaacttttagtaagtccattgctttatcccttattactgtgctctgcttttagtccc
aaatcccagcatgtgttcttaggaggggtaaaagtgttttacttttgattgaattttcaa
tgagctgaagttatctcttaaatattttttcttggttgaaagcaatgttgcttgaccga
ataatctaaaggagctttaagtgtcttttttagtaacttggtgtgtctcagaaattgcgtgaat
agtcctgatatagccactagaaacaaccacaaccattttttatataacttggtttcttctag
ATAACCTTAGATGAATTTGGAACAGCCAGAAGAAGTACAGTTGTTTCGTGGAATTTATTGA
TGCCTCACAAGAGGGGGCCCGGAGGTACACCTAGACCAATTGAAATGCATTCATATGA
CCCTTTGAG
gtatagtaatcagacagcagaagaggagtgtgatgtttttccaaggaaatcttctattaac
atttagttttcattag
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El cambio se encuentra en la tercera línea del intrón 8 (la **c** 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 strand										Donor splice sites, direct strand											
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pos	5'→3'	phase	strand	confidence	5'	exon	intron	3'		pos	5'→3'	phase	strand	confidence	5'	exon	intron	3'			
1287		0	+	0.72	GGCCCCGGAG		GTACACCTAG			1287		0	+	0.72	GGCCCCGGAG		GTACACCTAG				
Donor splice sites, complement strand										Donor splice sites, complement strand											
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pos	3'→5'	pos	5'→3'	phase	strand	confidence	5'	exon	intron	3'	pos	3'→5'	pos	5'→3'	phase	strand	confidence	5'	exon	intron	3'
887		519		1	-	0.32	TCAAGCCAAG		GTGGGAAGAC		887		519		1	-	0.32	TCAAGCCAAG		GTGGGAAGAC	
541		865		1	-	0.36	GAAGGCTGAG		GTAGGGGGAT		541		865		1	-	0.36	GAAGGCTGAG		GTAGGGGGAT	
Acceptor splice sites, direct strand										Acceptor splice sites, direct strand											
-----										-----											
pos	5'→3'	phase	strand	confidence	5'	intron	exon	3'		pos	5'→3'	phase	strand	confidence	5'	intron	exon	3'			
162		0	+	0.31	TCCCTACCAG		TTTCCTTCACC			162		0	+	0.31	TCCCTACCAG		TTTCCTTCACC				
1200		2	+	0.97	TTTCTTCTAG		ATATACCTTA			1200		2	+	0.97	TTTCTTCTAG		ATATACCTTA				
1211		1	+	0.41	TATACCTTAG		ATGAATTTGG			1211		1	+	0.41	TATACCTTAG		ATGAATTTGG				
1226		0	+	0.07	TTTGGAACAG		CCAGAAGAAG			1226		0	+	0.07	TTTGGAACAG		CCAGAAGAAG				
Acceptor splice sites, complement strand										Acceptor splice sites, complement strand											
-----										-----											
pos	3'→5'	pos	5'→3'	phase	strand	confidence	5'	intron	exon	3'	pos	3'→5'	pos	5'→3'	phase	strand	confidence	5'	intron	exon	3'
1293		113		2	-	0.15	ATTGGTCTAG		GTGTACCTCC		1293		113		2	-	0.15	ATTGGTCTAG		GTGTACCTCC	

Splice Site Prediction by Neural Network (NNSplice)

Donor site predictions for 10.42.3.123.574134.0 :

Start	End	Score	Exon	Intron
232	246	0.71	taaaaag	gtatttgc
698	712	0.84	attgcag	gtgtgaac
1280	1294	0.59	cccggag	gtacacct
1323	1337	0.49	ctttgag	gtatagta

Donor site predictions for 10.42.0.139.574146.0 :

Start	End	Score	Exon	Intron
232	246	0.71	taaaaag	gtatttgc
698	712	0.84	attgcag	gtgtgaac
1280	1294	0.59	cccggag	gtacacct
1323	1337	0.49	ctttgag	gtatagta

Acceptor site predictions for 10.42.3.123.574134.0 :

Start	End	Score	Intron	Exon
118	158	0.74	ccaaattgtcctatttcccc	agttaaaagctttccttccta
142	182	0.60	aaagctttccttcctacc	agttccttcaccttagaaagcc
156	196	0.90	ctaccagttccttcacctt	agaaagcctttccatgcctttt
188	228	0.56	atgccttttcaattataat	aggtgacttcttaatacatgtt
432	472	0.74	cttttattttttctgagac	agggcttctgtctgtggctcag
526	566	0.40	caagtgatccccctacctc	agccttcgagtagctgggact
618	658	0.74	gggtttcattattttgccc	aggtggtctcaaactcctggg
1107	1147	0.52	ttaagtacttggttgtctc	agaaattgcgtgaatagtctga
1180	1220	0.90	tatataacttgtttcttct	agatataccttagatgaatttg

Acceptor site predictions for 10.42.0.139.574146.0 :


Start	End	Score	Intron	Exon
118	158	0.80	ccaaattgttctatttcccc	agttaaaagctttccttccta
142	182	0.60	aaagctttccttcctacc	agttccttcaccttagaaagcc
156	196	0.90	ctaccagttccttcacctt	agaaagcctttccatgcctttt
188	228	0.56	atgccttttcaattataat	aggtgacttcttaatacatgtt
432	472	0.74	cttttattttttctgagac	agggcttctgtctgtggctcag
526	566	0.40	caagtgatccccctacctc	agccttcgagtagctgggact
618	658	0.74	gggtttcattattttgccc	aggtggtctcaaactcctggg
1107	1147	0.52	ttaagtacttggttgtctc	agaaattgcgtgaatagtctga
1180	1220	0.90	tatataacttgtttcttct	agatataccttagatgaatttg

Uno de los sitios *acceptor* se ve fortalecido (tiene mayor nivel de confianza) con la presencia de la mutación. Si esto estuviera ocurriendo y el *spliceosome* lo detectara antes que al *acceptor* normal del exón, se produciría la inclusión de un exón críptico de 1062 pb (siempre que el sitio *donor* normal del exón se siga usando).

Spliceman

Point mutation	Wildtype (wt)	Mutation (mt)	L1 distance	Ranking (L1)
attgt(c/t)ctatt	attgtc	attgtt	28890	67%

Human Splicing Finder

 No significant impact on splicing signals.	No significant impact on splicing signals.
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SVM-BPfinder

seq_id	agez	ss_dist	bp_seq	bp_scr	y_cont	ppt_off	ppt_len	ppt_scr	svm_scr		
wt	18	75	tttttataa		-4.47851256423	0.657142857143	17	18	28	28	-1.9547615
wt	18	72	ttataaatt		-1.65516640541	0.671641791045	14	18	28	28	-0.65471024
wt	18	67	aattgaatc		-0.574569926339	0.693548387097	9	18	28	28	0.091961141
wt	18	40	cagttaaaa		-3.55232070434	0.714285714286	5	29	54	54	-0.57186623
wt	18	39	agttaaaaag		-1.3499002872	0.735294117647	4	29	54	54	0.36056889
mut	18	75	tttttataa		-4.47851256423	0.657142857143	17	18	29	29	-1.9454456
mut	18	72	ttataaatt		-1.65516640541	0.671641791045	14	18	29	29	-0.6453944
mut	18	67	aattgaatc		-0.574569926339	0.693548387097	9	18	29	29	0.10127699
mut	18	40	cagttaaaa		-3.55232070434	0.714285714286	5	29	54	54	-0.57186623
mut	18	39	agttaaaaag		-1.3499002872	0.735294117647	4	29	54	54	0.36056889

Variant Effect Predictor tool

ENST00000455146.7:c.789-1074C>T	13:39686429-39686429	T	intron_variant, NMD_transcript_variant	COG6	ENSG00000133103	Transcript	ENST00000356576.8	nonsense_mediated_decay	COSV62997589
ENST00000455146.7:c.789-1074C>T	13:39686429-39686429	T	intron_variant	COG6	ENSG00000133103	Transcript	ENST00000416691.5	protein_coding	COSV62997589
ENST00000455146.7:c.789-1074C>T	13:39686429-39686429	T	intron_variant	COG6	ENSG00000133103	Transcript	ENST00000455146.8	protein_coding	COSV62997589
ENST00000455146.7:c.789-1074C>T	13:39686429-39686429	T	upstream_gene_variant	COG6	ENSG00000133103	Transcript	ENST00000460701.1	processed_transcript	COSV62997589
ENST00000455146.7:c.789-1074C>T	13:39686429-39686429	T	downstream_gene_variant	COG6	ENSG00000133103	Transcript	ENST00000465775.1	processed_transcript	COSV62997589
ENST00000455146.7:c.789-1074C>T	13:39686429-39686429	T	intron_variant, NMD_transcript_variant	COG6	ENSG00000133103	Transcript	ENST00000536488.5	nonsense_mediated_decay	COSV62997589
ENST00000455146.7:c.789-1074C>T	13:39686429-39686429	T	intron_variant, non_coding_transcript_variant	COG6	ENSG00000133103	Transcript	ENST00000537156.1	processed_transcript	COSV62997589

ESEfinder

La única predicción para la secuencia WT que tiene puntuación positiva es en las matrices 3', pero, cuando se comparan las puntuaciones con las de la secuencia mutante, aumentan muy ligeramente, por lo que no se tendrán en cuenta.

$\begin{matrix} 124 \\ (-1282) \end{matrix} \left \begin{matrix} \text{tgttctattccccagttaaaagctttcctt} \end{matrix} \right \begin{matrix} -8.13820 \\ \end{matrix}$	$\begin{matrix} 124 \\ (-1282) \end{matrix} \left \begin{matrix} \text{tgttctattccccagttaaaagctttcctt} \end{matrix} \right \begin{matrix} 6.73020 \\ \end{matrix}$	$\begin{matrix} 124 \\ (-1282) \end{matrix} \left \begin{matrix} \text{tgttctattccccagttaaaagctttcctt} \end{matrix} \right \begin{matrix} -9.32030 \\ \end{matrix}$	$\begin{matrix} 124 \\ (-1282) \end{matrix} \left \begin{matrix} \text{tgttctattccccagttaaaagctttcctt} \end{matrix} \right \begin{matrix} 6.92590 \\ \end{matrix}$
$\begin{matrix} 124 \\ (-1282) \end{matrix} \left \begin{matrix} \text{tgttctattccccagttaaaagctttcctt} \end{matrix} \right \begin{matrix} -8.91400 \\ \end{matrix}$	$\begin{matrix} 124 \\ (-1282) \end{matrix} \left \begin{matrix} \text{tgttctattccccagttaaaagctttcctt} \end{matrix} \right \begin{matrix} 7.13530 \\ \end{matrix}$	$\begin{matrix} 124 \\ (-1282) \end{matrix} \left \begin{matrix} \text{tgttctattccccagttaaaagctttcctt} \end{matrix} \right \begin{matrix} -10.06760 \\ \end{matrix}$	$\begin{matrix} 124 \\ (-1282) \end{matrix} \left \begin{matrix} \text{tgttctattccccagttaaaagctttcctt} \end{matrix} \right \begin{matrix} 7.31040 \\ \end{matrix}$