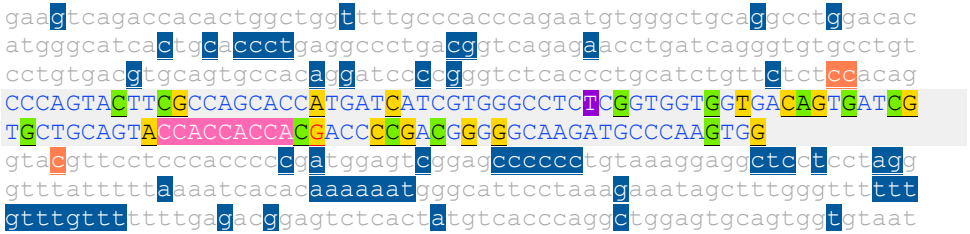
**Ejemplo comparación de resultados predictores in sillico**

Cambio de estudio CHRNA7 c.961G>T (chr15:32163306 G/T,  COSV60967947 o NM_000746.6: c.961G>T)

Exón 9 e intrones adyacentes:



El cambio se encuentra en segunda posición justo antes del exón 7 (la **g** en color rojo subrayada en amarillo).

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' |
|---|---|---|---|---|
| 287 | 2 | + | 0.71 | AGATGCCCAA^GTGGGTACGT |
| 291 | 0 | + | 0.96 | GCCCAAGTGG^GTACGTTCCT H |
| 295 | 1 | + | 0.06 | AAGTGGGTAC^GTTCCTCCCA |

Donor splice sites, complement strand

| pos 3'->5' | pos 5'->3' | phase | strand | confidence | 5' exon intron 3' |
|---|---|---|---|---|---|
| 158 | 313 | 2 | - | 0.99 | CAGATGCAGG^GTGAGACCCG H |

Acceptor splice sites, direct strand

| pos 5'->3' | phase | strand | confidence | 5' intron exon 3' |
|---|---|---|---|---|
| 36 | 2 | + | 0.00 | GCCCACCCAG^AATGTGGGCT |
| 134 | 0 | + | 0.17 | TGACGTGCAG^TGCCACAGGA |
| 142 | 2 | + | 0.30 | AGTGCCACAG^GATCCCCGGG |
| 180 | 1 | + | 0.97 | CTCTCCACAG^CCCAGTACTT |
| 185 | 0 | + | 0.19 | CACAGCCCAG^TACTTCGCCA |
| 196 | 2 | + | 0.17 | ACTTCGCCAG^CACCATGATC |

Acceptor splice sites, complement strand

| pos 3'->5' | pos 5'->3' | phase | strand | confidence | 5' intron exon 3' |
|---|---|---|---|---|---|
| 299 | 172 | 1 | - | 0.17 | GGGGTGGGAG^GAACGTACCC |
| 243 | 228 | 2 | - | 0.19 | GGTACTGCAG^CACGATCACT |

Donor splice sites, direct strand

| pos 5'->3' | phase | strand | confidence | 5' exon intron 3' |
|---|---|---|---|---|
| 287 | 2 | + | 0.71 | AGATGCCCAA^GTGGGTACGT |
| 291 | 0 | + | 0.96 | GCCCAAGTGG^GTACGTTCCT H |
| 295 | 1 | + | 0.06 | AAGTGGGTAC^GTTCCTCCCA |

Donor splice sites, complement strand

| pos 3'->5' | pos 5'->3' | phase | strand | confidence | 5' exon intron 3' |
|---|---|---|---|---|---|
| 158 | 313 | 2 | - | 0.99 | CAGATGCAGG^GTGAGACCCG H |

Acceptor splice sites, direct strand

| pos 5'->3' | phase | strand | confidence | 5' intron exon 3' |
|---|---|---|---|---|
| 36 | 2 | + | 0.00 | GCCCACCCAG^AATGTGGGCT |
| 134 | 0 | + | 0.17 | TGACGTGCAG^TGCCACAGGA |
| 142 | 2 | + | 0.30 | AGTGCCACAG^GATCCCCGGG |
| 180 | 1 | + | 0.97 | CTCTCCACAG^CCCAGTACTT |
| 185 | 0 | + | 0.19 | CACAGCCCAG^TACTTCGCCA |
| 196 | 2 | + | 0.17 | ACTTCGCCAG^CACCATGATC |

Acceptor splice sites, complement strand

| pos 3'->5' | pos 5'->3' | phase | strand | confidence | 5' intron exon 3' |
|---|---|---|---|---|---|
| 260 | 211 | 1 | - | 0.19 | GTCGGGGTAG^TGGTGGTGGT |
| 243 | 228 | 2 | - | 0.25 | GGTACTGCAG^CACGATCACT |
| 218 | 253 | 0 | - | 0.17 | CACCACCGAG^AGGCCCACGA |
| 216 | 255 | 2 | - | 0.17 | CCACCGAGAG^GCCCACGATG |

**Splice Site Prediction by Neural Network (NNSplice)**

### Donor site predictions for 10.42.2.148.572569.0 :

| Start | End | Score | Exon | Intron |
|---|---|---|---|---|
| 284 | 298 | 0.95 | caagtgg | **gt**acgttc |

### Donor site predictions for 10.42.3.123.572581.0 :

| Start | End | Score | Exon | Intron |
|---|---|---|---|---|
| 284 | 298 | 0.95 | caagtgg | **gt**acgttc |

### Acceptor site predictions for 10.42.2.148.572569.0 :

| Start | End | Score | Intron | Exon |
|---|---|---|---|---|
| 16 | 56 | 0.59 | tggctggttttgcccaccc | **ag**aatgtgggctgcaggcctgg |
| 160 | 200 | 0.92 | ctgcatctgttctctccac | **ag**cccagtacttcgccagcacc |
| 405 | 445 | 0.64 | ttttttgtttgttttttttg | **ag**acggagtctcactatgtcac |

### Acceptor site predictions for 10.42.3.123.572581.0 :

| Start | End | Score | Intron | Exon |
|---|---|---|---|---|
| 16 | 56 | 0.59 | tggctggttttgcccaccc | **ag**aatgtgggctgcaggcctgg |
| 160 | 200 | 0.92 | ctgcatctgttctctccac | **ag**cccagtacttcgccagcacc |
| 405 | 445 | 0.64 | ttttttgtttgttttttttg | **ag**acggagtctcactatgtcac |

**Spliceman**

| Point mutation | Wildtype (wt) | Mutation (mt) | L1 distance | Ranking (L1) |
|---|---|---|---|---|
| accac(g/t)acccc | gacccc | tacccc | 28016 | 61% |

**CRYP-SKIP**

Parece que hay un sitio críptico de *splicing* dentro del propio exón, pero el cambio de interés (la primera **a** en minúsculas detrás de las mayúsculas, que indican el exón) no lo toma en consideración, por lo que no debe considerar que tenga algún efecto en el *splicing*.

**Human Splicing Finder**

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

| Algorithm/Matix | position | sequence |
|---|---|---|
| ESE_SC35 (New ESE Site) | chr15:32163300 | CACCACTA |
| ESE_ASF (ESE Site Broken) | chr15:32163301 | ACCACGA |
| Fas ESS (New ESS Site) | chr15:32163302 | CCACTA |
| ESE_SRp40 (New ESE Site) | chr15:32163302 | CCACTAC |
| EIE (New ESE Site) | chr15:32163303 | CACTAC |
| Sironi_motif3 (New ESS Site) | chr15:32163304 | ACTACCCC |
| ESE_SRp40 (New ESE Site) | chr15:32163305 | CTACCCC |
| ESE_SC35 (ESE Site Broken) | chr15:32163305 | CGACCCCG |
| PESE (ESE Site Broken) | chr15:32163305 | CGACCCCG |
| ESE_SC35 (ESE Site Broken) | chr15:32163306 | GACCCCGA |
| Sironi_motif3 (New ESS Site) | chr15:32163306 | TACCCCGA |

**SVM-BPfinder**

| seq_id | agez | ss_dist | bp_seq | bp_scr | y_cont | ppt_off | ppt_len | ppt_scr | svm_scr |
|---|---|---|---|---|---|---|---|---|---|
| wt | 37 | 88 | cagtgatcg | 1.02825084502 | 0.518072289157 | 57 | 16 | 27 | -2.3847677 |
| mut | 37 | 88 | cagtgatcg | 1.02825084502 | 0.530120481928 | 14 | 16 | 17 | 0.24779291 |

## Variant Effect Predictor tool

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | missense_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000306901.9 | protein_coding | 9/10 | 1041 | 961 | 321 | D/Y | GAC/TAC | COSV60967947 |
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | 3_prime_UTR_variant, NMD_transcript_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000437966.3 | nonsense_mediated_decay | 7/8 | 810 | - | - | - | - | COSV60967947 |
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | missense_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000454250.7 | protein_coding | 9/10 | 1155 | 1048 | 350 | D/Y | GAC/TAC | COSV60967947 |
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | downstream_gene_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000635722.1 | nonsense_mediated_decay | - | - | - | - | - | - | COSV60967947 |
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | downstream_gene_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000635883.1 | protein_coding | - | - | - | - | - | - | COSV60967947 |
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | intron_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000635884.1 | protein_coding | - | - | - | - | - | - | COSV60967947 |
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | downstream_gene_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000635978.1 | protein_coding | - | - | - | - | - | - | COSV60967947 |
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | downstream_gene_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000636044.1 | nonsense_mediated_decay | - | - | - | - | - | - | COSV60967947 |
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | non_coding_transcript_exon_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000636245.1 | processed_transcript | 5/6 | 656 | - | - | - | - | COSV60967947 |
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | missense_variant, NMD_transcript_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000636271.1 | nonsense_mediated_decay | 8/10 | 803 | 805 | 269 | D/Y | GAC/TAC | COSV60967947 |
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | non_coding_transcript_exon_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000636292.1 | retained_intron | 6/7 | 1331 | - | - | - | - | COSV60967947 |
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | downstream_gene_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000636440.1 | protein_coding | - | - | - | - | - | - | COSV60967947 |
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | downstream_gene_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000636521.1 | retained_intron | - | - | - | - | - | - | COSV60967947 |
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | missense_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000636603.1 | protein_coding | 9/10 | 1042 | 775 | 259 | D/Y | GAC/TAC | COSV60967947 |
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | downstream_gene_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000636647.1 | retained_intron | - | - | - | - | - | - | COSV60967947 |
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | non_coding_transcript_exon_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000636709.1 | retained_intron | 1/1 | 7320 | - | - | - | - | COSV60967947 |
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | downstream_gene_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000636850.1 | nonsense_mediated_decay | - | - | - | - | - | - | COSV60967947 |
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | downstream_gene_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000636898.1 | retained_intron | - | - | - | - | - | - | COSV60967947 |
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | downstream_gene_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000636957.1 | processed_transcript | - | - | - | - | - | - | COSV60967947 |
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | missense_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000637033.1 | protein_coding | 9/10 | 947 | 775 | 259 | D/Y | GAC/TAC | COSV60967947 |
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | missense_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000637183.1 | protein_coding | 8/9 | 905 | 724 | 242 | D/Y | GAC/TAC | COSV60967947 |
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | non_coding_transcript_exon_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000637189.1 | retained_intron | 4/5 | 1101 | - | - | - | - | COSV60967947 |
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | missense_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000637348.1 | protein_coding | 3/4 | 189 | 190 | 64 | D/Y | GAC/TAC | COSV60967947 |
| ENST00000306901.8:c.961G>T | 15:32163306-32163306 | T | 3_prime_UTR_variant, NMD_transcript_variant | CHRNA7 | ENSG00000175344 | Transcript | ENST00000637350.1 | nonsense_mediated_decay | 9/11 | 1088 | - | - | - | - | COSV60967947 |

**ESEfinder**

Se obtienen dos resultados con puntuaciones positivas, uno para las matrices 5’ (259) y otro para las matrices 3’ (234):

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 234 (-237) | GTGATCGTGCTGCAGTACCACCACCACGAC | -32.02050 | 234 (-237) | GTGATCGTGCTGCAGTACCACCACCACGAC | 4.88400 | 234 (-237) | GTGATCGTGCTGCAGTACCACCACCACGAC | -28.50130 | 234 (-237) | GTGATCGTGCTGCAGTACCACCACCACGAC | 4.78730 |
| 259 (-212) | ACGACCCCGACGGGGGCAAGATGCCCAAGT | 3.26020 | 259 (-212) | ACGACCCCGACGGGGGCAAGATGCCCAAGT | -22.34250 | 259 (-212) | ACGACCCCGACGGGGGCAAGATGCCCAAGT | 2.89940 | 259 (-212) | ACGACCCCGACGGGGGCAAGATGCCCAAGT | -20.84660 |

Si comparamos las puntuaciones con las de la secuencia mutante, todas descienden un poco.

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 234 (-237) | GTGATCGTGCTGCAGTACCACCACCACTAC | -32.12360 | 234 (-237) | GTGATCGTGCTGCAGTACCACCACCACTAC | 4.14220 | 234 (-237) | GTGATCGTGCTGCAGTACCACCACCACTAC | -28.72120 | 234 (-237) | GTGATCGTGCTGCAGTACCACCACCACTAC | 4.11680 |
| 259 (-212) | ACTACCCCGACGGGGGCAAGATGCCCAAGT | 2.64030 | 259 (-212) | ACTACCCCGACGGGGGCAAGATGCCCAAGT | -20.68080 | 259 (-212) | ACTACCCCGACGGGGGCAAGATGCCCAAGT | 2.25950 | 259 (-212) | ACTACCCCGACGGGGGCAAGATGCCCAAGT | -19.27790 |

Se obtienen resultados contradictorios, por lo que no se puede dar ningún tipo de conclusión.

En cuanto a los ESE, hay cambios significativos en las matrices:

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 255 (-216) | CACCACG | -0.23271 | 255 (-216) | CACCACG | 0.99812 | 255 (-216) | CACCACGA | 0.81998 | 255 (-216) | CACCACG | 0.99982 |
| 256 (-215) | ACCACGA | 2.35845 | 256 (-215) | ACCACGA | 1.58513 | 256 (-215) | ACCACGAC | -4.23322 | 256 (-215) | ACCACGA | -2.80850 |
| 257 (-214) | CCACGAC | -3.82842 | 257 (-214) | CCACGAC | -0.86193 | 257 (-214) | CCACGACC | -3.70270 | 257 (-214) | CCACGAC | 2.00268 |
| 258 (-213) | CACGACC | -3.62759 | 258 (-213) | CACGACC | -1.30485 | 258 (-213) | CACGACCC | -2.01287 | 258 (-213) | CACGACC | -1.56714 |
| 259 (-212) | ACGACCC | -2.67216 | 259 (-212) | ACGACCC | -1.86784 | 259 (-212) | ACGACCCC | -5.24682 | 259 (-212) | ACGACCC | -1.78827 |
| 260 (-211) | CGACCCC | -1.94607 | 260 (-211) | CGACCCC | 0.45661 | 260 (-211) | CGACCCCG | 2.76802 | 260 (-211) | CGACCCC | 2.26093 |
| 261 (-210) | GACCCCG | 0.71371 | 261 (-210) | GACCCCG | 0.01423 | 261 (-210) | GACCCCGA | 3.32091 | 261 (-210) | GACCCCG | -2.88480 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 255 (-216) | CACCACT | 0.14084 | 255 (-216) | CACCACT | 1.20579 | 255 (-216) | CACCACTA | 3.13998 | 255 (-216) | CACCACT | -1.38191 |
| 256 (-215) | ACCACTA | 0.23842 | 256 (-215) | ACCACTA | 0.04182 | 256 (-215) | ACCACTAC | -5.04693 | 256 (-215) | ACCACTA | -4.83105 |
| 257 (-214) | CCACTAC | -5.74803 | 257 (-214) | CCACTAC | -2.46579 | 257 (-214) | CCACTACC | -3.56426 | 257 (-214) | CCACTAC | 4.31152 |
| 258 (-213) | CACTACC | -3.16988 | 258 (-213) | CACTACC | -1.24566 | 258 (-213) | CACTACCC | 0.45443 | 258 (-213) | CACTACC | -2.22745 |
| 259 (-212) | ACTACCC | -4.73594 | 259 (-212) | ACTACCC | -3.55543 | 259 (-212) | ACTACCCC | -3.50818 | 259 (-212) | ACTACCC | -0.22675 |
| 260 (-211) | CTACCCC | -2.62138 | 260 (-211) | CTACCCC | -0.27287 | 260 (-211) | CTACCCCG | 2.12034 | 260 (-211) | CTACCCC | 2.77042 |
| 261 (-210) | TACCCCG | -0.65947 | 261 (-210) | TACCCCG | -0.19262 | 261 (-210) | TACCCCGA | 1.27126 | 261 (-210) | TACCCCG | -0.38046 |

**EX-SKIP**

| Seq | PESS (count) | FAS-ESS hex2 (count) | FAS-ESS hex3 (count) | IIE (count) | IIE (sum) | NI-ESS trusted (count) | NI-ESS all (sum) | PESE (count) | RESCUE -ESE (count) | EIE (count) | EIE (sum) | NI-ESE trusted (count) | NI-ESE all (sum) | ESS (total) | ESE (total) | ESS/ESE (ratio) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| wt | 0 | 2 | 1 | 15 | 209.4238 | 3 | -5.2177 | 1 | 6 | 36 | 327.9889 | 27 | 49.4092 | 21 | 70 | 0.30 |
| mut | 0 | 3 | 1 | 15 | 209.4238 | 4 | -6.2177 | 1 | 6 | 37 | 355.9128 | 23 | 44.9522 | 23 | 67 | 0.34 |

**Allele mut has a higher chance of exon skipping than allele wt.**

**HOT-SKIP**

gaagtcagaccacactggctggttttgcccacccagaatgtgggctgcaggcctggacacatgggcatcactgcaccctg
aggccctgacggtcagagaacctgatcagggtgtgcctgtcctgtgacgtgcagtgccacaggatccccgggtctcaccc
tgcatctgttctctccacagCCCAGTACTTCGCCAGCACCATGATCATCGTGGGCCTCTCGGTGGTGGTGACAGTGATCG
TGCTGCAGTACCACCACCACGACCCCGACGGGGGCAAGATGCCCAAGTGGgtacgttcctcccacccccgatggagtcgg
agccccctgtaaaggaggctcctcctagggtttatttttaaaatcacacaaaaaatgggcattcctaaagaaatagctt
tgggtttttgtttgtttttttgagacggagtctcactatgtcacccaggctggagtgcagtggtgtaat