

## Ejemplo comparación de resultados predictores in silico

Cambio de estudio NF2 c.1488-1G>C (chr22:29681438 G/C, CS961644 o NM\_000268.4:c.1488-1G>C)

Exón 15 e intrones adyacentes:

```
cctcagccacccaaagtactgggattacagggtggagccacgctgcctggcctcctaact  
gttttattttaatectaaccacggccctgtgatcattatcccaattttaatgatgaggaaa  
oattcatagcatttaagtaccagcccaagctcccatggcctgtgagtggccaagtagag  
acgtgaaacccagggcctcaaaccctagatcgacaccaagcagcttgtgggccgagagc  
acctgagcggtgtctcactgtctgccaagccctgatgcatgatacccctctgcccgcag  
AGTGCATAACATGGAAAAGAGCAAGCATCTGCAGGAGCAGCTCAATGAACTCAAGACAGA  
AATCGAGGCCTTGAACTGAAAGAGAGGGAGACAGCTCTGGATATTCTGCACAATGAGAA  
CTCCGACAGGGGTGGCAGCAGCAAGCAATACCATTAAAAAG  
gtaccagggtctcttttcttgtattttgctgatcaggaccatcattaatgaaatgtgcgg  
ttggcatctggtttcctcagtagccaaagtcaactagactattggcatcttttgtatgtact  
tagttgaggaaattttttaacctatgccagaaagggaatttaattctgccaatgactatga  
acccattggctggatggaagttgagcttgtgtttaaagctctgatgtaatgaaccctgt  
tacagggtggatttttgagccctcatttagcagtaaagcataatgcccttttaagccaagg
```

El cambio se encuentra en la posición justo antes del exón 15 (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'
163		1	+	0.44	CCCATGGCCT	^	GTGAGTGGCC	
464		0	+	0.94	CATTA AAAAG	^	GTACCCAGGG	H

### Donor splice sites, complement strand

pos 3'→5'	pos 5'→3'	phase	strand	confidence	5'	exon	intron	3'
286	478	0	-	0.70	CGGCAAGAGG	^	GTATCATGCA	

### Acceptor splice sites, direct strand

pos	5'→3'	phase	strand	confidence	5'	intron	exon	3'
270		1	+	0.07	TCTGCCCAAG	^	CCCTGATGCA	
300		2	+	0.76	TTGCCGGCAG	^	AGTGGAAATAC	
302		1	+	0.34	GCCGGCAGAG	^	TGGAATACAT	
319		0	+	0.19	CATGGAAAAG	^	AGCAAGCATC	
334		0	+	0.07	GCATCTGCAG	^	GAGCAGCTCA	
337		0	+	0.07	TCTGCAGGAG	^	CAGCTCAATG	

### Acceptor splice sites, complement strand

pos 3'→5'	pos 5'→3'	phase	strand	confidence	5'	intron	exon	3'
477	287	2	-	0.07	TACAAGAAAG	^	AGACCCTGGG	
377	387	0	-	0.85	TCTCTTTCAG	^	TTTCAAGGCC	
370	394	1	-	0.17	CAGTTTCAAG	^	GCCTCGATTT	
161	603	1	-	0.27	CCACTCACAG	^	GCCATGGGAG	

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pos	5'→3'	phase	strand	confidence	5'	exon	intron	3'
163		1	+	0.44	CCCATGGCCT	^	GTGAGTGGCC	
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pos 3'→5'	pos 5'→3'	phase	strand	confidence	5'	exon	intron	3'
286	478	0	-	0.70	CGGCAAGAGG	^	GTATCATGCA	

### Acceptor splice sites, direct strand

pos	5'→3'	phase	strand	confidence	5'	intron	exon	3'
270		2	+	0.17	TCTGCCCAAG	^	CCCTGATGCA	
302		1	+	0.76	GCCGGCAGAG	^	TGGAATACAT	
319		0	+	0.19	CATGGAAAAG	^	AGCAAGCATC	
334		0	+	0.07	GCATCTGCAG	^	GAGCAGCTCA	

### Acceptor splice sites, complement strand

pos 3'→5'	pos 5'→3'	phase	strand	confidence	5'	intron	exon	3'
477	287	2	-	0.07	TACAAGAAAG	^	AGACCCTGGG	
377	387	0	-	0.85	TCTCTTTCAG	^	TTTCAAGGCC	
370	394	1	-	0.17	CAGTTTCAAG	^	GCCTCGATTT	
161	603	1	-	0.27	CCACTCACAG	^	GCCATGGGAG	

Se pierden dos sitios *acceptor* (en rojo) en la secuencia mutante. El primero coincide con el sitio *acceptor* del exón, mientras que el segundo está dentro del exón, por lo que no lo tendremos en cuenta. Se puede concluir que se pierde el sitio *acceptor* del exón 15 y pueden ocurrir dos cosas: que los otros sitios *acceptor* no sean útiles y se tenga que emplear el del otro exón, haciendo que se pierda el exón completo, o que sirvan los otros dos, lo que haría que se incluyeran 30 nt o se perdieran 2 (más probable por su nivel de *confidence* de 0.76), 19 o 34 nt, dependiendo de cuál se use.

Splice Site Prediction by Neural Network (NNSplice)

Donor site predictions for 85.53.15.54.29136.0 :

Start	End	Score	Exon	Intron
156	170	0.76	atggcct	<b>gt</b> gagtgg
457	471	0.90	taaaaag	<b>gt</b> accag

Donor site predictions for 85.53.15.54.29148.0 :

Start	End	Score	Exon	Intron
156	170	0.76	atggcct	<b>gt</b> gagtgg
457	471	0.90	taaaaag	<b>gt</b> accag

Acceptor site predictions for 85.53.15.54.29136.0 :

Start	End	Score	Intron	Exon
479	519	0.52	ttcttgatatttctgatc	<b>ag</b> gaccatcattaatgaaatgt
523	563	0.62	gttgcatctggtttcctc	<b>ag</b> tagccaagtcactagactat

Acceptor site predictions for 85.53.15.54.29148.0 :



Start	End	Score	Intron	Exon
479	519	0.52	ttcttgatatttctgatc	<b>ag</b> gaccatcattaatgaaatgt
523	563	0.62	gttgcatctggtttcctc	<b>ag</b> tagccaagtcactagactat

No hay cambios, por lo que la mutación no estará afectando al *splicing*.

Spliceman



Point mutation	Wildtype (wt)	Mutation (mt)	L1 distance	Ranking (L1)
cggca(g/c)agtgg	gcagag	gcacag	28384	64%

## Human Splicing Finder

 Broken WT Acceptor Site		Alteration of the WT Acceptor site, most probably affecting splicing	
Algorithm/Matix	position	sequences	variation
MaxEnt Acceptor site	chr22:29681419	- REF : TGATACCCTCTTGCCGGCAGAGT - ALT : TGATACCCTCTTGCCGGCACAGT	6.13 > -1.94 => -131.65%
HSF Acceptor site (matrix AG)	chr22:29681427	- REF : TCTTGCCGGCAGAG - ALT : TCTTGCCGGCACAG	82.88 > 55.01 => -33.63%
 New Acceptor splice site		Activation of a cryptic Acceptor site. Potential alteration of splicing	
Algorithm/Matix	position	sequences	variation
MaxEnt Acceptor site	chr22:29681421	- REF : ATACCCTCTTGCCGGCAGAGTGG - ALT : ATACCCTCTTGCCGGCACAGTGG	-13.23 > 5.21 => 139.38%
HSF Acceptor site (matrix AG)	chr22:29681429	- REF : TTGCCGGCAGAGTG - ALT : TTGCCGGCACAGTG	70.79 > 78.44 => 10.81%

wt	agez	ss_dist	bp_seq	bp_scr	y_cont	ppt_off	ppt_len	ppt_scr	svm_scr	seq_id	agez	ss_dist	bp_seq	bp_scr	y_cont	ppt_off	ppt_len	ppt_scr	svm_scr		
wt	22	484	tcattatcc		-2.23084160406	0.469728601253	1	10	17	-0.22492245	mut	22	484	tcattatcc		-2.23084160406	0.471816283925	1	10	17	-0.22424819
wt	22	473	attttaagt		-4.32054431873	0.463675213675	143	17	24	-9.9682456	mut	22	473	attttaagt		-4.32054431873	0.465811965812	143	17	24	-9.9675555
wt	22	472	ttttaatga		-0.243107202799	0.464668094218	142	17	24	-8.3081172	mut	22	472	ttttaatga		-0.243107202799	0.466809421842	142	17	24	-8.3074257
wt	22	469	taatgatga		0.123146405177	0.465517241379	139	17	24	-7.9745424	mut	22	469	taatgatga		0.123146405177	0.467672413793	139	17	24	-7.9738464
wt	22	466	tgatgagga		-2.43834989897	0.466377440347	136	17	24	-8.7873166	mut	22	466	tgatgagga		-2.43834989897	0.468546637744	136	17	24	-8.786616
wt	22	455	cattcatag		-0.353004124316	0.468888888889	125	17	24	-7.2737124	mut	22	455	cattcatag		-0.353004124316	0.471111111111	125	17	24	-7.2729947
wt	22	446	catttaagt		-3.28084521259	0.467120181406	116	17	24	-7.8509869	mut	22	446	catttaagt		-3.28084521259	0.469387755102	116	17	24	-7.8502546
wt	22	445	atttaagtg		-1.48843476894	0.468181818182	115	17	24	-7.0855324	mut	22	445	atttaagtg		-1.48843476894	0.470454545455	115	17	24	-7.0847983
wt	22	441	aagtgacca		1.35364911614	0.470183486239	111	17	24	-5.7188824	mut	22	441	aagtgacca		1.35364911614	0.47247706422	111	17	24	-5.7181416
wt	22	415	ctgtgagtg		-0.269837797909	0.463414634146	85	17	24	-4.7109841	mut	22	415	ctgtgagtg		-0.269837797909	0.465835658537	85	17	24	-4.7181963
wt	22	395	acgtgaacc		-0.686368508003	0.471794871795	65	17	24	-3.6054025	mut	22	395	acgtgaacc		-0.686368508003	0.474358974359	65	17	24	-3.6045744
wt	22	382	gcctcaaac		0.362615440381	0.466843501326	52	17	24	-2.3733967	mut	22	382	gcctcaaac		0.362615440381	0.46949602122	52	17	24	-2.37254
wt	22	335	acctgagcc		0.63893134935	0.466666666667	5	17	24	0.70975802	mut	22	335	acctgagcc		0.63893134935	0.469699696969	5	17	24	0.71073672
wt	22	324	gtctcactg		1.93922110443	0.460815047022	1	10	15	1.3863437	mut	22	324	gtctcactg		1.93922110443	0.46394984323	1	10	15	1.3873562
wt	22	305	ccctgatgc		3.15844546017	0.45	8	12	20	1.4637256	mut	22	305	ccctgatgc		3.15844546017	0.453333333333	8	12	20	1.4648022
wt	22	298	gcattgatac		-0.237507075742	0.450511945392	1	12	20	0.57730336	mut	22	298	gcattgatac		-0.237507075742	0.453924914676	1	12	20	0.57840565
wt	22	237	agctcaatg		-0.130224516128	0.461206896552	130	20	41	-7.347087	mut	22	237	agctcaatg		-0.130224516128	0.461206896552	130	20	41	-7.347087
wt	22	233	caatgaact		0.256311162308	0.464912280702	126	20	41	-6.94135	mut	22	233	caatgaact		0.256311162308	0.464912280702	126	20	41	-6.94135
wt	22	228	aactcaaga		-1.14477480684</																

## Variant Effect Predictor tool (cambio afectando al sitio *acceptor*, cambios en el *splicing*)

ENST00000403435.5:c.1488-1G>C	<a href="#">22-29681438-29681438</a>	C	 splice_acceptor_variant	NF2	<a href="#">ENSG00000186575</a>	Transcript	<a href="#">ENST00000334961.11</a>	protein_coding	<a href="#">CS961644</a> , <a href="#">CS982294</a> , <a href="#">COSV58520503</a> , <a href="#">COSV58526843</a>
ENST00000403435.5:c.1488-1G>C	<a href="#">22-29681438-29681438</a>	C	 splice_acceptor_variant	NF2	<a href="#">ENSG00000186575</a>	Transcript	<a href="#">ENST00000338641.10</a>	protein_coding	<a href="#">CS961644</a> , <a href="#">CS982294</a> , <a href="#">COSV58520503</a> , <a href="#">COSV58526843</a>
ENST00000403435.5:c.1488-1G>C	<a href="#">22-29681438-29681438</a>	C	 splice_acceptor_variant	NF2	<a href="#">ENSG00000186575</a>	Transcript	<a href="#">ENST00000353887.8</a>	protein_coding	<a href="#">CS961644</a> , <a href="#">CS982294</a> , <a href="#">COSV58520503</a> , <a href="#">COSV58526843</a>
ENST00000403435.5:c.1488-1G>C	<a href="#">22-29681438-29681438</a>	C	 splice_acceptor_variant	NF2	<a href="#">ENSG00000186575</a>	Transcript	<a href="#">ENST00000361166.9</a>	protein_coding	<a href="#">CS961644</a> , <a href="#">CS982294</a> , <a href="#">COSV58520503</a> , <a href="#">COSV58526843</a>
ENST00000403435.5:c.1488-1G>C	<a href="#">22-29681438-29681438</a>	C	 splice_acceptor_variant	NF2	<a href="#">ENSG00000186575</a>	Transcript	<a href="#">ENST00000361452.8</a>	protein_coding	<a href="#">CS961644</a> , <a href="#">CS982294</a> , <a href="#">COSV58520503</a> , <a href="#">COSV58526843</a>
ENST00000403435.5:c.1488-1G>C	<a href="#">22-29681438-29681438</a>	C	 splice_acceptor_variant	NF2	<a href="#">ENSG00000186575</a>	Transcript	<a href="#">ENST00000361676.8</a>	protein_coding	<a href="#">CS961644</a> , <a href="#">CS982294</a> , <a href="#">COSV58520503</a> , <a href="#">COSV58526843</a>
ENST00000403435.5:c.1488-1G>C	<a href="#">22-29681438-29681438</a>	C	 splice_acceptor_variant	NF2	<a href="#">ENSG00000186575</a>	Transcript	<a href="#">ENST00000397789.3</a>	protein_coding	<a href="#">CS961644</a> , <a href="#">CS982294</a> , <a href="#">COSV58520503</a> , <a href="#">COSV58526843</a>
ENST00000403435.5:c.1488-1G>C	<a href="#">22-29681438-29681438</a>	C	 splice_acceptor_variant	NF2	<a href="#">ENSG00000186575</a>	Transcript	<a href="#">ENST00000403435.5</a>	protein_coding	<a href="#">CS961644</a> , <a href="#">CS982294</a> , <a href="#">COSV58520503</a> , <a href="#">COSV58526843</a>
ENST00000403435.5:c.1488-1G>C	<a href="#">22-29681438-29681438</a>	C	 splice_acceptor_variant	NF2	<a href="#">ENSG00000186575</a>	Transcript	<a href="#">ENST00000403999.7</a>	protein_coding	<a href="#">CS961644</a> , <a href="#">CS982294</a> , <a href="#">COSV58520503</a> , <a href="#">COSV58526843</a>
ENST00000403435.5:c.1488-1G>C	<a href="#">22-29681438-29681438</a>	C	 intron_variant	NF2	<a href="#">ENSG00000186575</a>	Transcript	<a href="#">ENST00000413209.6</a>	protein_coding	<a href="#">CS961644</a> , <a href="#">CS982294</a> , <a href="#">COSV58520503</a> , <a href="#">COSV58526843</a>
ENST00000403435.5:c.1488-1G>C	<a href="#">22-29681438-29681438</a>	C	 intron_variant, NMD_transcript_variant	NF2	<a href="#">ENSG00000186575</a>	Transcript	<a href="#">ENST00000432151.5</a>	nonsense_mediated_decay	<a href="#">CS961644</a> , <a href="#">CS982294</a> , <a href="#">COSV58520503</a> , <a href="#">COSV58526843</a>
ENST00000403435.5:c.1488-1G>C	<a href="#">22-29681438-29681438</a>	C	 splice_acceptor_variant, NMD_transcript_variant	NF2	<a href="#">ENSG00000186575</a>	Transcript	<a href="#">ENST00000672461.1</a>	nonsense_mediated_decay	<a href="#">CS961644</a> , <a href="#">CS982294</a> , <a href="#">COSV58520503</a> , <a href="#">COSV58526843</a>
ENST00000403435.5:c.1488-1G>C	<a href="#">22-29681438-29681438</a>	C	 splice_acceptor_variant, NMD_transcript_variant	NF2	<a href="#">ENSG00000186575</a>	Transcript	<a href="#">ENST00000672805.1</a>	nonsense_mediated_decay	<a href="#">CS961644</a> , <a href="#">CS982294</a> , <a href="#">COSV58520503</a> , <a href="#">COSV58526843</a>
ENST00000403435.5:c.1488-1G>C	<a href="#">22-29681438-29681438</a>	C	 splice_acceptor_variant	NF2	<a href="#">ENSG00000186575</a>	Transcript	<a href="#">ENST00000672896.1</a>	protein_coding	<a href="#">CS961644</a> , <a href="#">CS982294</a> , <a href="#">COSV58520503</a> , <a href="#">COSV58526843</a>
ENST00000403435.5:c.1488-1G>C	<a href="#">22-29681438-29681438</a>	C	 splice_acceptor_variant, NMD_transcript_variant	NF2	<a href="#">ENSG00000186575</a>	Transcript	<a href="#">ENST00000673312.1</a>	nonsense_mediated_decay	<a href="#">CS961644</a> , <a href="#">CS982294</a> , <a href="#">COSV58520503</a> , <a href="#">COSV58526843</a>

ESEfinder

Solo se obtiene un resultado positivo con la posición de interés en las matrices 3'SS:

<div>286 (-478)</div>	ccctcttgccggcagAGTGGAAATACATGGA	-22.58050	<div>286 (-478)</div>	ccctcttgccggcagAGTGGAAATACATGGA	7.19800	<div>286 (-478)</div>	ccctcttgccggcagAGTGGAAATACATGGA	-21.12290	<div>286 (-478)</div>	ccctcttgccggcagAGTGGAAATACATGGA	7.43930
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Sin embargo, estas puntuaciones en la secuencia mutante son negativas:

<div>286 (-478)</div>	ccctcttgccggcacAGTGGAAATACATGGA	-27.44950	<div>286 (-478)</div>	ccctcttgccggcacAGTGGAAATACATGGA	-3.08740	<div>286 (-478)</div>	ccctcttgccggcacAGTGGAAATACATGGA	-25.94500	<div>286 (-478)</div>	ccctcttgccggcacAGTGGAAATACATGGA	-2.74910
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Por lo tanto, la mutación está generando la pérdida de un sitio *acceptor*, afectando al *splicing*.