Ejemplo comparación de resultados predictores in sillico
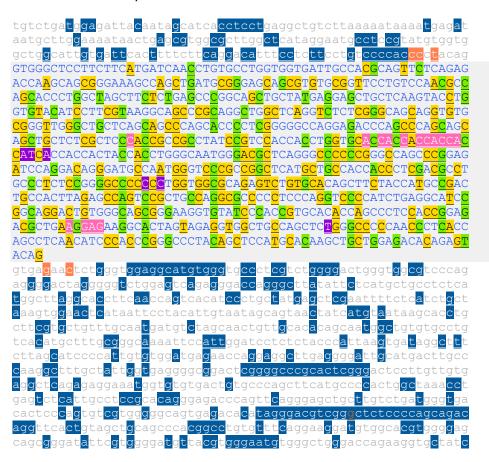
Cambio de estudio CACNA1G c.1924+645G>A (chr17:50576971 G/A, COSV61731312 o NM_018896.5:c.1924+645G>A)

Exón 8 e intrones adyacentes:

El cambio se encuentra en la antepenúltima línea del intrón 8 (la **g** en color naranja subrayada de 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'
           374        1     +        0.32      TACATCCTTC^GTAAGGCAGC
           416        1     +        0.91      CGGGCAGCAG^GTGTGCGGGT H
           965        1     +        1.00      CAGAGTACAG^GTGAGAACTC H
           988        0     +        0.60      GTGGAGGCAT^GTGGGTGCCC
          1401        1     +        0.34      TTTGCTATTG^GTGAGGGGCG

Donor splice sites, complement strand
-----------------------------------
  pos 3'->5'  pos 5'->3'  phase strand  confidence  5'     exon intron    3'
     1201        544        0     -        0.31      CACGAAGCAG^GTGCTTATTA
      899        846        1     -        0.65      GTTGAGGCTG^GTGAGGGTTG

Acceptor splice sites, direct strand
-----------------------------------
        pos 5'->3'  phase strand  confidence  5'     intron exon    3'
           148        1     +        0.55      TTTTCTTCAG^GACATTTCCT
           180        0     +        1.00      ACCCCTACAG^GTGGGCTCCT H
           462        0     +        0.19      CGGGGGCCAG^GAGACCCAGC
           465        0     +        0.20      GGGCCAGGAG^ACCCAGCCCA
           471        2     +        0.20      GGAGACCCAG^CCCAGCAGCA
           476        1     +        0.19      CCCAGCCCAG^CAGCAGCTGC
           479        1     +        0.19      AGCCCAGCAG^CAGCTGCTCT
           482        1     +        0.19      CCAGCAGCAG^CTGCTCTCGC
           761        0     +        0.56      CCCCTCCCAG^GTCCCCATCT
           774        0     +        0.18      CCCATCTGAG^GCATCCGGCA
           785        2     +        0.19      CATCCGGCAG^GACTGTGGGC
           797        2     +        0.19      CTGTGGGCAG^CGGGAAGGTG
           804        0     +        0.17      CAGCGGGAAG^GTGTATCCCA
          1526        0     +        0.33      CTCCGCACAG^GGAGACCCAG
          1622        1     +        0.25      TCCCCAGCAG^ACAGGTTCAC
          1626        2     +        0.14      CAGCAGACAG^GTTCACTGTA
          1637        1     +        0.17      TTCACTGTAG^CTGCAGCCCA
          1643        1     +        0.17      GTAGCTGCAG^CCCACGGCCT
          1662        2     +        0.00      TGTGTTTCAG^GAAGGATGTG

Acceptor splice sites, complement strand
-----------------------------------
  pos 3'->5'  pos 5'->3'  phase strand  confidence  5'     intron exon    3'
     1652         93        0     -        0.00      TGAAACACAG^GCCGTGGGCT
     1481        264        0     -        0.07      GGGCATGAAG^CTGGGCACAG
     1471        274        1     -        0.18      CTGGGCACAG^TCACACACCA
     1448        297        0     -        0.14      CCTCTCTGAG^CCTCACAACA
      946        799        0     -        0.56      GTGTCTCCAG^CAGCTTGTGC
      943        802        0     -        0.43      TCTCCAGCAG^CTTGTGCATG
      872        873        1     -        0.17      GAGCTGGCAG^CCACCTCTAC
      859        886        2     -        0.19      CCTCTACTAG^TGCCTTCTCC
      844        901        2     -        0.87      TCTCCTTCAG^CGTCTCCGGT
      830        915        1     -        0.19      TCCGGTGGAG^GGCTGGTGTG
      651       1094        0     -        0.07      AGGCGTCGAG^GGTGGTGGCA
      640       1105        2     -        0.19      GTGGTGGCAG^CATGAGCCGG
      395       1350        2     -        0.07      GAGACCTGAG^CCAGCCTGCG
      391       1354        0     -        0.07      CCTGAGCCAG^CCTGCGGGCT
      336       1409        0     -        0.17      CTCCTCATAG^CAGCTGCCGG
      320       1425        1     -        0.17      CCGGGCTCAG^AGAAGCTAGC
      318       1427        0     -        0.17      GGGCTCAGAG^AAGCTAGCCA
      315       1430        0     -        0.17      CTCAGAGAAG^CTAGCCAGGG
      311       1434        1     -        0.17      GAGAAGCTAG^CCAGGGTGCT
      307       1438        2     -        0.17      AGCTAGCCAG^GGTGCTGGCG
      289       1456        2     -        0.07      CGTTGGACAG^GAACCGCACA
```

```
Donor splice sites, direct strand
-----------------------------------
        pos 5'->3'  phase strand  confidence  5'     exon intron    3'
           374        1     +        0.32      TACATCCTTC^GTAAGGCAGC
           416        1     +        0.91      CGGGCAGCAG^GTGTGCGGGT H
           965        1     +        1.00      CAGAGTACAG^GTGAGAACTC H
           988        0     +        0.60      GTGGAGGCAT^GTGGGTGCCC
          1401        1     +        0.34      TTTGCTATTG^GTGAGGGGCG

Donor splice sites, complement strand
-----------------------------------
  pos 3'->5'  pos 5'->3'  phase strand  confidence  5'     exon intron    3'
     1201        544        0     -        0.31      CACGAAGCAG^GTGCTTATTA
      899        846        1     -        0.65      GTTGAGGCTG^GTGAGGGTTG

Acceptor splice sites, direct strand
-----------------------------------
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           148        1     +        0.55      TTTTCTTCAG^GACATTTCCT
           180        0     +        1.00      ACCCCTACAG^GTGGGCTCCT H
           462        0     +        0.19      CGGGGGCCAG^GAGACCCAGC
           465        0     +        0.20      GGGCCAGGAG^ACCCAGCCCA
           471        2     +        0.20      GGAGACCCAG^CCCAGCAGCA
           476        1     +        0.19      CCCAGCCCAG^CAGCAGCTGC
           479        1     +        0.19      AGCCCAGCAG^CAGCTGCTCT
           482        1     +        0.19      CCAGCAGCAG^CTGCTCTCGC
           761        0     +        0.56      CCCCTCCCAG^GTCCCCATCT
           774        0     +        0.18      CCCATCTGAG^GCATCCGGCA
           785        2     +        0.19      CATCCGGCAG^GACTGTGGGC
           797        2     +        0.19      CTGTGGGCAG^CGGGAAGGTG
           804        0     +        0.17      CAGCGGGAAG^GTGTATCCCA
          1526        0     +        0.33      CTCCGCACAG^GGAGACCCAG
          1619        1     +        0.07      CTCTCCCCAG^CAGACAGGTT
          1622        1     +        0.17      TCCCCAGCAG^ACAGGTTCAC
          1626        2     +        0.14      CAGCAGACAG^GTTCACTGTA
          1637        1     +        0.18      TTCACTGTAG^CTGCAGCCCA
          1643        1     +        0.17      GTAGCTGCAG^CCCACGGCCT
          1662        2     +        0.00      TGTGTTTCAG^GAAGGATGTG

Acceptor splice sites, complement strand
-----------------------------------
  pos 3'->5'  pos 5'->3'  phase strand  confidence  5'     intron exon    3'
     1652         93        0     -        0.00      TGAAACACAG^GCCGTGGGCT
     1481        264        0     -        0.07      GGGCATGAAG^CTGGGCACAG
     1471        274        1     -        0.18      CTGGGCACAG^TCACACACCA
     1448        297        0     -        0.14      CCTCTCTGAG^CCTCACAACA
      946        799        0     -        0.56      GTGTCTCCAG^CAGCTTGTGC
      943        802        0     -        0.43      TCTCCAGCAG^CTTGTGCATG
      872        873        1     -        0.17      GAGCTGGCAG^CCACCTCTAC
      859        886        2     -        0.19      CCTCTACTAG^TGCCTTCTCC
      844        901        2     -        0.87      TCTCCTTCAG^CGTCTCCGGT
      830        915        1     -        0.19      TCCGGTGGAG^GGCTGGTGTG
      651       1094        0     -        0.07      AGGCGTCGAG^GGTGGTGGCA
      640       1105        2     -        0.19      GTGGTGGCAG^CATGAGCCGG
      395       1350        2     -        0.07      GAGACCTGAG^CCAGCCTGCG
      391       1354        0     -        0.07      CCTGAGCCAG^CCTGCGGGCT
      336       1409        0     -        0.17      CTCCTCATAG^CAGCTGCCGG
      320       1425        1     -        0.17      CCGGGCTCAG^AGAAGCTAGC
      318       1427        0     -        0.17      GGGCTCAGAG^AAGCTAGCCA
      315       1430        0     -        0.17      CTCAGAGAAG^CTAGCCAGGG
      311       1434        1     -        0.17      GAGAAGCTAG^CCAGGGTGCT
      307       1438        2     -        0.17      AGCTAGCCAG^GGTGCTGGCG
      289       1456        2     -        0.07      CGTTGGACAG^GAACCGCACA
```

Se produce la activación de un sitio *acceptor* nuevo en la secuencia mutante. Este sitio se encuentra en el intrón 8, lo que provocaría la inclusión de un exón críptico si se empleara junto a un *donor* posterior a él o junto al del exón siguiente (de 1208 pb en este segundo caso).

Splice Site Prediction by Neural Network (NNSplice)

Donor site predictions for 10.42.3.123.574794.0 :

Start	End	Score	Exon	Intron
174	188	0.88	cctacag**gt**gggctc	
367	381	0.75	atccttc**gt**aaggca	
409	423	0.62	gcagcag**gt**gtgcgg	
958	972	0.99	agtacag**gt**gagaac	
1394	1408	0.71	gctattg**gt**gagggg	
1456	1470	0.43	ggaaatg**gt**gtgtga	

Donor site predictions for 10.42.0.139.574806.0 :

Start	End	Score	Exon	Intron
174	188	0.88	cctacag**gt**gggctc	
367	381	0.75	atccttc**gt**aaggca	
409	423	0.62	gcagcag**gt**gtgcgg	
958	972	0.99	agtacag**gt**gagaac	
1394	1408	0.71	gctattg**gt**gagggg	
1456	1470	0.43	ggaaatg**gt**gtgtga	

Acceptor site predictions for 10.42.3.123.574794.0 :

Start	End	Score	Intron	Exon
80	120	0.56	agcgtggcgcttggctcat**ag**gaatgcctccgtatgtggtg	
128	168	0.87	ttgtgattcacttttcttc**ag**gacatttcctcttcctgtcc	
160	200	0.93	ttcctgtccccacccctac**ag**gtgggctccttcttcatgat	
741	781	0.85	ctgccaggcgcccctccc**ag**gtccccatctgaggcatccg	
1072	1112	0.54	atgctgcctctcatggctt**ag**gcaccttcaaccagtcacat	
1506	1546	0.85	agtctcattgcctccgcac**ag**ggagacccagttcagggagc	
1642	1682	0.80	agcccacggcctgtgtttc**ag**gaaggatgtggcacgtgggg	

Acceptor site predictions for 10.42.0.139.574806.0 :

Start	End	Score	Intron	Exon
80	120	0.56	agcgtggcgcttggctcat**ag**gaatgcctccgtatgtggtg	
128	168	0.87	ttgtgattcacttttcttc**ag**gacatttcctcttcctgtcc	
160	200	0.93	ttcctgtccccacccctac**ag**gtgggctccttcttcatgat	
741	781	0.85	ctgccaggcgcccctccc**ag**gtccccatctgaggcatccg	
1072	1112	0.54	atgctgcctctcatggctt**ag**gcaccttcaaccagtcacat	
1506	1546	0.85	agtctcattgcctccgcac**ag**ggagacccagttcagggagc	
1642	1682	0.80	agcccacggcctgtgtttc**ag**gaaggatgtggcacgtgggg	

Spliceman

Point mutation	Wildtype (wt)	Mutation (mt)	L1 distance	Ranking (L1)
gtcgg(g/a)ctctc	gggctc	ggactc	28624	65%

Human Splicing Finder

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

Algorithm/Matix	position	sequence
ESE_ASF (New ESE Site)	chr17:50576965	CGTCGGA
ESE_ASFB (New ESE Site)	chr17:50576968	CGGACTC
ESE_SRp40 (New ESE Site)	chr17:50576971	ACTCTCC

SVM-BPfinder

seq_id	agez	ss_dist	bp_seq	bp_scr	y_cont	ppt_off	ppt_len	ppt_scr	svm_scr
wt	18	81	gggtgacac		1.54597972963	0.473684210526	76	0	0	-3.6505843
wt	18	55	cagtgagac		-0.412338424886	0.48	50	0	0	-2.7695622
mut	18	81	gggtgacac		1.54597972963	0.473684210526	76	0	0	-3.6505843
mut	18	55	cagtgagac		-0.412338424886	0.48	50	0	0	-2.7695622

Variant Effect Predictor tool

ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000442258.6	protein_coding	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000502264.5	protein_coding	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant, NMD_transcript_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000503436.5	nonsense_mediated_decay	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000503485.5	protein_coding	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant, NMD_transcript_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000503607.5	nonsense_mediated_decay	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant, NMD_transcript_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000504076.5	nonsense_mediated_decay	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000505165.5	protein_coding	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant, NMD_transcript_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000506406.5	nonsense_mediated_decay	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000507336.5	protein_coding	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000507510.6	protein_coding	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000507609.5	protein_coding	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000507896.5	protein_coding	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000510115.5	protein_coding	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000510366.5	protein_coding	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant, NMD_transcript_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000511765.5	nonsense_mediated_decay	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant, NMD_transcript_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000511768.5	nonsense_mediated_decay	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000512389.5	protein_coding	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000513689.6	protein_coding	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000513964.5	protein_coding	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000514079.5	protein_coding	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000514181.5	protein_coding	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000514717.5	protein_coding	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000515165.5	protein_coding	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000515411.5	protein_coding	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	intron_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000515765.5	protein_coding	-	-	rs1184748182, COSV61731312
ENST00000515165.5:c.1924+645G>A	17:50576971-50576971	A	downstream_gene_variant	CACNA1G	ENSG00000006283	Transcript	ENST00000570567.1	protein_coding	-	-	rs1184748182, COSV61731312

ESEfinder

Si buscamos las puntuaciones positivas para la secuencia WT, veríamos que tiene puntuaciones positivas para 5'SS en un resultado (1589) y dos resultados para las matrices 3'SS (1605 y 1608):

1589 (−156)	gagacacatagggacgtcgggctctcccca	0.91340

1589 (−156)	gagacacatagggacgtcgggctctcccca	-19.76040

1589 (−156)	gagacacatagggacgtcgggctctcccca	0.87580

1589 (−156)	gagacacatagggacgtcgggctctcccca	-20.78070

1605 (−140)	tcgggctctccccagcagacaggttcactg	-18.60220
1606 (−139)	cgggctctccccagcagacaggttcactgt	-27.21540
1607 (−138)	gggctctccccagcagacaggttcactgta	-14.19570
1608 (−137)	ggctctccccagcagacaggttcactgtag	-6.34780

1605 (−140)	tcgggctctccccagcagacaggttcactg	6.97220
1606 (−139)	cgggctctccccagcagacaggttcactgt	-23.35800
1607 (−138)	gggctctccccagcagacaggttcactgta	-30.37220
1608 (−137)	ggctctccccagcagacaggttcactgtag	6.06680

1605 (−140)	tcgggctctccccagcagacaggttcactg	-15.03000
1606 (−139)	cgggctctccccagcagacaggttcactgt	-25.77830
1607 (−138)	gggctctccccagcagacaggttcactgta	-10.76400
1608 (−137)	ggctctccccagcagacaggttcactgtag	-7.09560

1605 (−140)	tcgggctctccccagcagacaggttcactg	6.98790
1606 (−139)	cgggctctccccagcagacaggttcactgt	-20.90460
1607 (−138)	gggctctccccagcagacaggttcactgta	-31.84310
1608 (−137)	ggctctccccagcagacaggttcactgtag	6.51650

Si comparamos estas puntuciones con las de los resultados equivalentes para la secuencia mutante, se observa que descienden un poco en todos los casos:

1589 (−156)	gagacacatagggacgtcggactctcccca	0.59940

1589 (−156)	gagacacatagggacgtcggactctcccca	-20.10260

1589 (−156)	gagacacatagggacgtcggactctcccca	0.48110

1589 (−156)	gagacacatagggacgtcggactctcccca	-21.05260

1605 (−140)	tcggactctccccagcagacaggttcactg	-18.54560
1606 (−139)	cggactctccccagcagacaggttcactgt	-27.44880
1607 (−138)	ggactctccccagcagacaggttcactgta	-14.44090
1608 (−137)	gactctccccagcagacaggttcactgtag	-6.34840

1605 (−140)	tcggactctccccagcagacaggttcactg	6.35220
1606 (−139)	cggactctccccagcagacaggttcactgt	-23.82180
1607 (−138)	ggactctccccagcagacaggttcactgta	-30.84410
1608 (−137)	gactctccccagcagacaggttcactgtag	5.66790

1605 (−140)	tcggactctccccagcagacaggttcactg	-14.96320
1606 (−139)	cggactctccccagcagacaggttcactgt	-25.96620
1607 (−138)	ggactctccccagcagacaggttcactgta	-10.99880
1608 (−137)	gactctccccagcagacaggttcactgtag	-7.10910

1605 (−140)	tcggactctccccagcagacaggttcactg	6.24890
1606 (−139)	cggactctccccagcagacaggttcactgt	-21.51220
1607 (−138)	ggactctccccagcagacaggttcactgta	-32.47240
1608 (−137)	gactctccccagcagacaggttcactgtag	5.92230

El caso más significativo podría ser 1608, por lo que se podría decir que se está debilitando un sitio 3'SS en la secuencia mutante, lo cual no tendría efecto en el *splicing* porque no es un sitio que se use habitualmente.