Ejemplo comparación de resultados predictores in sillico

Cambio de estudio GLA c.636+919G>A (chrX:100654735 C/T, rs199473684 o NM_000169:c.636+919G>A)

Exón 4 e intrones adyacentes:

```
gtgagccaagatcgcaccactgcactccaccctggatgacagactgaaccccatctctcaaa
aaattaaaataaaataaaataaaataactatatatatagcccagctggaaattcattttc
tttcccttatttttacccattgttttctcatacag
GTTTATAAGCACATGTCCTTGGCCCTGAATAGGACTGGCAGAAGCATTGTGTACTCCTGTG
AGTGGCCTCTTTTATATGTGGCCCTTTCAAAAG
gtgagatagtgagcccagaatccaatagaactgtactgatagatagaacttgacaacaaa
ggaaaccaaggtctccttcaaagtccaacgttacttactatcatcctaccatctctccca
ggttccaaccacttctcaccatccccactgctgtaattatagcctaagctaccatcacct
ggaaagtcatccttgtgtcttcccctttatttcaccattcatgtcctgtctatcaacagt
ccttccaccagtatctctaaaatatctcctgaatcagcccacttccttccatcttcacta
catgcaccctggccttccaagctactatcggctctcaccagactgctgggaccaccctga
tctctctgcttccactctgtctcaacccccatctattttccaagcagcactagagttatc
atattaaaatgtaaatatcagttttttttttttaaagaaaaaaaccctgagacttaacagag
ttataaaaaatataaatgtcatcatcagttccctgcttaaaacccttaactcgcttccaa
ttgcacttggaatgaaaccaaactgcactgatccagcccttgcctgcctccccaaagtcc
aagggggtcatggctctttcctgctacactggttttctttctgtccctcaacactgcaa
gctctattgctgccccagggcctttacacttgcttttttttctgcctagacagttcttccc
caaagatttttaaagggcggggctccttaacattgaagtcgcagaccaaacgccacatat
gcagacagttcttctctaactacttaaaatagcctctgtccattcattcttcatcaca
ttaacctgtttaattttcttctcagagctccacactattggaagtattttgttgacttgt
taccatgtctccccactagagtgtaagtttcatgagggcagggaccttgtctgactttga
ctgtatctctcgcatatggttaagtgttgaatagttattatggaatgaatccctattat
tccctcattatctctgcaaaatagtcttttttctcaacatcttaaacctgatatcccacc
```

Se ha descrito que este cambio causa la inclusión *in*-frame de un exón críptico de 57 pb. La mutación elimina el motivo que une la proteína silenciadora de splicing (hnRNPA1/A2). Esto lleva al reconocimiento y la inclusión de una secuencia de pseudoexón[1].

El cambio se encuentra en el intrón 4 (la **g** en color rojo 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.

Nota: en Ensembl está anotada como c. 640-801G>A

1. Palhais B, Dembic M, Sabaratnam R, Nielsen KS, Doktor TK, Bruun GH, Andresen BS (2016) The prevalent deep intronic c. 639+919G>A GLA mutation causes pseudoexon activation and Fabry diseaseby abolishing the binding of hnRNPA1 and hnRNPA2/B1to a splicing silencer. Mol Genet Metab 119(3):258–269

NetGene2

```
Donor splice sites, direct strand
---------------------------------
            pos 5'->3'  phase strand  confidence  5'      exon intron    3'
               212         1     +        0.47        GTGTACTCCT^GTGAGTGGCC
               247         0     +        0.49        CTTTCAAAAG^GTGAGATAGT

Donor splice sites, complement strand
-------------------------------------
   pos 3'->5'  pos 5'->3'  phase strand  confidence  5'      exon intron    3'
      1149        178         0     -        0.32        GGGAGACATG^GTAACAAGTC
       355        972         1     -        0.44        GGGAGAGATG^GTAGGATGAT
       344        983         0     -        0.56        TAGGATGATA^GTAAGTAACG

Acceptor splice sites, direct strand
-------------------------------------
            pos 5'->3'  phase strand  confidence  5'      intron exon    3'
               154         1     +        0.80        TCTCATACAG^GTTATAAGCA
               367         2     +        0.53        TCTCTCCCAG^GTTCCAACCA
               701         0     +        0.56        TTTTTTAAAG^AAAAAAACCC
               953         0     +        0.16        TTCTGCCTAG^AACAGTTCTT
              1111         1     +        0.56        TTCTTCTCAG^AGCTCCACAC

Acceptor splice sites, complement strand
-----------------------------------------
   pos 3'->5'  pos 5'->3'  phase strand  confidence  5'      intron exon    3'
       178        1149         1     -        0.26        TCCTATTCAG^GGCCAAGGAC
```

```
Donor splice sites, direct strand
---------------------------------
            pos 5'->3'  phase strand  confidence  5'      exon intron    3'
               212         1     +        0.47        GTGTACTCCT^GTGAGTGGCC
               247         0     +        0.49        CTTTCAAAAG^GTGAGATAGT
              1169         1     +        0.32        CCACTAAAGT^GTAAGTTTCA

Donor splice sites, complement strand
-------------------------------------
   pos 3'->5'  pos 5'->3'  phase strand  confidence  5'      exon intron    3'
      1149        178         0     -        0.32        GGGAGACATG^GTAACAAGTC
       355        972         1     -        0.44        GGGAGAGATG^GTAGGATGAT
       344        983         0     -        0.56        TAGGATGATA^GTAAGTAACG

Acceptor splice sites, direct strand
-------------------------------------
            pos 5'->3'  phase strand  confidence  5'      intron exon    3'
               154         1     +        0.80        TCTCATACAG^GTTATAAGCA
               367         2     +        0.53        TCTCTCCCAG^GTTCCAACCA
               701         0     +        0.56        TTTTTTAAAG^AAAAAAACCC
               953         0     +        0.16        TTCTGCCTAG^AACAGTTCTT
              1111         1     +        0.56        TTCTTCTCAG^AGCTCCACAC

Acceptor splice sites, complement strand
-----------------------------------------
   pos 3'->5'  pos 5'->3'  phase strand  confidence  5'      intron exon    3'
       178        1149         1     -        0.26        TCCTATTCAG^GGCCAAGGAC
```

Aparece un nuevo sitio *donor* en la secuencia mutada (en rojo) si empleamos el acceptor anterior (en verde), se produciría la inclusión de un exón críptico de 57 pb. Si empleara el anterior, se produciría la inclusión de un exón críptico de 215 pb (aunque es menos probable porque tiene menos *confidence*.

Splice Site Prediction by Neural Network (NNSplice)

Donor site predictions for 85.53.15.54.14582.0 :

Start	End	Score	Exon	Intron
148	162	0.41	catacag**gt**tataag	
205	219	0.90	tactcct**gt**gagtgg	
240	254	0.98	tcaaaag**gt**gagata	
1162	1176	0.96	ctagagt**gt**aagttt	

Donor site predictions for 85.53.15.54.14594.0 :

Start	End	Score	Exon	Intron
148	162	0.41	catacag**gt**tataag	
205	219	0.90	tactcct**gt**gagtgg	
240	254	0.98	tcaaaag**gt**gagata	
1162	1176	0.98	ctaaagt**gt**aagttt	

Acceptor site predictions for 85.53.15.54.14582.0 :

Start	End	Score	Intron	Exon
134	174	0.98	acccattgtttctcatac**ag**gttataagcacatgtccttg	
165	205	0.71	catgtccttggccctgaat**ag**gactggcagaagcattgtgt	
347	387	0.90	tcatcctaccatctctccc**ag**gttccaaccacttctcacca	
681	721	0.83	atatcagtttttttttaa**ag**aaaaaaaccctgagacttaa	
933	973	0.98	acttgctttttttctgcct**ag**aacagttcttccccaaagat	
1091	1131	0.98	cctgtttaattttcttctc**ag**agctccacactatttggaag	
1145	1185	0.71	gttaccatgtctccccact**ag**agtgtaagtttcatgagggc	

Acceptor site predictions for 85.53.15.54.14594.0 :

Start	End	Score	Intron	Exon
134	174	0.98	acccattgtttctcatac**ag**gttataagcacatgtccttg	
165	205	0.71	catgtccttggccctgaat**ag**gactggcagaagcattgtgt	
347	387	0.90	tcatcctaccatctctccc**ag**gttccaaccacttctcacca	
681	721	0.83	atatcagtttttttttaa**ag**aaaaaaaccctgagacttaa	
933	973	0.98	acttgctttttttctgcct**ag**aacagttcttccccaaagat	
1091	1131	0.98	cctgtttaatttcttctc**ag**agctccacactatttggaag	

Desaparece un sitio aceptor de la secuencia wt a la mutada (en rojo). En el *splicing* normal, este sitio no se utiliza, por lo que no tiene por qué estar afectando al *splicing.*

GENSCAN → no da resultados para este cambio

```
Predicted genes/exons:                              Predicted genes/exons:



Gn.Ex Type S .Begin ...End .Len Fr Ph I/Ac Do/T CodRg P.... Tscr..  Gn.Ex Type S .Begin ...End .Len Fr Ph I/Ac Do/T CodRg P.... Tscr..

----- ---- - ------ ------ ---- -- -- ---- ---- ----- ----- ------  ----- ---- - ------ ------ ---- -- -- ---- ---- ----- ----- ------



NO EXONS/GENES PREDICTED IN SEQUENCE                 NO EXONS/GENES PREDICTED IN SEQUENCE
```

MaxEntScan

```
MAXENT: -34.50  MDD: -24.86     MM: -22.93     WMM: -19.58     MAXENT: -7.01          MM: -6.49          WMM: -7.51
```

Spliceman

Point mutation	Wildtype (wt)	Mutation (mt)	L1 distance	Ranking (L1)
cacta(g/a)agtgt	tagagt	taaagt	27888	61%

En el análisis del efecto del cambio, se obtiene una puntuación elevada (61%) para el cambio, por lo que puede estar afectando al *splicing*.

CRYP-SKIP

Se emplea para el exón y las regiones flanqueantes a este, por lo que este predictor no va a ser útil para una variante intrónica profunda.

Human Splicing Finder

Type	Interpretation
🔴 Alteration of auxiliary sequences	Significant alteration of ESE / ESS motifs ratio (2)

Algorithm/Matix	position	sequence
EIE (ESE Site Broken)	chrX:101399747	GAGTGT
IIE (ESS Site Broken)	chrX:101399748	AGAGTG
Sironi_motif2 (ESS Site Broken)	chrX:101399748	AGAGTGT
IIE (ESS Site Broken)	chrX:101399749	TAGAGT

Type	Interpretation
🔴 New Acceptor splice site	Activation of a cryptic Acceptor site. Potential alteration of splicing (cryptic exon activation)

Algorithm/Matix	position	sequences	variation
MaxEnt Acceptor site	chrX:101399764	- REF : ACCATGTCTCCCCACTAGAGTGT - ALT : ACCATGTCTCCCCACTAAAGTGT	-8.54 > 4.08 => 147.78%

SVM-BPfinder

wt:

seq_id	agez	ss_dist	bp_seq	bp_scr	y_cont	ppt_off	ppt_len	ppt_scr	svm_scr
wt	36	349	tttttaaag	-4.30072364603	0.587209302326	57	18	34	-4.3837729
wt	36	348	ttttaaagg	-1.53245739368	0.588921282799	56	18	34	-3.2360146
wt	36	332	tccttaaca	-1.36843041922	0.593272171254	40	18	34	-2.1576119
wt	36	331	ccttaacat	1.05271534081	0.59509202454	39	18	34	-1.1457329
wt	36	325	cattgaagt	-0.26992727141	0.596875	33	18	34	-1.2832442
wt	36	282	ctctaacta	2.4742265856	0.610108303249	15	24	42	1.0093922
wt	36	274	actttaaaa	-5.48424977644	0.60594795539	7	24	42	-1.6016844
wt	36	273	ctttaaaat	-1.31248017832	0.608208955224	6	24	42	0.095789053
wt	36	253	cattcattc	-0.207235646277	0.604838709677	1	9	19	0.62968323
wt	36	246	tcttcatca	-1.29719370536	0.597510373444	10	19	37	-0.20145336
wt	36	243	tcatcacat	-1.53711726586	0.596638655462	7	19	37	-0.10578137
wt	36	238	acattaacc	-2.90230044658	0.596566523605	2	19	37	-0.32384679
wt	36	237	cattaacct	2.63671602189	0.599137931034	1	19	37	1.9090688
wt	36	229	tgtttaatt	-3.49488724098	0.59375	2	10	27	-0.64994069
wt	36	228	gtttaattt	-1.03861221981	0.596412556054	1	10	27	0.37596535
wt	36	217	ttctcagag	-0.233334927075	0.580188679245	22	8	14	-0.76434108
wt	36	186	tgttgactt	1.53476146582	0.591160220994	1	9	14	1.2607604
wt	36	179	ttgttacca	-0.815611761055	0.586206896552	4	12	20	0.20487857
wt	36	155	gtgtaagtt	-0.770933805808	0.586666666667	38	10	16	-1.9668857
wt	36	149	gtttcatga	-1.13166954383	0.583333333333	32	10	16	-1.7294174
wt	36	146	tcatgaggg	-2.18584999844	0.58865248227	29	10	16	-1.950566
wt	36	127	gtctgactt	2.55945786451	0.614754098361	10	10	16	1.1185444
wt	36	121	ctttgactg	1.34723665694	0.612068965517	4	10	16	1.0228253
wt	36	99	tggttaagt	-2.88629176587	0.606382978723	13	8	14	-1.2249542
wt	36	98	ggttaagtg	-0.608778875392	0.612903225806	12	8	14	-0.26779609
wt	36	91	tgttgaata	-0.787962435	0.627906976744	5	8	14	0.10997911
wt	36	84	tagttattt	-3.40315432481	0.645569620253	14	27	46	-1.1798655
wt	36	80	tatttatgg	-2.44529988651	0.64	10	27	46	-0.55342572
wt	36	72	gaatgaatc	-0.42868818176	0.686567164179	2	27	46	0.75759948
wt	36	62	ctattattc	-2.49906547085	0.684210526316	1	18	32	-0.12093569
wt	36	54	ccctcatta	0.224180695393	0.65306122449	1	10	17	0.79554588
wt	36	51	tcattatct	-2.33445410954	0.652173913043	15	11	30	-0.97163764
wt	36	25	ttctcaaca	0.330802281765	0.6	0	0	0	-0.5408814
wt	36	17	atcttaaac	-1.729610229	0.583333333333	12	0	0	-0.84662656
wt	36	16	tcttaaacc	-0.436349277476	0.636363636364	11	0	0	-0.25982823

mut:

seq_id	agez	ss_dist	bp_seq	bp_scr	y_cont	ppt_off	ppt_len	ppt_scr	svm_scr
mut	36	349	tttttaaag	-4.30072364603	0.587209302326	57	18	34	-4.3837729
mut	36	348	ttttaaagg	-1.53245739368	0.588921282799	56	18	34	-3.2360146
mut	36	332	tccttaaca	-1.36843041922	0.593272171254	40	18	34	-2.1576119
mut	36	331	ccttaacat	1.05271534081	0.59509202454	39	18	34	-1.1457329
mut	36	325	cattgaagt	-0.26992727141	0.596875	33	18	34	-1.2832442
mut	36	282	ctctaacta	2.4742265856	0.610108303249	15	24	42	1.0093922
mut	36	274	actttaaaa	-5.48424977644	0.60594795539	7	24	42	-1.6016844
mut	36	273	ctttaaaat	-1.31248017832	0.608208955224	6	24	42	0.095789053
mut	36	253	cattcattc	-0.207235646277	0.604838709677	1	9	19	0.62968323
mut	36	246	tcttcatca	-1.29719370536	0.597510373444	10	19	37	-0.20145336
mut	36	243	tcatcacat	-1.53711726586	0.596638655462	7	19	37	-0.10578137
mut	36	238	acattaacc	-2.90230044658	0.596566523605	2	19	37	-0.32384679
mut	36	237	cattaacct	2.63671602189	0.599137931034	1	19	37	1.9090688
mut	36	229	tgtttaatt	-3.49488724098	0.59375	2	10	27	-0.64994069
mut	36	228	gtttaattt	-1.03861221981	0.596412556054	1	10	27	0.37596535
mut	36	217	ttctcagag	-0.233334927075	0.580188679245	22	8	14	-0.76434108
mut	36	186	tgttgactt	1.53476146582	0.591160220994	1	9	14	1.2607604
mut	36	179	ttgttacca	-0.815611761055	0.586206896552	4	12	20	0.20487857
mut	36	162	==cactaaagt==	1.02645622809	0.573248407643	45	10	16	==-1.7105445==
mut	36	155	gtgtaagtt	-0.770933805808	0.586666666667	38	10	16	-1.9668857
mut	36	149	gtttcatga	-1.13166954383	0.583333333333	32	10	16	-1.7294174
mut	36	146	tcatgaggg	-2.18584999844	0.58865248227	29	10	16	-1.950566
mut	36	127	gtctgactt	2.55945786451	0.614754098361	10	10	16	1.1185444
mut	36	121	ctttgactg	1.34723665694	0.612068965517	4	10	16	1.0228253
mut	36	99	tggttaagt	-2.88629176587	0.606382978723	13	8	14	-1.2249542
mut	36	98	ggttaagtg	-0.608778875392	0.612903225806	12	8	14	-0.26779609
mut	36	91	tgttgaata	-0.787962435	0.627906976744	5	8	14	0.10997911
mut	36	84	tagttattt	-3.40315432481	0.645569620253	14	27	46	-1.1798655
mut	36	80	tatttatgg	-2.44529988651	0.64	10	27	46	-0.55342572
mut	36	72	gaatgaatc	-0.42868818176	0.686567164179	2	27	46	0.75759948
mut	36	62	ctattattc	-2.49906547085	0.684210526316	1	18	32	-0.12093569
mut	36	54	ccctcatta	0.224180695393	0.65306122449	1	10	17	0.79554588
mut	36	51	tcattatct	-2.33445410954	0.652173913043	15	11	30	-0.97163764
mut	36	25	ttctcaaca	0.330802281765	0.6	20	0	0	-0.5408814
mut	36	17	atcttaaac	-1.729610229	0.583333333333	12	0	0	-0.84662656
mut	36	16	tcttaaacc	-0.436349277476	0.636363636364	11	0	0	-0.25982823

Aparición nuevo BP en la secuencia mutante, posible alteración del *splicing.*
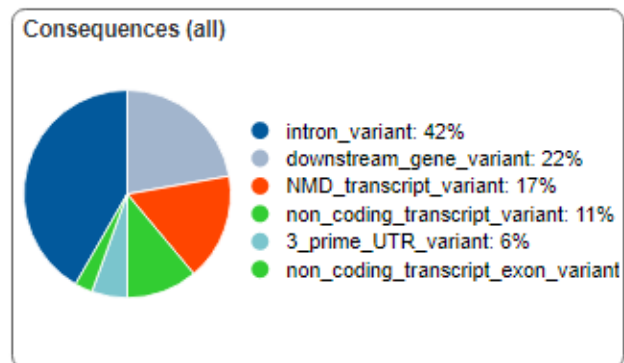
IntSplice

Prediction	Genomic Mutation	Ensembl 64 Transcript ID and Exon No.

Variant Effect Predictor tool

Category	Count
Variants processed	1
Variants filtered out	0
Novel / existing variants	0 (0.0) / 1 (100.0)
Overlapped genes	3
Overlapped transcripts	26
Overlapped regulatory features	0

Consequences (all)
- intron_variant: 42%
- downstream_gene_variant: 22%
- NMD_transcript_variant: 17%
- non_coding_transcript_variant: 11%
- 3_prime_UTR_variant: 6%
- non_coding_transcript_exon_variant

Se trata de una variante que está afectando al sitio de *splicing*, por lo que va a provocar que se altere el *splicing* normal. Es una variante *downstream* que está afectando al 3'UTR del gen y es una *non coding transcript exon variant*, es decir, una variante que se encuentra en un transcrito que no se suele transcribir porque no es el mayoritario. El método de NMD se encarga de degradar estos transcritos[2], dado que aparece un codón de parada.

2. Lewis BP, Green RE, Brenner SE. Evidence for the widespread coupling of alternative splicing and nonsense-mediated mRNA decay in humans. Proc Natl Acad Sci USA. 2003;100:189–192

Uploaded variant	Location	Allele	Consequence	Symbol	Gene	Feature type	Feature	Biotype
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	intron_variant	GLA	ENSG00000102393	Transcript	ENST00000218516.4	protein_coding
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	downstream_gene_variant	RPL36A	ENSG00000241343	Transcript	ENST00000372849.8	nonsense_mediated_decay
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	downstream_gene_variant	RPL36A	ENSG00000241343	Transcript	ENST00000392994.7	protein_coding
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	intron_variant	RPL36A-HNRNPH2	ENSG00000257529	Transcript	ENST00000409170.3	protein_coding
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	intron_variant	RPL36A-HNRNPH2	ENSG00000257529	Transcript	ENST00000409338.5	protein_coding
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	downstream_gene_variant	RPL36A	ENSG00000241343	Transcript	ENST00000427805.6	protein_coding
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	downstream_gene_variant	RPL36A	ENSG00000241343	Transcript	ENST00000465744.5	retained_intron
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	intron_variant, non_coding_transcript_variant	GLA	ENSG00000102393	Transcript	ENST00000466414.2	retained_intron
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	intron_variant, non_coding_transcript_variant	GLA	ENSG00000102393	Transcript	ENST00000468823.2	retained_intron
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	downstream_gene_variant	RPL36A	ENSG00000241343	Transcript	ENST00000471855.1	protein_coding
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	intron_variant, non_coding_transcript_variant	GLA	ENSG00000102393	Transcript	ENST00000479445.2	retained_intron
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	intron_variant, NMD_transcript_variant	GLA	ENSG00000102393	Transcript	ENST00000480513.6	nonsense_mediated_decay
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	intron_variant, NMD_transcript_variant	GLA	ENSG00000102393	Transcript	ENST00000486121.6	nonsense_mediated_decay
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	downstream_gene_variant	RPL36A	ENSG00000241343	Transcript	ENST00000489407.1	retained_intron
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	3_prime_UTR_variant, NMD_transcript_variant	GLA	ENSG00000102393	Transcript	ENST00000493905.6	nonsense_mediated_decay
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	downstream_gene_variant	RPL36A	ENSG00000241343	Transcript	ENST00000553110.8	protein_coding
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	downstream_gene_variant	RPL36A	ENSG00000241343	Transcript	ENST00000614077.4	protein_coding
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	intron_variant	GLA	ENSG00000102393	Transcript	ENST00000649178.1	protein_coding
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	3_prime_UTR_variant, NMD_transcript_variant	GLA	ENSG00000102393	Transcript	ENST00000674127.1	nonsense_mediated_decay
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	intron_variant, non_coding_transcript_variant	GLA	ENSG00000102393	Transcript	ENST00000674142.1	retained_intron
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	intron_variant	GLA	ENSG00000102393	Transcript	ENST00000674634.1	protein_coding
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	intron_variant	GLA	ENSG00000102393	Transcript	ENST00000675592.1	protein_coding
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	intron_variant, NMD_transcript_variant	GLA	ENSG00000102393	Transcript	ENST00000675799.1	nonsense_mediated_decay
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	non_coding_transcript_exon_variant	GLA	ENSG00000102393	Transcript	ENST00000675968.1	retained_intron
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	intron_variant	GLA	ENSG00000102393	Transcript	ENST00000676156.1	protein_coding
ENST00000218516.3:c.639+919G>A	X:101399747-101399747	T	intron_variant, NMD_transcript_variant	GLA	ENSG00000102393	Transcript	ENST00000676372.1	nonsense_mediated_decay

ESEfinder

Cuando se buscan los posibles sitios de *splicing*, solo se obtiene un resultado con la posición de interés con puntuación positiva en las matrices de 5'SS: tctccccactagagtgtaagtttcatgagg (6.52100y 6.49500). Si buscamos los resultados equivalentes en las predicciones para la secuencia mutada, vemos que las puntuaciones suben bastante (6.70680 y 6.63480), por lo que puede que no tenga un papel importante en el *splicing*. Por otro lado, hay un resultado con puntuación positiva en las matrices 3'SS: atgtctccccactagagtgtaagtttcatg (4.49150 y 4.60490). Su equivalente en la secuencia mutada tiene puntuaciones negativas (-9.49340 y -12.74310). Por lo tanto, se está perdiendo un sitio 3'SS, lo que hará que sea más probable la alteración del *splicing.*

EX-SKIP

Predictor para secuencias exónicas.

HOT-SKIP

Predictor para secuencias exónicas.