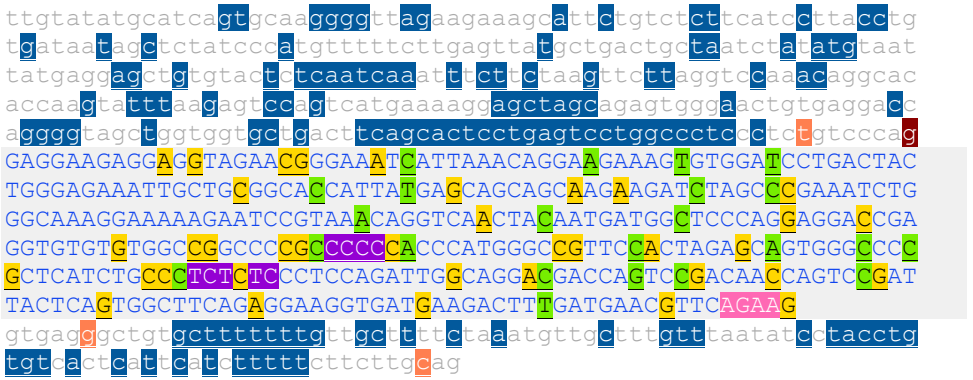
Ejemplo comparación de resultados predictores in sillico

Cambio de estudio CHD4 c.3820-1G>T (chr12:6583379 G/T, NM_001273.5:c.3820-1G>T)

Exón 25 e intrones adyacentes:



El cambio se encuentra en anterior posición al exón 25 (la **g** 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

pos 5'->3'	phase	strand	confidence	5' exon intron 3'	
482	1	+	0.90	GAGGACCGAG^GTGTGTGTGG	H
653	1	+	0.00	CGTTCAGAAG^GTGAGGGCTG	

Donor splice sites, complement strand

pos 3'->5'	pos 5'->3'	phase	strand	confidence	5' exon intron 3'
709	34	2	-	0.00	AGTGACACAG^GTAGGATATT
57	686	0	-	0.00	ATTATCACAG^GTAAGGATGA

Acceptor splice sites, direct strand

pos 5'->3'	phase	strand	confidence	5' intron exon 3'	
166	1	+	0.33	AAGTTCTTAG^GTCCAAACAG	
300	0	+	0.97	TCTGTCCCAG^GAGGAAGAGG	
303	0	+	0.25	GTCCCAGGAG^GAAGAGGAGG	
307	1	+	0.31	CAGGAGGAAG^AGGAGGTAGA	
309	0	+	0.25	GGAGGAAGAG^GAGGTAGAAC	
316	1	+	0.20	GAGGAGGTAG^AACGGGAAAT	
336	0	+	0.17	CATTAAACAG^GAAGAAAGTG	
340	1	+	0.07	AAACAGGAAG^AAAGTGTGGA	
344	2	+	0.07	AGGAAGAAAG^TGTGGATCCT	
565	1	+	1.00	CTCCCTCCAG^ATTGGCAGGA	H
573	0	+	0.77	AGATTGGCAG^GACGACCAGT	
582	0	+	0.19	GGACGACCAG^TCCGACAACC	
594	0	+	0.07	CGACAACCAG^TCCGATTACT	

Acceptor splice sites, complement strand

pos 3'->5'	pos 5'->3'	phase	strand	confidence	5' intron exon 3'
453	290	1	-	0.07	ATCATTGTAG^TTGACCTGTT
418	325	0	-	0.85	CTTTGCCCAG^ATTTCGGGCT
406	337	0	-	0.18	TTCGGGCTAG^ATCTTCTTGC

Donor splice sites, direct strand

pos 5'->3'	phase	strand	confidence	5' exon intron 3'	
482	1	+	0.90	GAGGACCGAG^GTGTGTGTGG	H
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Acceptor splice sites, direct strand

pos 5'->3'	phase	strand	confidence	5' intron exon 3'	
166	1	+	0.33	AAGTTCTTAG^GTCCAAACAG	
303	0	+	0.20	GTCCCATGAG^GAAGAGGAGG	
307	1	+	0.20	CATGAGGAAG^AGGAGGTAGA	
309	0	+	0.20	TGAGGAAGAG^GAGGTAGAAC	
312	0	+	0.20	GGAAGAGGAG^GTAGAACGGG	
316	1	+	0.20	GAGGAGGTAG^AACGGGAAAT	
336	0	+	0.18	CATTAAACAG^GAAGAAAGTG	
340	1	+	0.17	AAACAGGAAG^AAAGTGTGGA	
344	2	+	0.07	AGGAAGAAAG^TGTGGATCCT	
565	1	+	1.00	CTCCCTCCAG^ATTGGCAGGA	H
573	0	+	0.77	AGATTGGCAG^GACGACCAGT	
582	0	+	0.19	GGACGACCAG^TCCGACAACC	
594	0	+	0.07	CGACAACCAG^TCCGATTACT	

Acceptor splice sites, complement strand

pos 3'->5'	pos 5'->3'	phase	strand	confidence	5' intron exon 3'
453	290	1	-	0.07	ATCATTGTAG^TTGACCTGTT
418	325	0	-	0.85	CTTTGCCCAG^ATTTCGGGCT
406	337	0	-	0.18	TTCGGGCTAG^ATCTTCTTGC

Hay tres resultados (en verde, azul y amarillo) que son la misma región pero cambiando el nucleótido de estudio (de G a T). El resultado en 300 (en rojo) se pierde en la secuencia mutante, mientras que el cambio en 312 (en granate) no se encuentra en la secuencia WT. La predicción con 0.97 de *confidence* (en rojo) es la que predice correctamente la separación entre el intrón 24 y el exón 2. Al desaparecer, parece que provoca que se active un sitio críptico en el interior del exón (en granate), lo que provocaría la pérdida de los primeros 12 nucleótidos del exón (GAGGAAGAGGAG), que van desde el final del *acceptor* que se pierde hasta el final del *acceptor* que se gana. Si, por el contrario, se usara el *acceptor* anterior (en 166), se produciría la inclusión de 134 nt en el exón.

Splice Site Prediction by Neural Network (NNSplice)

Donor site predictions for 89.130.114.18.17973.0 :

Start	End	Score	Exon	Intron
306	320	0.50	agaggag	**gt**agaacg
475	489	0.95	gaccgag	**gt**gtgtgt
646	660	0.99	tcagaag	**gt**gagggc

Donor site predictions for 89.130.114.18.17995.0 :

Start	End	Score	Exon	Intron
306	320	0.50	agaggag	**gt**agaacg
475	489	0.95	gaccgag	**gt**gtgtgt
646	660	0.99	tcagaag	**gt**gagggc

Acceptor site predictions for 89.130.114.18.17973.0 :

Start	End	Score	Intron	Exon
146	186	0.98	aaatttcttctaagttctt	**ag**gtccaaacaggcacaccaag
280	320	0.93	ctggccctccctctgtccc	**ag**gaggaagaggaggtagaacg
545	585	1.00	atctgccctctctccctcc	**ag**attggcaggacgaccagtcc

Acceptor site predictions for 89.130.114.18.17995.0 :

Start	End	Score	Intron	Exon
146	186	0.98	aaatttcttctaagttctt	**ag**gtccaaacaggcacaccaag
545	585	1.00	atctgccctctctccctcc	**ag**attggcaggacgaccagtcc

Se pierde un sitio *acceptor* (en rojo) en la secuencia mutante, que coincide con el sitio *acceptor* del exón 25. Si en vez de este sitio se empleara el anterior (en 146), se produciría la inclusión de 134 pb o bien si se utilizara el siguiente (en 545) con el *donor* normal, se perderían los primeros 265 nt del exón.

MaxEntScan (solo realizamos el análisis del 3'SS porque está por delante del exón y, si afecta a un sitio de *splicing*, será de este tipo)

MAXENT: -47.41 MM: -38.85 WMM: -30.84

Spliceman

>sec
ttgtatatgcatcagtgcaaggggttagaagaaagcattctgtctcttcatccttacctgtgataatagctctatcccatgtttttcttgagttatgctgactgctaatctatatgtaattatgaggagctgtgtactctcaatcaaatttcttctaagttcttaggtccaaacaggcacaccaagtatttaagagtccagtcatgaaaaggagctagcagagtgggaactgtgaggaccaggggtagctggtggtgctgacttcagcactcctgagtcctggccctccctctg

Point mutation	Wildtype (wt)	Mutation (mt)	L1 distance	Ranking (L1)
tccca(g/t)gagga	cccagg	cccatg	31519	84%

Human Splicing Finder

Type	Interpretation
No significant impact on splicing signals.	No significant impact on splicing signals.

SVM-BPfinder

seq_id	agez	ss_dist	bp_seq	bp_scr	y_cont	ppt_off	ppt_len	ppt_scr	svm_scr			
wt	54	451	tgctgactt		2.98414993368	0.479820627803	17	21	36	0.98448057		
wt	54	446	acttcagca		-2.03765209265	0.47619047619	12	21	36	-0.66647307		
wt	54	436	tcctgagtc		1.03287939407	0.473317865429	2	21	36	1.1678405		
wt	54	384	aaatcatta		-2.95778833902	0.480211081794	168	17	23	-11.021095		
wt	54	381	tcattaaac		-2.65867129466	0.478723404255	165	17	23	-10.714563		
wt	54	380	cattaaaca		0.074864610367	0.48	164	17	23	-9.5805434		
wt	54	356	tcctgacta		2.23204488833	0.492877492877	140	17	23	-7.2125866		
wt	54	326	ccattatga		-1.86610195703	0.495327102804	110	17	23	-6.917464		
wt	54	323	ttatgagca		-1.3793032155	0.496855345912	107	17	23	-6.5364708		
wt	54	269	ccgtaaaca		0.322632548342	0.534090909091	53	17	23	-2.4399471		
wt	54	261	aggtcaact		-1.75441694288	0.5390625	45	17	23	-2.7452179		
wt	54	251	caatgatgg		1.01000098867	0.544715447154	35	17	23	-1.0280087		
wt	54	167	cgctcatct		1.79711376744	0.549382716049	1	18	38	1.5735711		
wt	54	110	cgattactc		0.0983919010237	0.52380952381	61	21	45	-2.8325065		
wt	54	106	tactcagtg		0.595671380279	0.514851485149	57	21	45	-2.387498		
wt	54	97	gcttcagag		-1.98697642417	0.510869565217	48	21	45	-2.8303278		
wt	54	86	aggtgatga		-0.12167486303	0.567901234568	37	21	45	-1.385273		
wt	54	83	tgatgaaga		-1.89740434115	0.576923076923	34	21	45	-1.8877463		
wt	54	74	ctttgatga		-0.0624952665958	0.594202898551	25	21	45	-0.59402688		
wt	54	71	tgatgaacg		-0.15695213482	0.606060606061	22	21	45	-0.43728654		
wt	54	64	cgttcagaa		-1.26063962107	0.610169491525	15	21	45	-0.42501709		
wt	54	56	aggtgaggg		-1.84603057797	0.686274509804	7	21	45	-0.12325904		
wt	54	27	ttctaaatg		1.09241064895	0.681818181818	4	11	22	1.0014709		

seq_id	agez	ss_dist	bp_seq	bp_scr	y_cont	ppt_off	ppt_len	ppt_scr	svm_scr			
mut	54	451	tgctgactt		2.98414993368	0.482062780269	17	23	37	0.99452056		
mut	54	446	acttcagca		-2.03765209265	0.478458049887	12	23	37	-0.65642487		
mut	54	436	tcctgagtc		1.03287939407	0.475638051044	2	23	37	1.1779057		
mut	54	410	ccatgagga		-2.13041375446	0.459259259259	194	17	23	-12.349662		
mut	54	384	aaatcatta		-2.95778833902	0.480211081794	168	17	23	-11.021095		
mut	54	381	tcattaaac		-2.65867129466	0.478723404255	165	17	23	-10.714563		
mut	54	380	cattaaaca		0.074864610367	0.48	164	17	23	-9.5805434		
mut	54	356	tcctgacta		2.23204488833	0.492877492877	140	17	23	-7.2125866		
mut	54	326	ccattatga		-1.86610195703	0.495327102804	110	17	23	-6.917464		
mut	54	323	ttatgagca		-1.3793032155	0.496855345912	107	17	23	-6.5364708		
mut	54	269	ccgtaaaca		0.322632548342	0.534090909091	53	17	23	-2.4399471		
mut	54	261	aggtcaact		-1.75441694288	0.5390625	45	17	23	-2.7452179		
mut	54	251	caatgatgg		1.01000098867	0.544715447154	35	17	23	-1.0280087		
mut	54	167	cgctcatct		1.79711376744	0.549382716049	1	18	38	1.5735711		
mut	54	110	cgattactc		0.0983919010237	0.52380952381	61	21	45	-2.8325065		
mut	54	106	tactcagtg		0.595671380279	0.514851485149	57	21	45	-2.387498		
mut	54	97	gcttcagag		-1.98697642417	0.510869565217	48	21	45	-2.8303278		
mut	54	86	aggtgatga		-0.12167486303	0.567901234568	37	21	45	-1.385273		
mut	54	83	tgatgaaga		-1.89740434115	0.576923076923	34	21	45	-1.8877463		
mut	54	74	ctttgatga		-0.0624952665958	0.594202898551	25	21	45	-0.59402688		
mut	54	71	tgatgaacg		-0.15695213482	0.606060606061	22	21	45	-0.43728654		
mut	54	64	cgttcagaa		-1.26063962107	0.610169491525	15	21	45	-0.42501709		
mut	54	56	aggtgaggg		-1.84603057797	0.686274509804	7	21	45	-0.12325904		
mut	54	27	ttctaaatg		1.09241064895	0.681818181818	4	11	22	1.0014709		

El único cambio es que aparece un nuevo BP en la secuencia mutante, aunque tiene una puntuación negativa muy baja, por lo que no lo tendremos en cuenta.

Variant Effect Predictor tool

Se trata de una variante que está afectando al sitio de *splicing*, concretamente al *acceptor* (21/39 resultados para CHD4).

ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_acceptor_variant	CHD4	ENSG00000111642	Transcript	ENST00000357008.7	protein_coding	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	non_coding_transcript_exon_variant	AC006064.2	ENSG00000247853	Transcript	ENST00000501075.2	lncRNA	2/2	836	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	upstream_gene_variant	SCARNA11	ENSG00000251898	Transcript	ENST00000516089.1	scaRNA	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_acceptor_variant, non_coding_transcript_variant	CHD4	ENSG00000111642	Transcript	ENST00000540960.2	retained_intron	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_acceptor_variant	CHD4	ENSG00000111642	Transcript	ENST00000544040.7	protein_coding	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_acceptor_variant	CHD4	ENSG00000111642	Transcript	ENST00000544484.6	protein_coding	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_acceptor_variant	CHD4	ENSG00000111642	Transcript	ENST00000642594.1	protein_coding	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_acceptor_variant, NMD_transcript_variant	CHD4	ENSG00000111642	Transcript	ENST00000642637.1	nonsense_mediated_decay	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_acceptor_variant, NMD_transcript_variant	CHD4	ENSG00000111642	Transcript	ENST00000642810.1	nonsense_mediated_decay	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	upstream_gene_variant	CHD4	ENSG00000111642	Transcript	ENST00000642860.1	retained_intron	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_acceptor_variant	CHD4	ENSG00000111642	Transcript	ENST00000642879.1	protein_coding	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_region_variant, intron_variant	CHD4	ENSG00000111642	Transcript	ENST00000643335.1	protein_coding	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	upstream_gene_variant	CHD4	ENSG00000111642	Transcript	ENST00000643367.1	processed_transcript	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_acceptor_variant, NMD_transcript_variant	CHD4	ENSG00000111642	Transcript	ENST00000643538.1	nonsense_mediated_decay	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_region_variant, intron_variant	CHD4	ENSG00000111642	Transcript	ENST00000643815.1	protein_coding	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	upstream_gene_variant	CHD4	ENSG00000111642	Transcript	ENST00000644077.1	protein_coding	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_acceptor_variant, NMD_transcript_variant	CHD4	ENSG00000111642	Transcript	ENST00000644137.1	nonsense_mediated_decay	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_acceptor_variant	CHD4	ENSG00000111642	Transcript	ENST00000644289.1	protein_coding	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_acceptor_variant	CHD4	ENSG00000111642	Transcript	ENST00000644352.1	protein_coding	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_acceptor_variant, non_coding_transcript_variant	CHD4	ENSG00000111642	Transcript	ENST00000644356.1	retained_intron	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_acceptor_variant, NMD_transcript_variant	AC006064.6	ENSG00000285238	Transcript	ENST00000644480.2	nonsense_mediated_decay	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	upstream_gene_variant	CHD4	ENSG00000111642	Transcript	ENST00000644652.1	nonsense_mediated_decay	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_acceptor_variant, NMD_transcript_variant	CHD4	ENSG00000111642	Transcript	ENST00000644801.1	nonsense_mediated_decay	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_acceptor_variant	CHD4	ENSG00000111642	Transcript	ENST00000645005.1	protein_coding	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_acceptor_variant	CHD4	ENSG00000111642	Transcript	ENST00000645022.1	protein_coding	-	-	-

ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_acceptor_variant	CHD4	ENSG00000111642	Transcript	ENST00000645095.1	protein_coding	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	upstream_gene_variant	CHD4	ENSG00000111642	Transcript	ENST00000645199.1	processed_transcript	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_acceptor_variant	CHD4	ENSG00000111642	Transcript	ENST00000645645.1	protein_coding	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	upstream_gene_variant	CHD4	ENSG00000111642	Transcript	ENST00000645991.1	nonsense_mediated_decay	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	downstream_gene_variant	CHD4	ENSG00000111642	Transcript	ENST00000646070.1	processed_transcript	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	upstream_gene_variant	CHD4	ENSG00000111642	Transcript	ENST00000646145.1	retained_intron	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	intron_variant, NMD_transcript_variant	CHD4	ENSG00000111642	Transcript	ENST00000646268.1	nonsense_mediated_decay	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	intron_variant, NMD_transcript_variant	AC006064.6	ENSG00000285238	Transcript	ENST00000646322.1	nonsense_mediated_decay	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_acceptor_variant, non_coding_transcript_variant	CHD4	ENSG00000111642	Transcript	ENST00000646360.1	retained_intron	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_acceptor_variant, non_coding_transcript_variant	CHD4	ENSG00000111642	Transcript	ENST00000646366.1	processed_transcript	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	upstream_gene_variant	CHD4	ENSG00000111642	Transcript	ENST00000646462.1	protein_coding	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_acceptor_variant	CHD4	ENSG00000111642	Transcript	ENST00000646608.1	protein_coding	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	upstream_gene_variant	CHD4	ENSG00000111642	Transcript	ENST00000646609.1	processed_transcript	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_acceptor_variant	CHD4	ENSG00000111642	Transcript	ENST00000646806.1	protein_coding	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	upstream_gene_variant	CHD4	ENSG00000111642	Transcript	ENST00000647112.1	processed_transcript	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	downstream_gene_variant	CHD4	ENSG00000111642	Transcript	ENST00000647333.1	retained_intron	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	upstream_gene_variant	CHD4	ENSG00000111642	Transcript	ENST00000647394.1	retained_intron	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	splice_region_variant, intron_variant	CHD4	ENSG00000111642	Transcript	ENST00000647483.1	protein_coding	-	-	-
ENST00000642879.1:c.3820-1G>T	12:6583379-6583379	A	non_coding_transcript_exon_variant	CHD4	ENSG00000111642	Transcript	ENST00000647535.1	retained_intron	1/15	749	-

ESEfinder

Solo se obtiene un resultado positivo donde se encuentre la posición de estudio (g antes de las mayúsculas del exón) para las matrices 5'SS:

298 (-445)	cagGAGGAAGAGGAGGTAGAACGGGAAATC	5.85330	298 (-445)	cagGAGGAAGAGGAGGTAGAACGGGAAATC	-12.40900	298 (-445)	cagGAGGAAGAGGAGGTAGAACGGGAAATC	5.58090	298 (-445)	cagGAGGAAGAGGAGGTAGAACGGGAAATC	-13.57770

Si buscamos el resultado equivalente para la secuencia mutante, se observa que las puntuaciones se mantienen prácticamente las mismas:

298 (-445)	catGAGGAAGAGGAGGTAGAACGGGAAATC	5.23340	298 (-445)	catGAGGAAGAGGAGGTAGAACGGGAAATC	-10.74730	298 (-445)	catGAGGAAGAGGAGGTAGAACGGGAAATC	4.94100	298 (-445)	catGAGGAAGAGGAGGTAGAACGGGAAATC	-12.00900

Por lo tanto, esta región no estará alterando al *splicing*.

Sin embargo, hay dos resultados positivos para las matrices 3'SS (286 y 289):

286 (-457)	ctccctctgtcccagGAGGAAGAGGAGGTA	-10.05210	286 (-457)	ctccctctgtcccagGAGGAAGAGGAGGTA	12.52000	286 (-457)	ctccctctgtcccagGAGGAAGAGGAGGTA	-6.79730	286 (-457)	ctccctctgtcccagGAGGAAGAGGAGGTA	12.37840
287 (-456)	tccctctgtcccagGAGGAAGAGGAGGTAG	-21.69230	287 (-456)	tccctctgtcccagGAGGAAGAGGAGGTAG	-9.18820	287 (-456)	tccctctgtcccagGAGGAAGAGGAGGTAG	-19.92170	287 (-456)	tccctctgtcccagGAGGAAGAGGAGGTAG	-7.13990
288 (-455)	ccctctgtcccagGAGGAAGAGGAGGTAGA	-10.34150	288 (-455)	ccctctgtcccagGAGGAAGAGGAGGTAGA	-27.49110	288 (-455)	ccctctgtcccagGAGGAAGAGGAGGTAGA	-8.59920	288 (-455)	ccctctgtcccagGAGGAAGAGGAGGTAGA	-29.05540
289 (-454)	cctctgtcccagGAGGAAGAGGAGGTAGAA	-10.97690	289 (-454)	cctctgtcccagGAGGAAGAGGAGGTAGAA	2.64250	289 (-454)	cctctgtcccagGAGGAAGAGGAGGTAGAA	-7.77060	289 (-454)	cctctgtcccagGAGGAAGAGGAGGTAGAA	1.95420

Si buscamos el resultado equivalente para la secuencia mutante, se observa que las puntuaciones para el primer resultado se reducen drásticamente, mientras que para el segundo se mantienen similares:

286 (-457)	ctccctctgtcccatGAGGAAGAGGAGGTA	-14.02190	286 (-457)	ctccctctgtcccatGAGGAAGAGGAGGTA	0.43240	286 (-457)	ctccctctgtcccatGAGGAAGAGGAGGTA	-10.72050	286 (-457)	ctccctctgtcccatGAGGAAGAGGAGGTA	-0.57500
287 (-456)	tccctctgtcccatGAGGAAGAGGAGGTAG	-21.76050	287 (-456)	tccctctgtcccatGAGGAAGAGGAGGTAG	-4.62030	287 (-456)	tccctctgtcccatGAGGAAGAGGAGGTAG	-19.92640	287 (-456)	tccctctgtcccatGAGGAAGAGGAGGTAG	-5.48140
288 (-455)	ccctctgtcccatGAGGAAGAGGAGGTAGA	-11.43880	288 (-455)	ccctctgtcccatGAGGAAGAGGAGGTAGA	-21.07160	288 (-455)	ccctctgtcccatGAGGAAGAGGAGGTAGA	-9.62610	288 (-455)	ccctctgtcccatGAGGAAGAGGAGGTAGA	-22.12040
289 (-454)	cctctgtcccatGAGGAAGAGGAGGTAGAA	-11.43610	289 (-454)	cctctgtcccatGAGGAAGAGGAGGTAGAA	2.70890	289 (-454)	cctctgtcccatGAGGAAGAGGAGGTAGAA	-8.26440	289 (-454)	cctctgtcccatGAGGAAGAGGAGGTAGAA	1.95860

Por lo tanto, se está perdiendo un sitio 3'SS (*acceptor*) en la secuencia mutante, provocando una más que probable alteración del *splicing.*