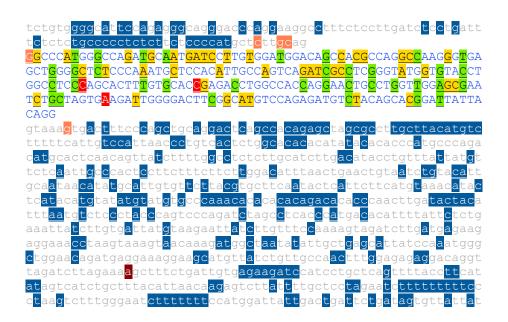**Ejemplo comparación de resultados predictores in sillico**

Cambio de estudio NTRK3 c.2133+614A>T (chr15:87928577 A/T, COSV62309620 o NM_001007156.3: c.2133+614A>T)

Exón 17 e intrones adyacentes:

```
tctgtggggcattccagagggcagggacccaggaaggcctttctccttgatctcctgatt
tctctctgccccctctcttctccccatgctcttgcag
CGCCCATGGGCCAGATGCAATGATCCTTGTGGATGGACAGCCACGCCAGGCCAAGGGTGA
GCTGGGGCTCTCCCAAATGCTCCACATTGCCAGTCAGATCGCCTCGGGTATGGTGTACCT
GGCCTCCCAGCACTTTGTGCACCGAGACCTGGCCACCAGGAACTGCCTGGTTGGAGCGAA
TCTGCTAGTGAAGATTGGGGACTTCGGCATGTCCAGAGATGTCTACAGCACGGATTATTA
CAGG
gtaaagtgactttcccagctgcaggactcagccacagagctagcgccttgcttacatgtc
tttttcattgtccattaaccctgtcactctggcacacacatatacacacccatgcccaga
catgcactcaacagttatcttttggcctctcttgcatcttgacatacctgtttattatgt
tctcaattgcccactccttctttcttcttggacatttaactgaactgtaatctgtacatt
gcaataacatatgcattgtgttcttacgtgcttcaatactcattcttcatgtaaacatac
tcatacatgtatatgtatgtgcccaaacacacacagacacaccaacttgatactaca
tttaatgtctccctaccagtcccagatctagcctcaccatgacacattttattctctg
aaattatcttgtgattatgtaagaattatcttgtttccaaaagtagatcttgatgagaag
aggaaacctaagtaaagtaacaaagatggctaatattgctgagcattatccaaatggg
ctggaacagatgaggaaaggaagcatgttatctgttgccaactttggagagaggacaggt
tagatcttagaaaagctttctgattgtgagaagatccatcctgctcagtttttaccttcat
atagtcatctgctttacattaacaagagtcttattttgctcctagaatcttttttttttcc
ctaagtctttgggaatctttttttttccatggattattgactgattctgatagtgttattat
```

El cambio se encuentra en la antepenúltima línea del intrón 17 (la **a** en color granate).

Se va a obtener los resultados que produce analizar esta variable con los diferentes 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'
          342         0      +       0.88       TTATTACAGG^GTAAAGTGAC H
          940         1      +       0.41       GAGAGGACAG^GTTAGATCTT
```

Donor splice sites, complement strand

```
 pos 3'->5'  pos 5'->3'  phase strand  confidence 5'     exon intron    3'
     995        127       0      -      0.34       CTATATGAAG^GTAAAACTGA
     739        383       1      -      0.41       TGTGTCATGG^GTGAGGCTAG
     608        514       2      -      0.83       ATTGAAGCAC^GTAAGAACAC
     508        614       2      -      0.81       TAATAAACAG^GTATGTCAAG
     215        907       0      -      0.65       GGGAGGCCAG^GTACACCATA
```

Acceptor splice sites, direct strand

```
          pos 5'->3'  phase strand  confidence 5'     intron exon    3'
          97          2      +       0.96       GCTCTTGCAG^GGCCCATGGG
          111         1      +       0.34       CATGGGCCAG^ATGCAATGAT
          137         0      +       0.19       GGATGGACAG^CCACGCCAGG
          146         0      +       0.07       GCCACGCCAG^GCCAAGGGTG
          784         2      +       0.17       ATTATGTAAG^AATTATCTTG
          804         1      +       0.07       TTTCCAAAAG^TAGATCTTGA
          807         1      +       0.31       CCAAAAGTAG^ATCTTGATGA
```

Acceptor splice sites, complement strand

```
 pos 3'->5'  pos 5'->3'  phase strand  confidence 5'     intron exon    3'
     882        240       0      -      0.27       TCTGTTCCAG^CCCATTTGGA
     641        481       0      -      0.07       CATGTATGAG^TATGTTTACA
     320        802       1      -      0.19       CGTGCTGTAG^ACATCTCTGG
     299        823       1      -      0.19       CATGCCGAAG^TCCCCAATCT
     282        840       0      -      0.19       TCTTCACTAG^CAGATTCGCT
     279        843       0      -      0.81       TCACTAGCAG^ATTCGCTCCA
     264        858       0      -      0.14       CTCCAACCAG^GCAGTTCCTG
```

Splice Site Prediction by Neural Network (NNSplice)

Donor site predictions for wt :

Start	End	Score	Exon Intron
147	161	0.89	gccaagg**gt**gagctg
335	349	0.76	ttacagg**gt**aaagtg
649	663	0.46	tgtatat**gt**atgtgc
773	787	0.69	tgattat**gt**aagaat
933	947	0.86	aggacag**gt**tagatc

Donor site predictions for mut :

Start	End	Score	Exon Intron
147	161	0.89	gccaagg**gt**gagctg
335	349	0.76	ttacagg**gt**aaagtg
649	663	0.46	tgtatat**gt**atgtgc
773	787	0.69	tgattat**gt**aagaat
933	947	0.86	aggacag**gt**tagatc

Acceptor site predictions for wt :

Start	End	Score	Intron Exon
77	117	0.97	cttctccccatgctcttgc**ag**ggcccatgggccagatgcaa
707	747	0.71	tgtctccctacccagtccc**ag**atctagcctcacccatgaca
985	1025	0.78	ctcagttttaccttcatat**ag**tcatctgctttacattaaca
1026	1066	0.49	agagtcttattttgctcct**ag**aatcttttttttttccctaag
1046	1086	0.57	gaatctttttttttcccta**ag**tctttgggaatctttttttc

Acceptor site predictions for mut :

Start	End	Score	Intron Exon
77	117	0.97	cttctccccatgctcttgc**ag**ggcccatgggccagatgcaa
707	747	0.71	tgtctccctacccagtccc**ag**atctagcctcacccatgaca
985	1025	0.78	ctcagttttaccttcatat**ag**tcatctgctttacattaaca
1026	1066	0.49	agagtcttattttgctcct**ag**aatcttttttttttccctaag
1046	1086	0.57	gaatctttttttttcccta**ag**tctttgggaatctttttttc

Spliceman

Point mutation	Wildtype (wt)	Mutation (mt)	L1 distance	Ranking (L1)
agaaa(a/t)gcttt	agcttt	tgcttt	29204	69%

Human Splicing Finder

🔴 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	77	aggttagat	-4.12420066332	0.583333333333	14	10	18	-1.7431334
wt	14	70	atcttagaa	-3.10373208072	0.584615384615	7	10	18	-0.90006934
wt	14	56	ttctgattg	1.52540888771	0.627450980392	12	12	20	0.62843286
wt	14	50	ttgtgagaa	-1.8483285963	0.644444444444	6	12	20	-0.30726649
wt	14	32	tgctcagtt	0.427335704084	0.703703703704	2	12	23	0.88404345
wt	14	26	gttttacct	-1.44575692241	0.714285714286	12	10	17	-0.53482143
wt	14	20	ccttcatat	-0.863509964966	0.666666666667	6	10	17	0.057566143
mut	14	77	aggttagat	-4.12420066332	0.597222222222	12	12	19	-1.6027352
mut	14	70	atcttagaa	-3.10373208072	0.6	5	12	19	-0.75918808
mut	14	56	ttctgattg	1.52540888771	0.627450980392	12	12	20	0.62843286
mut	14	50	ttgtgagaa	-1.8483285963	0.644444444444	6	12	20	-0.30726649
mut	14	32	tgctcagtt	0.427335704084	0.703703703704	2	12	23	0.88404345
mut	14	26	gttttacct	-1.44575692241	0.714285714286	12	10	17	-0.53482143
mut	14	20	ccttcatat	-0.863509964966	0.666666666667	6	10	17	0.057566143

Variant Effect Predictor tool

ENST00000394480.6:c.2133+614A>T	15:87928577-87928577	A	intron_variant	NTRK3	ENSG00000140538 Transcript	ENST00000355254.6	protein_coding	-	-	-	-	-	-	COSV62309620
ENST00000394480.6:c.2133+614A>T	15:87928577-87928577	A	intron_variant	NTRK3	ENSG00000140538 Transcript	ENST00000357724.6	protein_coding	-	-	-	-	-	-	COSV62309620
ENST00000394480.6:c.2133+614A>T	15:87928577-87928577	A	intron_variant	NTRK3	ENSG00000140538 Transcript	ENST00000360948.6	protein_coding	-	-	-	-	-	-	COSV62309620
ENST00000394480.6:c.2133+614A>T	15:87928577-87928577	A	intron_variant	NTRK3	ENSG00000140538 Transcript	ENST00000394480.6	protein_coding	-	-	-	-	-	-	COSV62309620
ENST00000394480.6:c.2133+614A>T	15:87928577-87928577	A	intron_variant	NTRK3	ENSG00000140538 Transcript	ENST00000542733.6	protein_coding	-	-	-	-	-	-	COSV62309620
ENST00000394480.6:c.2133+614A>T	15:87928577-87928577	A	intron_variant	NTRK3	ENSG00000140538 Transcript	ENST00000557856.5	protein_coding	-	-	-	-	-	-	COSV62309620
ENST00000394480.6:c.2133+614A>T	15:87928577-87928577	A	intron_variant	NTRK3	ENSG00000140538 Transcript	ENST00000558576.5	protein_coding	-	-	-	-	-	-	COSV62309620
ENST00000394480.6:c.2133+614A>T	15:87928577-87928577	A	3_prime_UTR_variant	NTRK3	ENSG00000140538 Transcript	ENST00000558676.5	protein_coding	14/14	2749	-	-	-	-	COSV62309620
ENST00000394480.6:c.2133+614A>T	15:87928577-87928577	A	downstream_gene_variant	NTRK3	ENSG00000140538 Transcript	ENST00000559680.1	processed_transcript	-	-	-	-	-	-	COSV62309620
ENST00000394480.6:c.2133+614A>T	15:87928577-87928577	A	intron_variant	NTRK3	ENSG00000140538 Transcript	ENST00000626019.2	protein_coding	-	-	-	-	-	-	COSV62309620
ENST00000394480.6:c.2133+614A>T	15:87928577-87928577	A	intron_variant	NTRK3	ENSG00000140538 Transcript	ENST00000629765.1	protein_coding	-	-	-	-	-	-	COSV62309620

ESEfinder

Se observa uno único resultado con puntuaciones positivas para las matrices 5'SS. Si comparamos esta con la equivalente en la secuencia mutante, la puntuación desciende muy ligeramente. Por lo tanto, podría estar debilitándose un sitio *acceptor*, pero es poco probable, además de que no tendrá efecto en el *splicing* porque está dentro del intrón y no se emplea para el *splicing* normal.

952 (-170)	aaaagctttctgattgtgagaagatccatc	3.68340	952 (-170)	aaaagctttctgattgtgagaagatccatc	-23.65650	952 (-170)	aaaagctttctgattgtgagaagatccatc	3.71810	952 (-170)	aaaagctttctgattgtgagaagatccatc	-25.85550
952 (-170)	aaatgctttctgattgtgagaagatccatc	3.26980	952 (-170)	aaatgctttctgattgtgagaagatccatc	-21.38780	952 (-170)	aaatgctttctgattgtgagaagatccatc	3.29250	952 (-170)	aaatgctttctgattgtgagaagatccatc	-23.47770