Ejemplo comparación de resultados predictores in sillico

Cambio de estudio RTTN c.5824-590G>A (chr18:70025438 G/A, COSV55350104 o NM_173630.4: c.5824-590G>A)

Exón 44 e intrones adyacentes:

```
ttcagtgcttacggagagtgttgaacattggaatcacttctcttctgccacttgatgct
atgtgaaatcgtatagggtgtgtgtatttatgtgcatgctcacgtgcataatcatgggtc
tacaaattacctcggaattcttgttcatttaaaatagatattgctgcacttaaacagcta
tatttatctgcaatattgtccagggtaactgatctttgtaataaagactctgagttac
ttcagaatttttcaatttatcataatatttagactgaattttatatattcaaattactca
aaaactgtaatttactttgagtatatacacacatacacagagagagagaggagagggag
agaggtggcaaagaggactcagagttgatcagaatgtttacttaaccagaaattctca
cttcttggccagttcatagcatttacaggaatttattcagtgttgtgagatatcactttg
ctgtttaatttacatgttacacaggctatttctatgcttcctaacttagtggtacatct
taaccacctggtgttgaagcgacgtcctgagattggtcaggctcgtggtcagggcagcc
atgtgtggagcccaatgggctgggcaggatgctgaagccatccacagggttctccttg
ggtcttatgttgatggctttctgttgttgtttgatttaatgttatattttcatatatt
cagactaatgctgtctttttccccttcctttcaacag
GAAGCAGCTCTTGAAGCTCACCTTGTCCCTGTCTTGCACTCTCTCTGGCCTTGGATTTTG
ATGGATGATTCATTGATGCAAATTTCTCTGCAGCTCCTTTGTGTCTATACTGCAAATTTT
CCAAATG
gtaatagggatccaatactgcctttcaataatataccaaaatactagtttttataaatgttg
ttaaggtggactggaaaaactaatacatattttgaagtattttctctgatttattgaggat
atgatgggcaaaggcaagctttctcgtaggtattatgagagcagacagatattttagtgt
gtttgttgacatgagagagtcattggcagcgcagggaatagagagggaggactggtctga
```

El cambio se encuentra en la tercera línea del intrón 43 (la **g** 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
---------------------------------
   No donor site predictions above threshold.

Donor splice sites, complement strand
-------------------------------------
   pos 3'->5'  pos 5'->3'  phase strand  confidence  5'      exon intron    3'
       776         347       2     -        0.62       CAGGGACAAG^GTGAGCTTCA
       421         702       1     -        0.34       TGGCCAAGAA^GTGAGAATTT
       130         993       1     -        0.36       GAATTCCGAG^GTAATTTGTA
        12        1111       -     -        0.00       AACACTCTCC^GTAAGCACTG

Acceptor splice sites, direct strand
------------------------------------
               pos 5'->3'  phase strand  confidence  5'    intron exon      3'
                   755       2     +        0.56       CCTTCAACAG^GAAGCAGCTC
                  1031       1     +        0.00       TTTCTCGTAG^GTATTATGAG

Acceptor splice sites, complement strand
----------------------------------------
   pos 3'->5'  pos 5'->3'  phase strand  confidence  5'    intron exon      3'
       664         459       2     -        0.07       TCAACATAAG^ACCCAAGGAG
       657         466       0     -        0.18       AAGACCCAAG^GAGAACCCTG
       654         469       0     -        0.18       ACCCAAGGAG^AACCCTGTGG
       634         489       2     -        0.07       ATGGCTTCAG^CATCCTGCCC
```

```
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       634         489       2     -        0.07       ATGGCTTCAG^CATCCTGCCC
```

Hay un cambio en uno de los sitios *acceptor* predichos (en azul) por la presencia de la mutacion. Sin embargo, este no se emplea en el *splicing* ni cambia su *score*, por lo que no se tendrá en cuenta.

Splice Site Prediction by Neural Network (NNSplice)

Donor site predictions for wt :

Start	End	Score	Exon Intron
71	85	0.55	gtatagggtgtgtgt
200	214	0.82	tcccagggtaactga
526	540	0.73	cttagtggtacatct
876	890	0.68	ccaaatggtaatagg

Donor site predictions for mut :

Start	End	Score	Exon Intron
71	85	0.55	gtatagggtgtgtgt
200	214	0.82	tcccagggtaactga
526	540	0.73	cttagtggtacatct
876	890	0.68	ccaaatggtaatagg

Acceptor site predictions for wt :

Start	End	Score	Intron Exon
137	177	0.45	attcttgttcatttaaaatagatattgctgcacttaaacag
735	775	0.99	ctttttcccttccttcaacaggaagcagctcttgaagctca
828	868	0.72	ttgatgcaaatttctctgcagctcctttgtgtctatactgc
1011	1051	0.85	caaaggcaagctttctcgtaggtattatgagagcagacaga

Acceptor site predictions for mut :

Start	End	Score	Intron Exon
137	177	0.52	attcttgttcatttaaaatagatattgctacacttaaacag
735	775	0.99	ctttttcccttccttcaacaggaagcagctcttgaagctca
828	868	0.72	ttgatgcaaatttctctgcagctcctttgtgtctatactgc
1011	1051	0.85	caaaggcaagctttctcgtaggtattatgagagcagacaga

Se produce un cambio en uno de los sitios *acceptor* predichos (en amarillo) por la presencia de la mutación. Este cambio hace que el *score* del sitio aumente, lo que podría llevar a que el complejo de *splicing,* lo reconociera alterando el mensajero resultante.

Spliceman

Point mutation	Wildtype (wt)	Mutation (mt)	L1 distance	Ranking (L1)
ttgct(g/a)cactt	tgctgc	tgctac	29650	72%

Human Splicing Finder

Alteration of auxiliary sequences	Significant alteration of ESE / ESS motifs ratio (3)

Algorithm/Matix	position	sequence
EIE (New ESE Site)	chr18:70025440	CTACAC
ESE_SRp40 (New ESE Site)	chr18:70025440	CTACACT
EIE (New ESE Site)	chr18:70025441	GCTACA
ESE_SRp55 (ESE Site Broken)	chr18:70025442	TGCTGC
EIE (New ESE Site)	chr18:70025442	TGCTAC

SVM-BPfinder

seq_id	agez	ss_dist	bp_seq	bp_scr	y_cont	ppt_off	ppt_len	ppt_scr	svm_scr
wt	23	52	aaattacct	-0.0698462321051	0.553191489362	9	13	27	0.23493732
wt	23	34	tgttcattt	-0.935860554115	0.48275862069	29	0	0	-1.6443902
wt	23	30	catttaaaa	-3.83973244712	0.44	25	0	0	-2.5420098
wt	23	29	atttaaaat	-1.95061244719	0.458333333333	24	0	0	-1.7331106
mut	23	52	aaattacct	-0.0698462321051	0.553191489362	9	13	27	0.23493732
mut	23	34	tgttcattt	-0.935860554115	0.48275862069	29	0	0	-1.6443902
mut	23	30	catttaaaa	-3.83973244712	0.44	25	0	0	-2.5420098
mut	23	29	atttaaaat	-1.95061244719	0.458333333333	24	0	0	-1.7331106

Variant Effect Predictor tool

ENST00000255674.11:c.5824-590G>A	18:70025438-70025438	T	intron_variant	RTTN	ENSG00000176225	Transcript	ENST00000255674.11	protein_coding	-	-	-	-	-	-	COSV55350104
ENST00000255674.11:c.5824-590G>A	18:70025438-70025438	T	intron_variant	RTTN	ENSG00000176225	Transcript	ENST00000578780.2	protein_coding	-	-	-	-	-	-	COSV55350104
ENST00000255674.11:c.5824-590G>A	18:70025438-70025438	T	intron_variant, NMD_transcript_variant	RTTN	ENSG00000176225	Transcript	ENST00000579986.6	nonsense_mediated_decay	-	-	-	-	-	-	COSV55350104
ENST00000255674.11:c.5824-590G>A	18:70025438-70025438	T	upstream_gene_variant	RTTN	ENSG00000176225	Transcript	ENST00000580034.2	retained_intron	-	-	-	-	-	-	COSV55350104
ENST00000255674.11:c.5824-590G>A	18:70025438-70025438	T	intron_variant, NMD_transcript_variant	RTTN	ENSG00000176225	Transcript	ENST00000581161.5	nonsense_mediated_decay	-	-	-	-	-	-	COSV55350104
ENST00000255674.11:c.5824-590G>A	18:70025438-70025438	T	intron_variant, NMD_transcript_variant	RTTN	ENSG00000176225	Transcript	ENST00000583043.5	nonsense_mediated_decay	-	-	-	-	-	-	COSV55350104
ENST00000255674.11:c.5824-590G>A	18:70025438-70025438	T	intron_variant, non_coding_transcript_variant	RTTN	ENSG00000176225	Transcript	ENST00000583765.1	processed_transcript	-	-	-	-	-	-	COSV55350104
ENST00000255674.11:c.5824-590G>A	18:70025438-70025438	T	intron_variant, non_coding_transcript_variant	RTTN	ENSG00000176225	Transcript	ENST00000639128.1	retained_intron	-	-	-	-	-	-	COSV55350104
ENST00000255674.11:c.5824-590G>A	18:70025438-70025438	T	intron_variant, NMD_transcript_variant	RTTN	ENSG00000176225	Transcript	ENST00000639487.1	nonsense_mediated_decay	-	-	-	-	-	-	COSV55350104
ENST00000255674.11:c.5824-590G>A	18:70025438-70025438	T	upstream_gene_variant	RTTN	ENSG00000176225	Transcript	ENST00000640525.1	retained_intron	-	-	-	-	-	-	COSV55350104
ENST00000255674.11:c.5824-590G>A	18:70025438-70025438	T	intron_variant	RTTN	ENSG00000176225	Transcript	ENST00000640769.2	protein_coding	-	-	-	-	-	-	COSV55350104
ENST00000255674.11:c.5824-590G>A	18:70025438-70025438	T	intron_variant	RTTN	ENSG00000176225	Transcript	ENST00000677824.1	protein_coding	-	-	-	-	-	-	COSV55350104
ENST00000255674.11:c.5824-590G>A	18:70025438-70025438	T	intron_variant, NMD_transcript_variant	RTTN	ENSG00000176225	Transcript	ENST00000679113.1	nonsense_mediated_decay	-	-	-	-	-	-	COSV55350104

ESEfinder

Se observan dos resultados con puntuaciones positivas para las matrices tres prima (143 y 163):

143 (-980)	gttcatttaaaatagatattgctgcactta	-8.48850	143 (-980)	gttcatttaaaatagatattgctgcactta	0.96960	143 (-980)	gttcatttaaaatagatattgctgcactta	-9.02510	143 (-980)	gttcatttaaaatagatattgctgcactta	0.64250
163 (-960)	gctgcacttaaacagctatattttatctgc	-3.36130	163 (-960)	gctgcacttaaacagctatattttatctgc	0.06840	163 (-960)	gctgcacttaaacagctatattttatctgc	-4.11040	163 (-960)	gctgcacttaaacagctatattttatctgc	0.02000

Cuando se comparan estas puntuaciones con las equivalentes en la secuencia mutante, se observa que, para ambos casos, las puntuaciones han bajado, haciendo que la puntuación para 163 pase a ser negativa:

143 (-980)	gttcatttaaaatagatattgctacactta	-8.72960	143 (-980)	gttcatttaaaatagatattgctacactta	0.58260	143 (-980)	gttcatttaaaatagatattgctacactta	-9.49530	143 (-980)	gttcatttaaaatagatattgctacactta	0.30530
163 (-960)	gctacacttaaacagctatattttatctgc	-3.59470	163 (-960)	gctacacttaaacagctatattttatctgc	-0.39540	163 (-960)	gctacacttaaacagctatattttatctgc	-4.29830	163 (-960)	gctacacttaaacagctatattttatctgc	-0.58760

Por lo tanto lo más probable es que os esté debilitando o se esté perdiendo un sitio *acceptor*.