

Ejemplo comparación de resultados predictores in silico

Cambio de estudio LMO7 c.3281G>A (chr13:75841932 G/A, rs148659145 o NM_001306080.2: c.3281G>A)

Exón 22 e intrones adyacentes:

```
agttacatctcagtcactcatgtgtagcttctttagtgttaaaaactcttaacatgtttt
ttgagtggagtcctgggccaactatagtttagtagcatctaaactgaatatatgtgtc
tatatgaattacggaaataaaacagatttagatggatttttttctcaggtcacctag
GAACTGATGGTCCTAAGCTCAAAACGCATGTCCTTGACCACACGGGAGCCCTCTCTTGCC
ACCTGGGAAGCTACCTGGAGTGAAGGGTCAAGTCTTCAGACAGAGAAGGAACCCGAGCA
GGACAAGAGGAGAGGAGACGCCACAAGAGGAAGTTGTTGATGAGGACCAGGAAAGAAG
CCGCAGGATCAGCTTGTTATTGAGAGAGAGAGAAATGGGAGCAACAGCTTCAGGAAGAG
CAAGAGCAAAAGCGGCTTCAGGCTGAGGCTGAGGAGCAGAAGCGTCCTGCCGAGGAGCAG
AAGCGCCAGGCAGAGATAGAGCGGGAACATCAGTCAGAATATACCAGTACAGGAG
gtatgtcccaagccagagggtacaaaggcttagctgtatccattcccacttcaaagg
caccgcttgctcttctgtttcctaaccaccattctccacacaagtgaataacaagccac
caaagagaacctttaaatataaaccttgccatgtcatcttgcttccacccataacac
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El cambio se encuentra en última línea del exón 22 (la **g** en color rojo y subrayada en amarillo).

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'
537		2	+	0.99	AGTACAGGAG	GTATGTCCCC	H	

Donor splice sites, complement strand

No donor site predictions above threshold.

Acceptor splice sites, direct strand

pos	5'→3'	phase	strand	confidence	5'	intron	exon	3'
180		1	+	0.31	GGTCACCTAG	GAAGT	GATGG	
474		0	+	0.07	TCCTGCGGAG	GAGCAGAAGC		
477		0	+	0.07	TGCGGAGGAG	CAGAAGCGCC		
480		0	+	0.07	GGAGGAGCAG	AAGCGCCAGG		

Acceptor splice sites, complement strand

pos 3'→5'	pos 5'→3'	phase	strand	confidence	5'	intron	exon	3'
467	250	2	-	0.97	TCCTCCGCAG	GACGCTTCTG		
255	462	0	-	0.25	TTCACCTCCAG	GTAGCTTCCC		
251	466	1	-	0.15	CTCCAGGTAG	CTTCCCAGGT		
243	474	0	-	0.23	AGCTTCCCAG	GTGGCAAGAG		

Donor splice sites, direct strand

pos	5'→3'	phase	strand	confidence	5'	exon	intron	3'
537		2	+	0.99	AGTACAGGAG	GTATGTCCCC	H	

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180		1	+	0.31	GGTCACCTAG	GAAGT	GATGG	
477		0	+	0.07	TGCGGAGGAG	CAGAAGCACC		
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Acceptor splice sites, complement strand

pos 3'→5'	pos 5'→3'	phase	strand	confidence	5'	intron	exon	3'
467	250	2	-	0.97	TCCTCCGCAG	GACGCTTCTG		
255	462	0	-	0.25	TTCACCTCCAG	GTAGCTTCCC		
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243	474	0	-	0.23	AGCTTCCCAG	GTGGCAAGAG		

Se pierde un sitio *acceptor* (en rojo) en la secuencia mutante. Este se encuentra dentro del exón y no participa en el *splicing* normal, por lo que es probable que la mutación no esté afectando al *splicing*.

Splice Site Prediction by Neural Network (NNSplice)

Donor site predictions for 10.42.3.123.573434.0 :

Start	End	Score	Exon	Intron
32	46	0.58	t t t a g t g	g t a a a a a c
530	544	1.00	a c a g g a g	g t a t g t c c

Donor site predictions for 10.42.0.139.573446.0 :

Start	End	Score	Exon	Intron
32	46	0.58	t t t a g t g	g t a a a a a c
530	544	1.00	a c a g g a g	g t a t g t c c

Acceptor site predictions for 10.42.3.123.573434.0 : Acceptor site predictions for 10.42.0.139.573446.0 :

Start	End	Score	Intron	Exon	Start	End	Score	Intron	Exon
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Spliceman

Point mutation	Wildtype (wt)	Mutation (mt)	L1 distance	Ranking (L1)
gaagc(g/a)ccagg	gccagg	accagg	29861	73%

CRYP-SKIP

Parece que hay un sitio críptico de *splicing* dentro del propio exón, pero el cambio de interés (la primera *a* en minúsculas detrás de las mayúsculas, que indican el exón) no lo toma en consideración, por lo que no debe considerar que tenga algún efecto en el *splicing*.

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	29	88	acatcagtc		-2.89413913121	0.469879518072	27	9	12	-2.1769216		
wt	29	84	cagtcagaa		-2.51429962648	0.46835443038	23	9	12	-1.7754958		
wt	29	31	ggcttagct		-1.12253480167	0.615384615385	5	17	28	0.10535571		
mut	29	88	acatcagtc		-2.89413913121	0.469879518072	27	9	12	-2.1769216		
mut	29	84	cagtcagaa		-2.51429962648	0.46835443038	23	9	12	-1.7754958		
mut	29	31	ggcttagct		-1.12253480167	0.615384615385	5	17	28	0.10535571		

Variant Effect Predictor tool

ENST00000321797.12:c.3281G>A	13:75841932-75841932	A	missense_variant	LMO7	ENSG00000136153	Transcript	ENST00000321797.12	protein_coding	22/29	4002	3281	1094	R/H	CGC/CAC	rs148659145
ENST00000321797.12:c.3281G>A	13:75841932-75841932	A	missense_variant	LMO7	ENSG00000136153	Transcript	ENST00000341547.8	protein_coding	23/30	4394	3134	1045	R/H	CGC/CAC	rs148659145
ENST00000321797.12:c.3281G>A	13:75841932-75841932	A	missense_variant	LMO7	ENSG00000136153	Transcript	ENST00000357063.7	protein_coding	25/32	5396	4091	1364	R/H	CGC/CAC	rs148659145
ENST00000321797.12:c.3281G>A	13:75841932-75841932	A	missense_variant	LMO7	ENSG00000136153	Transcript	ENST00000377499.9	protein_coding	22/24	3313	2978	993	R/H	CGC/CAC	rs148659145
ENST00000321797.12:c.3281G>A	13:75841932-75841932	A	missense_variant	LMO7	ENSG00000136153	Transcript	ENST00000377534.8	protein_coding	24/31	4308	3980	1327	R/H	CGC/CAC	rs148659145
ENST00000321797.12:c.3281G>A	13:75841932-75841932	A	missense_variant	LMO7	ENSG00000136153	Transcript	ENST00000447038.5	protein_coding	16/18	3006	3008	1003	R/H	CGC/CAC	rs148659145
ENST00000321797.12:c.3281G>A	13:75841932-75841932	A	missense_variant	LMO7	ENSG00000136153	Transcript	ENST00000465261.6	protein_coding	21/27	4041	3281	1094	R/H	CGC/CAC	rs148659145
ENST00000321797.12:c.3281G>A	13:75841932-75841932	A	downstream_gene_variant	LMO7	ENSG00000136153	Transcript	ENST00000485987.1	processed_transcript	-	-	-	-	-	-	rs148659145
ENST00000321797.12:c.3281G>A	13:75841932-75841932	A	downstream_gene_variant	LMO7	ENSG00000136153	Transcript	ENST00000524651.5	protein_coding	-	-	-	-	-	-	rs148659145
ENST00000321797.12:c.3281G>A	13:75841932-75841932	A	downstream_gene_variant	LMO7	ENSG00000136153	Transcript	ENST00000525107.6	protein_coding	-	-	-	-	-	-	rs148659145
ENST00000321797.12:c.3281G>A	13:75841932-75841932	A	missense_variant	LMO7	ENSG00000136153	Transcript	ENST00000525914.5	protein_coding	7/8	786	788	263	R/H	CGC/CAC	rs148659145
ENST00000321797.12:c.3281G>A	13:75841932-75841932	A	missense_variant	LMO7	ENSG00000136153	Transcript	ENST00000526202.5	protein_coding	19/26	2992	2912	971	R/H	CGC/CAC	rs148659145
ENST00000321797.12:c.3281G>A	13:75841932-75841932	A	downstream_gene_variant	LMO7	ENSG00000136153	Transcript	ENST00000533809.2	protein_coding	-	-	-	-	-	-	rs148659145

ESEfinder

No se detectan cambios para las matrices 5' y 3' SS ni cambios destacables en las ESE.

458 (-259)	AGAAGCG	-2.58197	458 (-259)	AGAAGCG	-1.49224	458 (-259)	AGAAGCGT	-4.48709	458 (-259)	AGAAGCG	0.02403
459 (-258)	GAAGCGT	-0.56005	459 (-258)	GAAGCGT	-1.20976	459 (-258)	GAAGCGTC	-0.79349	459 (-258)	GAAGCGT	-4.28968
460 (-257)	AAGCGTC	-2.08666	460 (-257)	AAGCGTC	-1.69754	460 (-257)	AAGCGTCC	-3.50014	460 (-257)	AAGCGTC	-5.02047
461 (-256)	AGCGTCC	-6.58000	461 (-256)	AGCGTCC	-4.03985	461 (-256)	AGCGTCCT	-1.66240	461 (-256)	AGCGTCC	-1.06062
462 (-255)	GCGTCCT	-2.34175	462 (-255)	GCGTCCT	-2.09415	462 (-255)	GCGTCCTG	1.87716	462 (-255)	GCGTCCT	-5.59770
463 (-254)	CGTCCTG	-0.00851	463 (-254)	CGTCCTG	1.24579	463 (-254)	CGTCCTGC	-2.45497	463 (-254)	CGTCCTG	0.33936
464 (-253)	GTCCTGC	-1.83466	464 (-253)	GTCCTGC	-1.57053	464 (-253)	GTCCTGCG	3.48499	464 (-253)	GTCCTGC	0.02409
458 (-259)	AGAAGCG	-2.58197	458 (-259)	AGAAGCG	-1.49224	458 (-259)	AGAAGCGT	-4.48709	458 (-259)	AGAAGCG	0.02403
459 (-258)	GAAGCGT	-0.56005	459 (-258)	GAAGCGT	-1.20976	459 (-258)	GAAGCGTC	-0.79349	459 (-258)	GAAGCGT	-4.28968
460 (-257)	AAGCGTC	-2.08666	460 (-257)	AAGCGTC	-1.69754	460 (-257)	AAGCGTCC	-3.50014	460 (-257)	AAGCGTC	-5.02047
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462 (-255)	GCGTCCT	-2.34175	462 (-255)	GCGTCCT	-2.09415	462 (-255)	GCGTCCTG	1.87716	462 (-255)	GCGTCCT	-5.59770
463 (-254)	CGTCCTG	-0.00851	463 (-254)	CGTCCTG	1.24579	463 (-254)	CGTCCTGC	-2.45497	463 (-254)	CGTCCTG	0.33936
464 (-253)	GTCCTGC	-1.83466	464 (-253)	GTCCTGC	-1.57053	464 (-253)	GTCCTGCG	3.48499	464 (-253)	GTCCTGC	0.02409

EX-SKIP

Seq	PESS (count)	FAS-ESS hex2 (count)	FAS-ESS hex3 (count)	IIE (count)	IIE (sum)	NI-ESS trusted (count)	NI-ESS all (sum)	PESE (count)	RESCUE -ESE (count)	EIE (count)	EIE (sum)	NI-ESE trusted (count)	NI-ESE all (sum)	ESS (total)	ESE (total)	ESS/ESE (ratio)
wt	5	8	4	44	585.5495	20	-25.7392	49	77	172	2371.1191	203	238.9277	81	501	0.16
mut	5	8	4	44	585.5495	20	-25.7392	50	78	174	2392.5882	207	242.0713	81	509	0.16

Both alleles have a comparable chance of exon skipping.

HOT-SKIP

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agttacatctcagtcactcatgtgtagcttccttagtggtaaaaactcttaacatgttttttgagtggagtcctgggcca
actatagtttagtagcatctaaaaactgaatatatatgtgtctatatgaaattacggaaataaacagatttagatggatttc
ttttttcactggtcacctagGAACTGATGGTCCTAAGCTCAAACAGCATGTCTCTGACCACACGGGAGCCCTCTCTTGCC
ACCTGGGAAGCTACCTGGAGTGAGGGTCCAAGTCTTCAGACAGAGAAGGAACCCGAGCAGGAGAAGAGGAGAGGAGACA
GCCACAAGAGGAAGTTGTTTCATGAGGACCAAGGAAAGAAGCCGCAGGATCAGCTTGTTATTGAGAGAGAGAGGAATGGG
AGCAACAGCTTCAGGAAGAGCAAGAGCAAAAGCGGCTTCAGGCTGAGGCTGAGGAGCAGAAGCGTCCTGCGGAGGAGCAG
AAGCGCCAGGCAGAGATAGAGCGGGAAACATCAGTCAGAAATATACAGTACAGGAGgtatgtccccacagccagagggtg
caaaggcttagctgtatccattccccacttcaaaggcaccgcttgctcttcctgtttcctaccacccattctccacaca
agtgaaataaagccaccaaaagagaacctttaaatcataaaacctgcatgtcatcttgcttcaccacccataaacac

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