**Ejemplo comparación de resultados predictores in sillico**
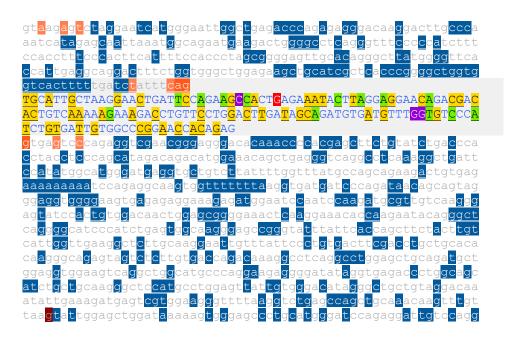
Cambio de estudio CES1 c.1318+724G>C (chr16:55809793 G/C, COSV62090951 o NM_001025195.2: c.1318+724G>C)

Exón 11 e intrones adyacentes:



El cambio se encuentra en la última fila del intrón 11 (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                              Donor splice sites, direct strand
---------------------------------                              ---------------------------------
       pos 5'->3'  phase strand  confidence 5'     exon intron    3'          pos 5'->3'  phase strand  confidence 5'     exon intron    3'
          411        1     +        0.99     AACCACAGAG^GTGAGTCCCA H              411        1     +        0.99     AACCACAGAG^GTGAGTCCCA H
          995        1     +        0.87     GGGGATATAG^GTGAGACCCT                995        1     +        0.88     GGGGATATAG^GTGAGACCCT

Donor splice sites, complement strand                          Donor splice sites, complement strand
-------------------------------------                          -------------------------------------
 pos 3'->5'  pos 5'->3'  phase strand  confidence 5'    exon intron    3'    pos 3'->5'  pos 5'->3'  phase strand  confidence 5'    exon intron    3'
    475        716         2     -        0.35     ATGCTGGGAG^GTAGGTGGGT     475        716         2     -        0.35     ATGCTGGGAG^GTAGGTGGGT
    228        963         1     -        0.58     CCAGCCCCGG^GTGAGCGATG     228        963         1     -        0.58     CCAGCCCCGG^GTGAGCGATG

Acceptor splice sites, direct strand                           Acceptor splice sites, direct strand
------------------------------------                           ------------------------------------
       pos 5'->3'  phase strand  confidence 5'    intron exon    3'          pos 5'->3'  phase strand  confidence 5'    intron exon    3'
          150        1     +        0.25     TCCACCCTAG^CGGGGAGTTG               150        1     +        0.25     TCCACCCTAG^CGGGGAGTTG
          262        0     +        0.95     TCTATTTCAG^TGCATTGCTA               262        0     +        0.95     TCTATTTCAG^TGCATTGCTA
          274        0     +        0.34     CATTGCTAAG^GAACTGATTC               274        0     +        0.34     CATTGCTAAG^GAACTGATTC
          287        1     +        0.34     CTGATTCCAG^AAGCCACTGA               287        1     +        0.34     CTGATTCCAG^AAGCCACTGA
          290        1     +        0.34     ATTCCAGAAG^CCACTGAGAA               290        1     +        0.34     ATTCCAGAAG^CCACTGAGAA
          298        0     +        0.20     AGCCACTGAG^AAATACTTAG               298        0     +        0.20     AGCCACTGAG^AAATACTTAG
          308        1     +        0.18     AAATACTTAG^GAGGAACAGA               308        1     +        0.18     AAATACTTAG^GAGGAACAGA
          311        1     +        0.18     TACTTAGGAG^GAACAGACGA               311        1     +        0.18     TACTTAGGAG^GAACAGACGA
          317        1     +        0.07     GGAGGAACAG^ACGACACTGT               317        1     +        0.07     GGAGGAACAG^ACGACACTGT
          576        0     +        0.16     TTTATGCCAG^CAGAAGACTG               576        0     +        0.16     TTTATGCCAG^CAGAAGACTG
          579        0     +        0.16     ATGCCAGCAG^AAGACTGTGA               579        0     +        0.16     ATGCCAGCAG^AAGACTGTGA
          918        0     +        0.18     TTGTGACCAG^ACAAAGGCCT               918        0     +        0.18     TTGTGACCAG^ACAAAGGCCT
          924        0     +        0.17     CCAGACAAAG^GCCTCAGGCC               924        0     +        0.17     CCAGACAAAG^GCCTCAGGCC
          931        1     +        0.07     AAGGCCTCAG^GCCTGGAGCT               931        1     +        0.07     AAGGCCTCAG^GCCTGGAGCT

Acceptor splice sites, complement strand                       Acceptor splice sites, complement strand
----------------------------------------                       ----------------------------------------
 pos 3'->5'  pos 5'->3'  phase strand  confidence 5'    intron exon    3'    pos 3'->5'  pos 5'->3'  phase strand  confidence 5'    intron exon    3'
   1060        131         1     -        0.33     TGTCCTACAG^CAGCCCTATG   1060        131         1     -        0.33     TGTCCTACAG^CAGCCCTATG
    884        307         2     -        0.16     TTGTGTGCAG^CAGGTCGAAG     884        307         2     -        0.16     TTGTGTGCAG^CAGGTCGAAG
    881        310         2     -        0.72     TGTGCAGCAG^GTCGAAGTCA     881        310         2     -        0.72     TGTGCAGCAG^GTCGAAGTCA
    785        406         2     -        0.27     TGCCACTCAG^ATGGGATGCC     785        406         2     -        0.27     TGCCACTCAG^ATGGGATGCC
    729        462         1     -        0.33     CCCGCTCCAG^TTGTCCACAG     729        462         1     -        0.33     CCCGCTCCAG^TTGTCCACAG
    584        607         0     -        0.68     TTTCTCACAG^TCTTCTGCTG     584        607         0     -        0.68     TTTCTCACAG^TCTTCTGCTG
```

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

### Donor site predictions for 10.42.0.139.574626.0 :

| Start | End | Score | Exon | Intron |
|-------|-----|-------|------|--------|
| 404 | 418 | 1.00 | cacagag**gt**gagtcc | |
| 988 | 1002 | 0.93 | gatatag**gt**gagacc | |

### Donor site predictions for 10.42.3.123.574614.0 :

| Start | End | Score | Exon | Intron |
|-------|-----|-------|------|--------|
| 404 | 418 | 1.00 | cacagag**gt**gagtcc | |
| 988 | 1002 | 0.93 | gatatag**gt**gagacc | |

### Acceptor site predictions for 10.42.0.139.574626.0 :

| Start | End | Score | Intron | Exon |
|-------|-----|-------|--------|------|
| 130 | 170 | 0.84 | ccacttcattttccaccct**ag**cggggagttgcacagggctt | |
| 242 | 282 | 0.89 | tcactttttgatctatttc**ag**tgcattgctaaggaactgat | |

### Acceptor site predictions for 10.42.3.123.574614.0 :

| Start | End | Score | Intron | Exon |
|-------|-----|-------|--------|------|
| 130 | 170 | 0.84 | ccacttcattttccaccct**ag**cggggagttgcacagggctt | |
| 242 | 282 | 0.89 | tcactttttgatctatttc**ag**tgcattgctaaggaactgat | |

**Spliceman**

| Point mutation | Wildtype (wt) | Mutation (mt) | L1 distance | Ranking (L1) |
|----------------|---------------|---------------|-------------|--------------|
| gttaa(g/c)tattg | agtatt | actatt | 26604 | 53% |

**Human Splicing Finder**

| Algorithm/Matix | position | sequence |
|---|---|---|
| Alteration of auxiliary sequences | Significant alteration of ESE / ESS motifs ratio (4) | |
| EIE (New ESE Site) | chr16:55809793 | CTATTG |
| ESE_SRp40 (New ESE Site) | chr16:55809793 | CTATTGG |
| EIE (New ESE Site) | chr16:55809794 | ACTATT |
| IIE (ESS Site Broken) | chr16:55809794 | AGTATT |
| ESE_SRp55 (ESE Site Broken) | chr16:55809796 | TAAGTA |
| ESS_hnRNPA1 (ESS Site Broken) | chr16:55809796 | TAAGTA |
| EIE (New ESE Site) | chr16:55809796 | TAACTA |
| PESS (New ESS Site) | chr16:55809796 | TAACTATT |
| PESS (ESS Site Broken) | chr16:55809797 | TTAAGTAT |
| PESS (New ESS Site) | chr16:55809797 | TTAACTAT |

**SVM-BPfinder**

| seq_id | agez | ss_dist | bp_seq | bp_scr | y_cont | ppt_off | ppt_len | ppt_scr | svm_scr |
|--------|------|---------|--------|--------|--------|---------|---------|---------|---------|
| wt | 13 | 59 | ttgttaagt | -3.37854056137 | 0.37037037037 | 54 | 0 | 0 | -4.219571 |
| wt | 13 | 58 | tgttaagta | -0.966770826387 | 0.377358490566 | 53 | 0 | 0 | -3.2096939 |
| wt | 13 | 41 | ggataaaaa | -2.2896137965 | 0.388888888889 | 36 | 0 | 0 | -2.647854 |
| mut | 13 | 59 | ttgttaact | -2.5629887778 | 0.388888888889 | 54 | 0 | 0 | -3.894263 |
| mut | 13 | 58 | tgttaacta | 1.15337884134 | 0.396226415094 | 53 | 0 | 0 | -2.3734613 |
| mut | 13 | 41 | ggataaaaa | -2.2896137965 | 0.388888888889 | 36 | 0 | 0 | -2.647854 |

Las diferencias se encuentran en la presencia de la mutación en el BP detectado. Las puntuaciones disminuyen de la secuencia WT a la mutante, pero siguen siendo negativas, por lo que no se tendrán en cuenta.

**Variant Effect Predictor tool**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ENST00000360526.7:c.1318+724G>C | 16:55809793-55809793 | G | intron_variant | CES1 | ENSG00000198848 | Transcript | ENST00000360526.8 | protein_coding | - | - | COSV62090951 |
| ENST00000360526.7:c.1318+724G>C | 16:55809793-55809793 | G | intron_variant | CES1 | ENSG00000198848 | Transcript | ENST00000361503.8 | protein_coding | - | - | COSV62090951 |
| ENST00000360526.7:c.1318+724G>C | 16:55809793-55809793 | G | intron_variant | CES1 | ENSG00000198848 | Transcript | ENST00000422046.6 | protein_coding | - | - | COSV62090951 |
| ENST00000360526.7:c.1318+724G>C | 16:55809793-55809793 | G | intron_variant | CES1 | ENSG00000198848 | Transcript | ENST00000563241.5 | protein_coding | - | - | COSV62090951 |
| ENST00000360526.7:c.1318+724G>C | 16:55809793-55809793 | G | intron_variant, NMD_transcript_variant | CES1 | ENSG00000198848 | Transcript | ENST00000565568.1 | nonsense_mediated_decay | - | - | COSV62090951 |
| ENST00000360526.7:c.1318+724G>C | 16:55809793-55809793 | G | downstream_gene_variant | CES1 | ENSG00000198848 | Transcript | ENST00000569260.1 | nonsense_mediated_decay | - | - | COSV62090951 |
| ENST00000360526.7:c.1318+724G>C | 16:55809793-55809793 | G | regulatory_region_variant | - | - | RegulatoryFeature | ENSR00000538209 | promoter_flanking_region | - | - | COSV62090951 |

**ESEfinder**

Solo se encuentra una puntuación positiva para las predicciones donde está presente la posición de interés y se da en las matrices 5'SS:

| 1110 (-81) | gccagctgcaaacaagtttgttaagtattg | 2.73340 |
|---|---|---|

| 1110 (-81) | gccagctgcaaacaagtttgttaagtattg | -13.48480 |
|---|---|---|

| 1110 (-81) | gccagctgcaaacaagtttgttaagtattg | 2.26180 |
|---|---|---|

| 1110 (-81) | gccagctgcaaacaagtttgttaagtattg | -16.81070 |
|---|---|---|

La misma posición pero en la secuencia mutante da puntuaciones ligeramente superiores:

| 1110 (-81) | gccagctgcaaacaagtttgttaactattg | 2.78070 |
|---|---|---|

| 1110 (-81) | gccagctgcaaacaagtttgttaactattg | -13.66540 |
|---|---|---|

| 1110 (-81) | gccagctgcaaacaagtttgttaactattg | 2.33500 |
|---|---|---|

| 1110 (-81) | gccagctgcaaacaagtttgttaactattg | -16.92580 |
|---|---|---|

Puede que se esté haciendo más fuerte un sitio *donor* en la secuencia mutante, pero es poco probable dada la diferencia de *score*.