

## Ejemplo comparación de resultados predictores in silico

Cambio de estudio RAB11A c.41-1373A>G (chr 15:65875959 A/G, COSV56041717 o NM\_001206836.2: c.41-1373A>G)

Exón 2 e intrones adyacentes:

```
ccacatccagctctcttagtaaggaatggcttaccccttggccttatttttgatatgt
ttgattttctagtcctgatgtctaaatcaattagctagtttagataagaagaagtccttag
tagtcaaaagtaggaacgaatgtaatgtttcttgaaatgctgataccaattttacaaag
gaaaaaatactagctcagattctataaacgtgtgaactcattttgaaactttaaatcag
atagaaaatgttttaaaacaacttaactgcctgtttctctcattgtttttgaaattcttaa
gctgaatacacacttttgagtgaagaaatctccatgtatctagttagtaataaactgct
gctatagaatacacaggaaagcaaatcatcctaacaagacctattttcaaaaaaatcat
ggagtcataagtgaagtatatgcccctcttctgtttgtttgtattttattttatttttt
tttaagacagagtcctcagtggtcgcccgaggtggagtgagtgacacaaatctcgccca
ctgcaacctctgcttcggggttcaagctattctcatgcctcaggccacacagtagttgg
gattacatgctgcaccaccacacctggctaatttttgtatttttatagagatggggttt
tcaccatgttgccaggtctagctcgaactcttgccagcaatccgcacaccttgcc
cttccaaaatgctgggattacaggtgtgagccaccacaaccggcacttgctttgttttg
actcacagtatgtcagaattattagtttctaaacacccctgttttatctgtagtggttg
ggtttttttggcagaggggtctctaactttgaaccaatctgataaatgcaactatttaaag
gtcatttttagtttctgtgatctgtatggtgatgtttataaacctatgcctcatatgtta
aatgtttttcattttgcttaggtctcagtcctgaggtcacaccagtccttgatgaaaag
aagtatattcattttgctcttccctaccactaacagcctttgtgttttcatttctgat
acattacattttaatatcataaattattcatttaggttaggatttatcttgaaagtgtgt
atcttctaagtagtaagattggcatagcattgacggtaattaatgcctgtcatcaatgc
caagggcatctgtgattaaaactgcatgtttaagacagggtagcgtatctgtgctaagca
gatgatatgtggaagtcatgcaggtagaaacaggtgaaaagaataaaatcggtttttattg
atttggaactggtcaagaacatgctgttcaacccccaaccccaattcctttttaaaagtc
atatacccttatttttcttgcctttattactctgaagccaacttcattctgttgaaagca
tagtgggtgtctgaatatgtttgcctcatctcatctgacattgaattctttgtcttccag
TTGTCCTTATTGGAGATTCTGGTGTGGAAAGAGTAATCTCTGTCTCGATTTACTCGAA
ATGAGTTTAAATCTGGAAAGCAAGAGCACCATTTGGAGTAGAGTTTGCAACAAGAGCATCC
AGGTGATGGA AAAACAATAAAGGCACAGATATGGGACACAGCAGGCAAGAGCATATC
GAGCTATAACATCAGC
gtaagtctcatggttttttaagttctgtgaaatgggttgccatcgagtgaattagctgact
tttgggtattggaaaaagaactgtttgtttttttcttttaagaattctagtagtaggaatcc
ttagaaattttattttaaagtatgttttttaaaactcatgatccatattttgagttcttct
```

El cambio se encuentra en la tercera línea del intrón 1 (la **a** 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'
1284	1	+	0.39	AGTCATGCAG	^GTAGAAACAG		
1623	0	+	0.55	AAGCATCCAG	^GTTGATGGAA		
1697	1	+	0.62	TAACATCAGC	^GTAAGTCTCA		

### Donor splice sites, complement strand

pos 3'→5'	pos 5'→3'	phase	strand	confidence	5'	exon	intron	3'
1386	491	2	-	0.51	GAAAAATAAG	^GTATATGACT		
624	1253	1	-	0.32	AATTAGCCAG	^GTGTGGTGGT		

### Acceptor splice sites, direct strand

pos 5'→3'	phase	strand	confidence	5'	intron	exon	3'
649	2	+	0.31	ATTTTTATAG	^AGATGGGGTT		
981	0	+	0.77	TTTTGCCTAG	^GTCTCAGTCC		
1500	1	+	0.96	GTCTTTCCAG	^TTGTCCTTAT		
1515	1	+	0.17	CTTATTGGAG	^ATTCTGGTGT		
1796	0	+	0.00	TTCTTTTAAG	^AATCTAGTA		

### Acceptor splice sites, complement strand

pos 3'→5'	pos 5'→3'	phase	strand	confidence	5'	intron	exon	3'
1572	305	0	-	0.65	TGCTTTCCAG	^ATTAAACTCA		
1542	335	0	-	0.07	ATCGAGACAG	^GAGATTACTC		
1519	358	2	-	0.48	CCAACACCAG	^AATCTCCAAT		

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1515	1	+	0.17	CTTATTGGAG	^ATTCTGGTGT		
1796	0	+	0.00	TTCTTTTAAG	^AATCTAGTA		

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1519	358	2	-	0.48	CCAACACCAG	^AATCTCCAAT		

## Splice Site Prediction by Neural Network (NNSplice)

### Donor site predictions for wt :

Start	End	Score	Exon	Intron
737	751	0.85	attacag	gtgtgagc
781	795	0.58	actcaca	gtatgtca
1170	1184	0.97	attgacg	gtaattaa
1277	1291	0.87	catgcag	gtagaaac
1690	1704	0.99	catcagc	gtaagtct

### Acceptor site predictions for wt :

Start	End	Score	Intron	Exon
52	92	0.64	tttgtatgtttgattttct	agtcctgatgtctaaatcaatt
466	506	0.91	tattttattttttttta	agacagagtctcagtgtgtcgc
564	604	0.84	caagctattctcatgcctc	agggccacaaagtagttgggatt
629	669	0.99	ctaatttttgatttttat	agagatggggttttcaccatgt
768	808	0.55	tgctttggttttgactcac	agtatgtcagaattattagttt
813	853	0.85	ataccccctgtttatctgt	agtggttggttttttggca
834	874	0.81	tggcttgggttttttggc	agaggggtctctaactttgaac
961	1001	0.99	aatgtttttcattttgcct	aggtctcagtcctgaggtcaca
1100	1140	0.51	taaattattcatttagttt	aggatttatctttgaagttgtt
1131	1171	0.52	tgaagttgttatcttctaa	agtagtaagattggcatagcat
1480	1520	0.89	ttgaattctttgtctttcc	agttgtccttattggagattct
1776	1816	0.98	ctgttgttttttctttta	agaattctagtattaggaatcc

### Donor site predictions for mut :

Start	End	Score	Exon	Intron
737	751	0.85	attacag	gtgtgagc
781	795	0.58	actcaca	gtatgtca
1170	1184	0.97	attgacg	gtaattaa
1277	1291	0.87	catgcag	gtagaaac
1690	1704	0.99	catcagc	gtaagtct

### Acceptor site predictions for mut :

Start	End	Score	Intron	Exon
52	92	0.64	tttgtatgtttgattttct	agtcctgatgtctaaatcaatt
466	506	0.91	tattttattttttttta	agacagagtctcagtgtgtcgc
564	604	0.84	caagctattctcatgcctc	agggccacaaagtagttgggatt
629	669	0.99	ctaatttttgatttttat	agagatggggttttcaccatgt
768	808	0.55	tgctttggttttgactcac	agtatgtcagaattattagttt
813	853	0.85	ataccccctgtttatctgt	agtggttggttttttggca
834	874	0.81	tggcttgggttttttggc	agaggggtctctaactttgaac
961	1001	0.99	aatgtttttcattttgcct	aggtctcagtcctgaggtcaca
1100	1140	0.51	taaattattcatttagttt	aggatttatctttgaagttgtt
1131	1171	0.52	tgaagttgttatcttctaa	agtagtaagattggcatagcat
1480	1520	0.89	ttgaattctttgtctttcc	agttgtccttattggagattct
1776	1816	0.98	ctgttgttttttctttta	agaattctagtattaggaatcc

Spliceman

Point mutation	Wildtype (wt)	Mutation (mt)	L1 distance	Ranking (L1)
gtcaa(a/g)agtag	tcaaaa	tcaaga	29141	69%

Human Splicing Finder

<div><div></div>Alteration of auxiliary sequences</div>		Significant alteration of ESE / ESS motifs ratio (3)	
Algorithm/Matix	position	sequence	
ESE_SRp40 (New ESE Site)	chr15:65875953	AGTCAAG	
RESCUE ESE (New ESE Site)	chr15:65875954	GTCAAG	
EIE (New ESE Site)	chr15:65875954	GTCAAG	
RESCUE ESE (New ESE Site)	chr15:65875955	TCAAGA	
EIE (New ESE Site)	chr15:65875955	TCAAGA	
ESE_SRp40 (ESE Site Broken)	chr15:65875955	TCAAAAG	
ESE_9G8 (New ESE Site)	chr15:65875956	CAAGAG	
RESCUE ESE (ESE Site Broken)	chr15:65875956	CAAAAG	
ESS_hnRNPA1 (New ESS Site)	chr15:65875957	AAGAGT	
EIE (ESE Site Broken)	chr15:65875957	AAAAGT	
ESE_9G8 (New ESE Site)	chr15:65875959	GAGTAG	

<div><div></div>New Acceptor splice site</div>		Activation of a cryptic Acceptor site. Potential alteration of splicing	
Algorithm/Matix	position	sequences	variation
HSF Acceptor site (matrix AG)	chr15:65875948	- REF : TTAGTAGTCAAAG - ALT : TTAGTAGTCAAGAG	47.27 > 75.14 => 58.96%

SVM-BPfinder

seq_id	agez	ss_dist	bp_seq	bp_scr	y_cont	ppt_off	ppt_len	ppt_scr	svm_scr			
wt	51	83	agtttagat		-4.65768376033		0.397435897436	44	8	17	-3.9203221	
wt	51	78	agataagaa		-3.09893903868		0.41095890411	39	8	17	-2.9891407	
wt	51	66	gtcttagta		-2.12606694945		0.426229508197	27	8	17	-1.8437035	
wt	51	59	tagtcaaaa		-2.7770588632		0.425925925926	20	8	17	-1.6556074	
wt	51	40	atgtaatgt		0.733804510822		0.542857142857	1	8	17	0.95949502	
wt	51	30	tcctgaaat		0.53027192505		0.48	15	7	15	-0.045306812	
wt	51	22	tgctgatac		2.27708398931		0.529411764706	7	7	15	1.1609978	
mut	51	83	agtttagat		-4.65768376033		0.397435897436	44	8	17	-3.9203221	
mut	51	78	agataagaa		-3.09893903868		0.41095890411	39	8	17	-2.9891407	
mut	51	66	gtcttagta		-2.12606694945		0.426229508197	27	8	17	-1.8437035	
mut	51	59	tagtcaaga		-2.81888211612		0.425925925926	20	8	17	-1.6719832	
mut	51	40	atgtaatgt		0.733804510822		0.542857142857	1	8	17	0.95949502	
mut	51	30	tcctgaaat		0.53027192505		0.48	15	7	15	-0.045306812	
mut	51	22	tgctgatac		2.27708398931		0.529411764706	7	7	15	1.1609978	

Variant Effect Predictor tool

ENST00000261890.6:c.41-1373A>G	<a href="#">15:65875959-65875959</a>	G	<a href="#">intron_variant</a>	RAB11A	<a href="#">ENSG00000103769</a>	Transcript	<a href="#">ENST00000261890.7</a>	protein_coding	-	-	-	-	-	-	<a href="#">COSV56041717</a>
ENST00000261890.6:c.41-1373A>G	<a href="#">15:65875959-65875959</a>	G	<a href="#">intron_variant</a>	RAB11A	<a href="#">ENSG00000103769</a>	Transcript	<a href="#">ENST00000564910.5</a>	protein_coding	-	-	-	-	-	-	<a href="#">COSV56041717</a>
ENST00000261890.6:c.41-1373A>G	<a href="#">15:65875959-65875959</a>	G	<a href="#">intron_variant</a>	RAB11A	<a href="#">ENSG00000103769</a>	Transcript	<a href="#">ENST00000565075.5</a>	protein_coding	-	-	-	-	-	-	<a href="#">COSV56041717</a>
ENST00000261890.6:c.41-1373A>G	<a href="#">15:65875959-65875959</a>	G	<a href="#">intron_variant</a>	RAB11A	<a href="#">ENSG00000103769</a>	Transcript	<a href="#">ENST00000566233.5</a>	protein_coding	-	-	-	-	-	-	<a href="#">COSV56041717</a>
ENST00000261890.6:c.41-1373A>G	<a href="#">15:65875959-65875959</a>	G	<a href="#">intron_variant</a>	RAB11A	<a href="#">ENSG00000103769</a>	Transcript	<a href="#">ENST00000567671.1</a>	protein_coding	-	-	-	-	-	-	<a href="#">COSV56041717</a>
ENST00000261890.6:c.41-1373A>G	<a href="#">15:65875959-65875959</a>	G	<a href="#">intron_variant, non_coding_transcript_variant</a>	RAB11A	<a href="#">ENSG00000103769</a>	Transcript	<a href="#">ENST00000568850.5</a>	processed_transcript	-	-	-	-	-	-	<a href="#">COSV56041717</a>
ENST00000261890.6:c.41-1373A>G	<a href="#">15:65875959-65875959</a>	G	<a href="#">intron_variant, non_coding_transcript_variant</a>	RAB11A	<a href="#">ENSG00000103769</a>	Transcript	<a href="#">ENST00000569304.1</a>	processed_transcript	-	-	-	-	-	-	<a href="#">COSV56041717</a>
ENST00000261890.6:c.41-1373A>G	<a href="#">15:65875959-65875959</a>	G	<a href="#">intron_variant</a>	RAB11A	<a href="#">ENSG00000103769</a>	Transcript	<a href="#">ENST00000569896.1</a>	protein_coding	-	-	-	-	-	-	<a href="#">COSV56041717</a>

## ESEfinder

Hay dos resultados con puntuaciones positivas para las matrices 5'SS (115 y 127):

115 (-1762)	tottagtagtcaaaagtaggaacgaatgta	4.52320	115 (-1762)	tottagtagtcaaaagtaggaacgaatgta	-11.64060	115 (-1762)	tottagtagtcaaaagtaggaacgaatgta	4.25820	115 (-1762)	tottagtagtcaaaagtaggaacgaatgta	-15.12310
127 (-1750)	aaagtaggaacgaatgtaatgtttcctgaa	3.72290	127 (-1750)	aaagtaggaacgaatgtaatgtttcctgaa	-19.54330	127 (-1750)	aaagtaggaacgaatgtaatgtttcctgaa	3.33270	127 (-1750)	aaagtaggaacgaatgtaatgtttcctgaa	-20.92380

Si comparamos estas puntuaciones con las equivalentes en la secuencia mutante, se observa que para 115 han bajado, mientras que para 127 se han mantenido igual:

115 (-1762)	tottagtagtcaagagtaggaacgaatgta	2.21770	115 (-1762)	tottagtagtcaagagtaggaacgaatgta	-28.21990	115 (-1762)	tottagtagtcaagagtaggaacgaatgta	1.89560	115 (-1762)	tottagtagtcaagagtaggaacgaatgta	-29.66040
127 (-1750)	agagtaggaacgaatgtaatgtttcctgaa	3.72350	127 (-1750)	agagtaggaacgaatgtaatgtttcctgaa	-19.14440	127 (-1750)	agagtaggaacgaatgtaatgtttcctgaa	3.34620	127 (-1750)	agagtaggaacgaatgtaatgtttcctgaa	-20.32960

Por lo tanto, lo más probable es que se estuviera debilitando un sitio *donor* en la secuencia mutante, lo que no tendría mucho efecto en el *splicing* debido a que este se encuentra dentro del intrón y se emplea de normal.