

## Ejemplo comparación de resultados predictores in silico

Cambio de estudio GADD45GIP c.350+644G>C (chr19:12956219 G/C, COSV57534895 o NM\_052850.4: c.350+644G>C)

Exón 1 e intrones adyacentes:

```
ggcctccaaagtgtgtgagattacaggcgtgagccacccgcccgaacaaaaatatgtatt
ttaagtgtcagtggtgcagaaaggagagaaaaaaagtgaattataaaatgactttaat
cagtaactgtttaactaaaatttaaaagtatacttggaacgtataaaacccatactatc
cccttcgdcgcccctccccgaactgaggcagcagggacacccctcgcctgccctggccc
acacccgggcccctcgcgctcaggcacccggcatggacagcccgccgggcccgtgtgaggacc
GCACAGCCAGATGGCGCGTCCGTCGACAGGCACGCAGCCTACTAGGTGTGGCGCGA
CCTGCCCCGGGTTCCTGGTACC GGCGCGGCCGCCGCCGCGCCGAGGC GGGA
CCCGGTGGCCAGACCCCGAGGACCTCTGACCCCGCGGTGGCAGCTGGGACCGCGCTACG
CGGCTAAGCAGTTCGCGCGTTACGGCGCCGCTCCGCGGTGTCGCCCGGTTCTGTTATGCC
CGTCCCGCAGCAGCTGCGGAGCTGGAGGCCGAAGAACCGGAATGGTACC CGAGCCTGG
CGACCATG CAGGAGTCGCTGCGGTTGAAG CAGCTGGCCGAAGAGCAGAGCGTCGGGAG
G
gtgcgtgcgtgcaggcagacgcggggctgccctctgtgcgcggggcgaactgggccc
aaaccgtgcctacccttcggggtcaggtcgcagcatctgpatgtttagatttatgtat
acgtcacctggccgcaaaacaaactcagaataacgtccatttagtcattcatcagcag
ttttgtttgtttgtttgtttgtttgtttgtttgtttgtttgtttgtttgtttgtttgttt
gagacaagagtcctcgtctgttgcccaggctg
gagtgcagtcgaagatcttggctcactgcaacctccgcctccccggttcaagcgattct
ccgccttagcctccggtgtagctgggattacaggcgtgcaccaccacgcccggcaaat
ttttagtatttagtagagacgggggttccacdatgttggccaggctggtctcaaacctcctt
acttcaagtgatccacctgcctcagcctcccaaagtgcgggattacaggtgtgagcat
cacgtccggccatcacaccaagtctttactgagcgcataacggtgtgdaagacctgtcc
caggccgggggcccgtgtgctcgtgcttgtaatcccagttatttgggaggccgaggcagggc
ccaaaaggcccttaatatcagatggcgacagctgacaggaataataccaatggtga
cagccgactagtaggaaggtataaagagtgaagagtgtgtgcctgaaggcagctttgttt
```

El cambio se encuentra en la penúltima línea del intrón 1 (la **g** en color naranja subrayada en azul).

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'
662	2	+	0.95	GTCGGGAGAG	^	GTGCGTGCGT	H
666	0	+	0.31	GGAGAGGTGC	^	GTGCGTGCAAG	
1340	2	+	0.00	TAGTAGGAAG	^	GTAAGAGT	

### Donor splice sites, complement strand

pos 3'→5'	pos 5'→3'	phase	strand	confidence	5'	exon	intron	3'
1182	200	2	-	0.47	CTTGACACAG	^	GTATGCGCTC	
789	593	1	-	0.46	TTGCGGCCAG	^	GTGAGCGTAT	
734	648	0	-	0.35	CCCCGAAGGG	^	GTAGGCACGG	

### Acceptor splice sites, direct strand

pos 5'→3'	phase	strand	confidence	5'	intron	exon	3'
306	1	+	0.07	CCCCGCACAG	^	CCAAGATGGC	
311	0	+	0.17	CACAGCCAAG	^	ATGGCGGCGT	
332	0	+	0.07	CGTGCGACAG	^	GCACGCAGCC	
440	0	+	0.07	AGACCCGAG	^	GACCTCCTGA	
464	0	+	0.18	GCGGTGGCAG	^	CTGGGACCGC	
551	0	+	0.07	GTCGCCGGAG	^	CAGCTGCGGG	
1283	0	+	0.14	TTAATATCAG	^	ATGGCGACAG	

### Acceptor splice sites, complement strand

pos 3'→5'	pos 5'→3'	phase	strand	confidence	5'	intron	exon	3'
633	749	2	-	0.33	CTTCGGCCAG	^	CTGCTTCACC	
618	764	2	-	0.14	TCACCCGCAG	^	CGACTCCTGC	

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Se pierde un sitio *acceptor* en la secuencia mutante. Este se encuentra en medio del intrón y no participa en el *splicing* normal, por lo que es probable que no se altere el proceso con la mutación presente.

Splice Site Prediction by Neural Network (NNSplice)

Donor site predictions for 10.42.2.148.574782.0 :

Start	End	Score	Exon	Intron
342	356	0.71	ctactag	gtgtggcg
655	669	0.96	gggagag	gtgctgctgc
1124	1138	0.85	attacag	gtgtgagc
1333	1347	0.72	taggaag	gtaaaaga

Acceptor site predictions for 10.42.2.148.574782.0 :

Start	End	Score	Intron	Exon
392	432	0.41	gcggccgccccgcgccgc	agccgggacccccgggtggccag
751	791	0.76	cgcagcatctgcatgttgt	agatttatgtatacgtcacct
951	991	0.53	caagcgattctccccctt	agcctccgggtgtagctgggatt
1017	1057	0.62	aaatttttagtagtttttagt	agagacgggggttccaccatggt

Donor site predictions for 10.42.1.119.574770.0 :

Start	End	Score	Exon	Intron
342	356	0.71	ctactag	gtgtggcg
655	669	0.96	gggagag	gtgctgctgc
1124	1138	0.85	attacag	gtgtgagc
1333	1347	0.72	taggaag	gtaaaaga


Acceptor site predictions for 10.42.1.119.574770.0 :

Start	End	Score	Intron	Exon
392	432	0.41	gcggccgccccgcgccgc	agccgggacccccgggtggccag
751	791	0.76	cgcagcatctgcatgttgt	agatttatgtatacgtcacct
951	991	0.53	caagcgattctccccctt	agcctccgggtgtagctgggatt
1017	1057	0.62	aaatttttagtagtttttagt	agagacgggggttccaccatggt

Spliceman

Point mutation	Wildtype (wt)	Mutation (mt)	L1 distance	Ranking (L1)
ggcaa(g/c)ataat	ggcaag	ggcaac	25862	48%

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	14	151	ctgtaatcc		0.955193859776	0.383561643836	7	6	13	0.57767833		
wt	14	142	cagttattt		-3.0095707119	0.36496350365	116	9	10	-7.9081856		
wt	14	106	cccttaata		-1.65548716115	0.346534653465	80	9	10	-5.1052103		
wt	14	105	ccttaatat		-0.0184574304476	0.35	79	9	10	-4.3998183		
wt	14	100	atatcagat		-3.73810709832	0.336842105263	74	9	10	-5.543995		
wt	14	85	agctgacag		2.09854707368	0.3375	59	9	10	-2.3089817		
wt	14	73	agataatac		-0.752671139213	0.352941176471	47	9	10	-2.6608014		
wt	14	61	tggtgacag		1.56541485441	0.339285714286	35	9	10	-0.99799165		
wt	14	39	aggtaaaag		-0.922297799811	0.352941176471	13	9	10	-0.57507536		
wt	14	31	gagtgaaaga		-1.43700571908	0.423076923077	5	9	10	-0.2475695		
wt	14	16	gcctgaggg		0.00743996436623	0.454545454545	11	0	0	-0.14478569		
mut	14	151	ctgtaatcc		0.955193859776	0.390410958904	7	6	13	0.57989046		
mut	14	142	cagttattt		-3.0095707119	0.372262773723	116	9	10	-7.9058281		
mut	14	106	cccttaata		-1.65548716115	0.356435643564	80	9	10	-5.1020126		
mut	14	105	ccttaatat		-0.0184574304476	0.36	79	9	10	-4.3965886		
mut	14	100	atatcagat		-3.73810709832	0.347368421053	74	9	10	-5.5405953		
mut	14	85	agctgacag		2.09854707368	0.35	59	9	10	-2.3049446		
mut	14	73	acataatac		-0.811717389342	0.352941176471	47	9	10	-2.6839208		
mut	14	61	tggtgacag		1.56541485441	0.339285714286	35	9	10	-0.99799165		
mut	14	39	aggtaaaag		-0.922297799811	0.352941176471	13	9	10	-0.57507536		
mut	14	31	gagtgaaaga		-1.43700571908	0.423076923077	5	9	10	-0.2475695		
mut	14	16	gcctgaggg		0.00743996436623	0.454545454545	11	0	0	-0.14478569		

## Variant Effect Predictor tool

ENST00000316939.2:c.350+644G>C [19:12956219-12956219](#) G [intron\\_variant](#) GADD45GIP1 [ENSG00000179271](#) Transcript [ENST00000316939.3](#) protein\_coding - - [COSV57534895](#)

## ESEfinder

Se encuentran dos resultados en la secuencia WT con puntuaciones positivas en las matrices 5'SS:

1286 (-96)	ggcgacagctgacaggcaagataataccaa	4.81330	1286 (-96)	ggcgacagctgacaggcaagataataccaa	-0.69970	1286 (-96)	ggcgacagctgacaggcaagataataccaa	4.02600	1286 (-96)	ggcgacagctgacaggcaagataataccaa	-0.64010
1303 (-79)	aagataataccaatggtgacagccgactag	5.37170	1303 (-79)	aagataataccaatggtgacagccgactag	-13.99350	1303 (-79)	aagataataccaatggtgacagccgactag	5.38000	1303 (-79)	aagataataccaatggtgacagccgactag	-15.55010

Si los comparamos con las predicciones equivalentes para la secuencia mutante, se observa que las puntuaciones en el primero descienden considerablemente, mientras que en el segundo ascienden muy ligeramente.

1286 (-96)	ggcgacagctgacaggcaacataataccaa	1.01040	1286 (-96)	ggcgacagctgacaggcaacataataccaa	-0.33300	1286 (-96)	ggcgacagctgacaggcaacataataccaa	0.24620	1286 (-96)	ggcgacagctgacaggcaacataataccaa	-0.32420
1303 (-79)	aacataataccaatggtgacagccgactag	5.49480	1303 (-79)	aacataataccaatggtgacagccgactag	-12.69940	1303 (-79)	aacataataccaatggtgacagccgactag	5.52360	1303 (-79)	aacataataccaatggtgacagccgactag	-14.28080

Por lo tanto, podría estar debilitándose un sitio 5'SS (*donor*) en la secuencia mutante, lo que podría afectar al *splicing* (aunque es poco probable dado que está en medio del intrón y no participa en el *splicing* normal).