

Ejemplo comparación de resultados predictores in silico

Cambio de estudio OXCT1 c.1248+5G>A (chr5: 41794100 G/A, rs1561081472 o NM_000436_ c.1248+5G>A)

Exón 12 y 13 e intrones adyacentes:

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cataaaacCGGTCTGAGGTAACAAAAAGGGGACTGCTGCTGTAGAGAATTTCCAAACAAT
aagaaaactaaAGTGGTCTGCTTTCATTATCATCATGCAAAGGCAAGAGCAATGTGAG
TGACAAAATGCTTTCACCAACAAAAGGTTCTACTGTGTCCCCCACCTGCTATGTCACT
gcatTTTGGTAAAGGGAGCTTAAGTTCTTTTGGGAATGGAGAAGATGTGGACCTCTGTTT
acCATGATACCTTTAGAAATCTAATAGCCTTTCTTTCTTTTGTGTGTGTGTGTGTGTAG
GCAAGCAAAAGCAGTTACTATTCTTCCAGGAGCCTCTTTTTTCTCCAGCGATGAATCATTG
CAATGATTAGAGG
GTATGTAATAACAGAGGCCCTCTCATGTTTCAGACAGTATGGAATGAGCTGAGTCATCGTCT
GAGTGTGTTAGCAAGGCTCTGAAACACAGCCACATTTCCAGCCAGAAAACATCAGGAG
TTTGTTCGGCTGCAGGGTGTGAAATTATTCACATCGAATGTTGGGCAATTGAATATCCAGC
CCACTGTTAGGAAAAGAACCCCAAAATGGAATCCATGCCCTTGTCGAACCATACTGAA
ATAAACATTTCTAAGGTGCTTTCTGAGGATTCCTAACATTTCTTCAAACTGTGTTCACTT
TCTAGCTAGTGTGTTGTGTTCAAGTAGTCACATCTCTGCTCACTGAAGGAAACAGTCTTCT
TGTTGTAACCTGTTCATAGTTTGTATTGAAACAATGTATACTGTAACTTTTTTTCAAAA
AAGAAATTTTCTAGTATTATTTCTCTGTCTCAGCATGGAGCTGATCTATTAGGAAATCAGTGT
GTGGTGGGGGAAGCAAGTATATTTTGTGGTATGAAGTTATCTAATTGCTGGCAAGTT
CAAGCAAAGGGGACAAAAGCTTATGAGGTTCACTTATTCTTCTCTTTCTTTCTCTCAG
TGGACAAGTCCATCTGACAATGCTAGGAGCGATGCAGGTTTCCAAATATGGTGACCTGGC
TAACTGGATCATACCT
GTAAATACACATTTTCTTCTGAAATATGTTTGGATCAGAAATATATATTTTAAATAC
TAAGAAAAAGGGCCATGAGATCAGATCTTTAAAAATGAAATATTTTAAATATAATTTT
TTACTAGAAATCAATTTTAAATAGTAAGCACTGAGAAGTATTCAGCTTTGCCAATTTAC
TGTGCATTTGGGACTAAAAGTAGGCAAGGTTCAGAGCCGAAACCTCTTGCTTAGAAGAAAC
CAAGTTAGAGAAGACTTAAAAAACAGAAAAAGGGCTAATTAACCTGAAAAATAAGAAAT
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Se ha descrito que este cambio causa el *skipping* del exón 12 y 13. La mutación lleva a “parálisis del *splicing*” y la eliminación de todo el fragmento de 11 nucleótidos del intrón 13, incluidos los exones 12 y 13¹.

El cambio se encuentra en la quinta posición justo después del exón 13 (la **g** en color naranja).

Se va a obtener los resultados que produce analizar esta variable con los diferentes predictores y ver cuál de ellos es más preciso.

1. Hori T, Fukao T, Murase K, Sakaguchi N, Harding CO, Kondo N (2013) Molecular basis of two-exon skipping (exons 12 and 13) by c.1248+5g>a in OXCT1 gene: study on intermediates of OXCT1 transcripts in fibroblasts. Hum Mutat 34(3):473–480

NetGene2

The sequence: wt has the following composition:

Length: 1347 nucleotides.

30.6% A, 17.6% C, 20.4% G, 31.4% T, 0.0% X, 38.0% G+C

Donor splice sites, direct strand

pos 5'→3'	phase	strand	confidence	5'	exon	intron	3'
374	2	+	0.44	TGATTAGAGG	^	GTATGTAATA	
1048	0	+	0.81	GATGATACCT	^	GTAAGTAGAC	
1256	1	+	0.00	AGTAGGCAAG	^	GTCAGAGCCG	

Donor splice sites, complement strand

pos 3'→5'	pos 5'→3'	phase	strand	confidence	5'	exon	intron	3'
167	1181	1	-	0.37	GACATAGCAG	^	GTGGGGGGAC	

Acceptor splice sites, direct strand

pos 5'→3'	phase	strand	confidence	5'	intron	exon	3'
327	1	+	0.43	ATTCTTCCAG	^	GAGCCTCTTT	
823	2	+	0.36	TCTCTGTCAG	^	CATGGAGCTG	
971	2	+	0.53	TCTTTCTCAG	^	TGGACACGTC	

Acceptor splice sites, complement strand

pos 3'→5'	pos 5'→3'	phase	strand	confidence	5'	intron	exon	3'
1046	302	2	-	0.38	CTACTTACAG	^	GTATCATCCA	

The sequence: mut has the following composition:

Length: 1347 nucleotides.

30.7% A, 17.6% C, 20.3% G, 31.4% T, 0.0% X, 37.9% G+C

Donor splice sites, direct strand

pos 5'→3'	phase	strand	confidence	5'	exon	intron	3'
374	2	+	0.44	TGATTAGAGG	^	GTATGTAATA	
1256	1	+	0.00	AGTAGGCAAG	^	GTCAGAGCCG	

Donor splice sites, complement strand

pos 3'→5'	pos 5'→3'	phase	strand	confidence	5'	exon	intron	3'
167	1181	1	-	0.37	GACATAGCAG	^	GTGGGGGGAC	

Acceptor splice sites, direct strand

pos 5'→3'	phase	strand	confidence	5'	intron	exon	3'
327	1	+	0.43	ATTCTTCCAG	^	GAGCCTCTTT	
823	2	+	0.36	TCTCTGTCAG	^	CATGGAGCTG	
971	2	+	0.50	TCTTTCTCAG	^	TGGACACGTC	

Acceptor splice sites, complement strand

pos 3'→5'	pos 5'→3'	phase	strand	confidence	5'	intron	exon	3'
1046	302	2	-	0.48	CTATTTACAG	^	GTATCATCCA	

Al comparar las predicciones para ambas secuencias, se observa que se pierde un sitio *donor* en la secuencia mutante (marcado en rojo). Ese coincide con el sitio *donor* del exón 13, por lo que, al no poder utilizarlo, se producirá *skipping* de dicho exón.

Splice Site Prediction by Neural Network (NNSplice)

Donor site predictions for 85.53.81.70.14888.0 :

Start	End	Score	Exon	Intron
11	25	0.44	gtctgag	gtacaaaa
182	196	0.53	cattttg	gtaaaggg
367	381	0.99	ttagagg	gtatgtaa
877	891	0.61	tttggtg	gtatgaag
1041	1055	0.95	gatacct	gtaaagtag
1185	1199	0.90	taattaa	gt aagcac
1249	1263	0.82	aggcaag	gtcagagc

Donor site predictions for 85.53.81.70.14875.0 :

Start	End	Score	Exon	Intron
11	25	0.44	gtctgag	gtacaaaa
182	196	0.53	cattttg	gtaaaggg
367	381	0.99	ttagagg	gtatgtaa
877	891	0.61	tttggtg	gtatgaag
1185	1199	0.90	taattaa	gt aagcac
1249	1263	0.82	aggcaag	gtcagagc

Acceptor site predictions for 85.53.81.70.14888.0 :

Start	End	Score	Intron	Exon
307	347	0.46	aaacagttactattcttcc	aggagcctcttttttctccagc
326	366	0.90	aggagcctcttttttctcc	agcgatgaatcatttgcaatga
658	698	0.81	caaactgtgttcactttct	agctagtgtttgtgttcaagta
732	772	0.54	cctgtgtacacctgttcat	agtttgatttgaacaatgta
951	991	0.97	ttcttcctctttctttct	agtggaacacgtcgatctgacaa
1154	1194	0.85	ttaatatatatttcttact	agaatcaaattttaattaagta

Acceptor site predictions for 85.53.81.70.14875.0 :

Start	End	Score	Intron	Exon
307	347	0.46	aaacagttactattcttcc	aggagcctcttttttctccagc
326	366	0.90	aggagcctcttttttctcc	agcgatgaatcatttgcaatga
658	698	0.81	caaactgtgttcactttct	agctagtgtttgtgttcaagta
732	772	0.54	cctgtgtacacctgttcat	agtttgatttgaacaatgta
951	991	0.97	ttcttcctctttctttct	agtggaacacgtcgatctgacaa
1154	1194	0.85	ttaatatatatttcttact	agaatcaaattttaattaagta

Se pierde un sitio *donor* al analizar la secuencia mutada que coincide con el sitio *donor* del exón 13, por lo que está afectando al *splicing*, produciendo muy probablemente el *skipping* del exón 13.

GENSCAN → no da resultados para este cambio

Predicted genes/exons:

Gn.Ex Type S .Begin ...End .Len Fr Ph I/Ac Do/T CodRg P.... Tscr..

NO EXONS/GENES PREDICTED IN SEQUENCE

Predicted genes/exons:

Gn.Ex Type S .Begin ...End .Len Fr Ph I/Ac Do/T CodRg P.... Tscr..

NO EXONS/GENES PREDICTED IN SEQUENCE

MaxEntScan (solo se estudia 5' por la proximidad con el cambio)

>sec
TGATACCTgtaagtagacat MAXENT: -40.57 MDD: -21.91 MM: -25.99 WMM: -23.44

La posición no es muy susceptible a cambios, dado que no se encuentra en un sitio débil de *splicing* (valores muy negativos para los diferentes indicadores).

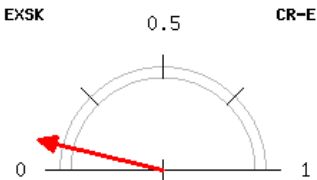
Spliceman

Point mutation	Wildtype (wt)	Mutation (mt)	L1 distance	Ranking (L1)
tgtaa(g/a)tagac	taagta	taaata	30390	76%

En el análisis de la región adyacente al cambio, se obtiene una puntuación elevada (76%) para el cambio G>A, por lo que puede estar afectando al *splicing*.

CRYP-SKIP


Exon length (bp)	73
PESS (≤ -2.62) density	4.11
NN 5'ss score density	0.00
SF2/ASF score density	2.82
FAS-ESS (hex2) density	2.74
EIE score density	327.08
Probability of cryptic splice site activation (P_{CR-E})	0.07



Parece que hay un sitio críptico de *splicing* dentro del propio exón, pero el cambio de interés no lo toma en consideración, por lo que no debe considerar que tenga algún efecto en el *splicing*.



Human Splicing Finder

Type	↑↓	Interpretation	↑↓
 Broken WT Donor Site		Alteration of the WT Donor site, most probably affecting splicing	
Algorithm/Matix		position	sequences
HSF Donor site (matrix GT)		chr5:41794005	- REF : CCTGTAA GT - ALT : CCTGTAA AT
MaxEnt Donor site		chr5:41794005	- REF : CCTGTAA GT - ALT : CCTGTAA AT
			variation
			83.32 > 73.75 => -11.49%
			7.52 > -0.64 => -108.51%

SVM-BPfinder

seq_id	agez	ss_dist	bp_seq	bp_scr	y_cont	ppt_off	ppt_len	ppt_scr	svm_scr		seq_id	agez	ss_dist	bp_seq	bp_scr	y_cont	ppt_off	ppt_len	ppt_scr	svm_scr	
wt	13	486	tagtgatta	-0.825866110956	0.47817047817	1	13	26	0.41176096		mut	13	486	tagtgatta	-0.825866110956	0.47817047817	1	13	26	0.41176096	
wt	13	483	tgattatttt	-2.92603331295	0.476987447699	1	10	22	-0.44819921		mut	13	483	tgattatttt	-2.92603331295	0.476987447699	1	10	22	-0.44819921	
wt	13	472	ctgtcagca	-1.4589611324	0.468950749465	52	6	13	-3.1884237		mut	13	472	ctgtcagca	-1.4589611324	0.468950749465	52	6	13	-3.1884237	
wt	13	460	agctgatct	1.34433483292	0.472527472527	40	6	13	-1.3300658		mut	13	460	agctgatct	1.34433483292	0.472527472527	40	6	13	-1.3300658	
wt	13	453	ctattagga	-4.0854488258	0.46875 33	6	13	-3.0142145		mut	13	453	ctattagga	-4.0854488258	0.46875 33	6	13	-3.0142145			
wt	13	445	aaatcagtt	-2.65403802592	0.472727272727	25	6	13	-1.9460784		mut	13	445	aaatcagtt	-2.65403802592	0.472727272727	25	6	13	-1.9460784	
wt	13	405	gtatgaagt	-1.67761648512	0.4825 3	7	15	-0.1494122		mut	13	405	gtatgaagt	-1.67761648512	0.4825 3	7	15	-0.1494122			
wt	13	400	aagttattc	-3.23910098873	0.483544303797	48	28	65	-3.1431021		mut	13	400	aagttattc	-3.23910098873	0.483544303797	48	28	65	-3.1431021	
wt	13	394	ttctaattg	1.59743849057	0.480719794344	42	28	65	-0.87049065		mut	13	394	ttctaattg	1.59743849057	0.480719794344	42	28	65	-0.87049065	
wt	13	379	agttcaagc	-1.61999791089	0.478609625668	27	28	65	-1.1814757		mut	13	379	agttcaagc	-1.61999791089	0.478609625668	27	28	65	-1.1814757	
wt	13	358	agcttatga	-1.49364139848	0.492917847025	6	28	65	0.20188468		mut	13	358	agcttatga	-1.49364139848	0.492917847025	6	28	65	0.20188468	
wt	13	355	ttatgaggt	-2.17505223734	0.494285714286	3	28	65	0.12541689		mut	13	355	ttatgaggt	-2.17505223734	0.494285714286	3	28	65	0.12541689	
wt	13	349	ggttcactt	-0.0296954416326	0.494186046512	1	24	59	1.0360948		mut	13	349	ggttcactt	-0.0296954416326	0.494186046512	1	24	59	1.0360948	
wt	13	344	actttattc	-4.35180452645	0.489675516224	1	19	50	-0.7415146		mut	13	344	actttattc	-4.35180452645	0.489675516224	1	19	50	-0.7415146	
wt	13	324	ttctcagtg	0.588237616788	0.460815047022	87	13	27	-4.4744957		mut	13	324	ttctcagtg	0.588237616788	0.460815047022	87	13	27	-4.4744957	
wt	13	306	atctgacaa	1.52420752116	0.461794019934	69	13	27	-2.9683333		mut	13	306	atctgacaa	1.52420752116	0.461794019934	69	13	27	-2.9683333	
wt	13	269	tggtgacct	2.63890969484	0.469696969697	32	13	27	-0.18728442		mut	13	269	tggtgacct	2.63890969484	0.469696969697	32	13	27	-0.18728442	
wt	13	260	ggctaaactg	3.0387153898	0.466666666667	23	13	27	0.53796464		mut	13	260	ggctaaactg	3.0387153898	0.466666666667	23	13	27	0.53796464	
wt	13	252	ggatgatac	-0.548226770123	0.46963562753	15	13	27	-0.35914728		mut	13	252	ggatgatac	-0.548226770123	0.46963562753	15	13	27	-0.35914728	
wt	13	243	ctgtaaagta	-0.856731725038	0.466386554622	6	13	27	0.088693989		mut	13	243	ctgtaaagta	-0.856731725038	0.466386554622	6	13	27	0.088693989	
wt	13	229	tttttattc	-3.75051124112	0.459821428571	31	6	13	-2.7593576		mut	13	229	tttttattc	-3.75051124112	0.459821428571	31	6	13	-2.7593576	
wt	13	223	ttctgaatt	0.584236905393	0.454128440367	25	6	13	-0.68414763		mut	13	223	ttctgaatt	0.584236905393	0.454128440367	25	6	13	-0.68414763	
wt	13	219	gaattatgt	-1.21990482251	0.453271028037	21	6	13	-1.137638		mut	13	219	gaattatgt	-1.21990482251	0.453271028037	21	6	13	-1.137638	
wt	13	208	ggatcagaa	-3.92207229714	0.448275862069	10	6	13	-1.5009961		mut	13	208	ggatcagaa	-3.92207229714	0.448275862069	10	6	13	-1.5009961	
wt	13	202	gaataatta	-0.763296081587	0.456852791878	4	6	13	0.11837415		mut	13	202	gaataatta	-0.763296081587	0.456852791878	4	6	13	0.11837415	
wt	13	199	taattatat	-2.22373023541	0.453608247423	1	6	13	-0.26460782		mut	13	199	taattatat	-2.22373023541	0.453608247423	1	6	13	-0.26460782	
wt	13	192	attttaaat	-4.84966382696	0.44385026738	32	6	17	-3.220921		mut	13	192	attttaaat	-4.84966382696	0.44385026738	32	6	17	-3.220921	
wt	13	191	ttttaaata	-1.80412339012	0.44623655914	31	6	17	-1.964379		mut	13	191	ttttaaata	-1.80412339012	0.44623655914	31	6	17	-1.964379	
wt	13	184	tactaagaa	-0.37090280647	0.446927374302	24	6	17	-0.95989401		mut	13	184	tactaagaa	-0.37090280647	0.446927374302	24	6	17	-0.95989401	
wt	13	169	ccatgagat	-1.50297489779	0.469512195122	9	6	17	-0.44638467		mut	13	169	ccatgagat	-1.50297489779	0.469512195122	9	6	17	-0.44638467	
wt	13	164	agatcacga	-1.86844269119	0.471698113208	4	6	17	-0.27228499		mut	13	164	agatcacga	-1.86844269119	0.471698113208	4	6	17	-0.27228499	
wt	13	154	tccttaaaa	-4.81345218081	0.456375838926	10	6	13	-1.8473974		mut	13	154	tccttaaaa	-4.81345218081	0.456375838926	10	6	13	-1.8473974	
wt	13	153	ctttaaaaa	-1.80921970188	0.459459459459	9	6	13	-0.60680412		mut	13	153	ctttaaaaa	-1.80921970188	0.459459459459	9	6	13	-0.60680412	
wt	13	147	aaatgaaat	-1.44061386659	0.471830985915	3	6	13	-0.078691939		mut	13	147	aaatgaaat	-1.44061386659	0.471830985915	3	6	13	-0.078691939	
wt	13	138	attttaata	-5.1666216293	0.466165413534	6	11	21	-1.6547981		mut	13	138	attttaata	-5.1666216293	0.466165413534	6	11	21	-1.6547981	
wt	13	137	ttttaatat	-0.636668955298	0.469696969697	5	11	21	0.18333137		mut	13	137	ttttaatat	-0.636668955298	0.469696969697	5	11	21	0.18333137	
wt	13	124	ttcttacta	-0.180744604499	0.445378151261	99	10	21	-5.5960491		mut	13	124	ttcttacta	-0.180744604499	0.445378151261	99	10	21	-5.5960491	
wt	13	115	gaatcaaat	-2.45300024531	0.445454545455	90	10	21	-5.9160351		mut	13	115	gaatcaaat	-2.45300024531	0.445454545455	90	10	21	-5.9160351	
wt	13	108	attttaatt	-4.8626284941	0.436893203883	83	10	21	-6.4191952		mut	13	108	attttaatt	-4.8626284941	0.436893203883	83	10	21	-6.4191952	
wt	13	107	ttttaatta	-1.2990955485	0.441176470588	82	10	21	-4.9592219		mut	13	107	ttttaatta	-1.2990955485	0.441176470588	82	10	21	-4.9592219	
wt	13	104	taattaagt	-2.56602645133	0.434343434343	79	10	21	-5.2675972		mut	13	104	taattaagt	-2.56602645133	0.434343434343	79	10	21	-5.2675972	
wt	13	103	aattaagta	-1.64706671047	0.438775510204	78	10	21	-4.8430513		mut	13	103	aattaagta	-1.64706671047	0.438775510204	78	10	21	-4.8430513	
wt	13	99	aagtaagca	-0.75750347952	0.446808510638	74	10	21	-4.2389575		mut	13	99	aagtaagca	-0.75750347952	0.446808510638	74	10	21	-4.2389575	
wt	13	92	cactgagaa	0.0494099468397	0.448275862069	67	10	21	-3.4794506		mut	13	92	cactgagaa	0.0494099468397	0.448275862069	67	10	21	-3.4794506	
wt	13	82	tattcagct	-0.427825035865	0.454545454545	57	10	21	-3.0313026		mut	13	82	tattcagct	-0.427825035865	0.454545454545	57	10	21	-3.0313026	
wt	13	68	aatttactg	-1.67657882597	0.412698412698	43	10	21	-2.6475876		mut	13	68	aatttactg	-1.67657882597	0.412698412698	43	10	21	-2.6475876	
wt	13	50	gactaaaag	0.665771398687	0.377777777778	25	10	21	-0.60235528		mut	13	50	gactaaaag	0.665771398687	0.377777777778	25	10	21	-0.60235528	
wt	13	35	aggtcagag	-2.6774474921	0.433333333333	10	10	21	-0.94396589		mut	13	35	aggtcagag	-2.6774474921	0.433333333333	10	10	21	-0.94396589	
wt	13	15	tgcttagaa	-2.25910295864	0.2 10	0	0	0	-1.0511568		mut	13	15	tgcttagaa	-2.25910295864	0.2 10	0	0	-1.0511568		

IntSplice

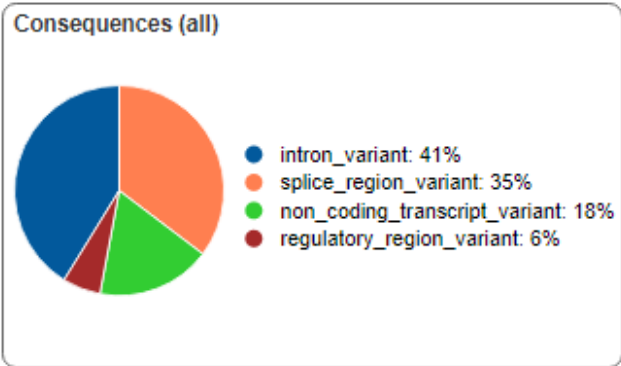
SNV at chr5:41794100 can't be predicted by IntSplice.

Prediction shows either Abnormal or Normal.

Prediction	Genomic Mutation	Ensembl 64 Transcript ID and Exon No.
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Variant Effect Predictor tool

Category	Count
Variants processed	1
Variants filtered out	0
Novel / existing variants	0 (0.0) / 1 (100.0)
Overlapped genes	1
Overlapped transcripts	7
Overlapped regulatory features	1



Se trata de una variante que está afectando al sitio de *splicing*, por lo que va a provocar que se altere el *splicing* normal. Además, es una *non coding transcript exon variant*, es decir, una variante que se encuentra en un transcrito que no se suele transcribir porque no es el mayoritario. El método de NMD se encarga de degradar estos transcritos², dado que aparece un codón de parada.

2. Lewis BP, Green RE, Brenner SE. Evidence for the widespread coupling of alternative splicing and nonsense-mediated mRNA decay in humans. Proc Natl Acad Sci USA. 2003;100:189–192

Uploaded variant	Location	Allele	Consequence	Symbol	Gene	Feature type
ENST00000196371.5:c.1248+5G>A	5:41793998-41793998	T	splice region variant, intron variant	OXCT1	ENSG00000083720	Transcript
ENST00000196371.5:c.1248+5G>A	5:41793998-41793998	T	splice region variant, intron variant, non coding transcript variant	OXCT1	ENSG00000083720	Transcript
ENST00000196371.5:c.1248+5G>A	5:41793998-41793998	T	splice region variant, intron variant	OXCT1	ENSG00000083720	Transcript
ENST00000196371.5:c.1248+5G>A	5:41793998-41793998	T	splice region variant, intron variant	OXCT1	ENSG00000083720	Transcript
ENST00000196371.5:c.1248+5G>A	5:41793998-41793998	T	splice region variant, intron variant	OXCT1	ENSG00000083720	Transcript
ENST00000196371.5:c.1248+5G>A	5:41793998-41793998	T	splice region variant, intron variant, non coding transcript variant	OXCT1	ENSG00000083720	Transcript
ENST00000196371.5:c.1248+5G>A	5:41793998-41793998	T	intron variant, non coding transcript variant	OXCT1	ENSG00000083720	Transcript
ENST00000196371.5:c.1248+5G>A	5:41793998-41793998	T	regulatory region variant	-	-	RegulatoryFeature

ESEfinder

1033 (-315)	AACTGGATGATACCTgtaagtagacatttt	7.04910	1033 (-315)	AACTGGATGATACCTgtaagtagacatttt	-29.50650	1033 (-315)	AACTGGATGATACCTgtaagtagacatttt	6.86130	1033 (-315)	AACTGGATGATACCTgtaagtagacatttt	-28.47620
1034 (-314)	ACTGGATGATACCTgtaagtagacattttt	-30.05710	1034 (-314)	ACTGGATGATACCTgtaagtagacattttt	-15.00510	1034 (-314)	ACTGGATGATACCTgtaagtagacattttt	-27.48490	1034 (-314)	ACTGGATGATACCTgtaagtagacattttt	-16.18560
1035 (-313)	CTGGATGATACCTgtaagtagacatttttt	-29.72170	1035 (-313)	CTGGATGATACCTgtaagtagacatttttt	-30.98720	1035 (-313)	CTGGATGATACCTgtaagtagacatttttt	-27.00970	1035 (-313)	CTGGATGATACCTgtaagtagacatttttt	-29.71210
1036 (-312)	TGGATGATACCTgtaagtagacatttttta	-28.53440	1036 (-312)	TGGATGATACCTgtaagtagacatttttta	-34.44910	1036 (-312)	TGGATGATACCTgtaagtagacatttttta	-27.25970	1036 (-312)	TGGATGATACCTgtaagtagacatttttta	-39.20510
1037 (-311)	GGATGATACCTgtaagtagacattttttat	0.00370	1037 (-311)	GGATGATACCTgtaagtagacattttttat	-13.90430	1037 (-311)	GGATGATACCTgtaagtagacattttttat	-0.54990	1037 (-311)	GGATGATACCTgtaagtagacattttttat	-17.25030

La posición de interés no aparece como el mejor resultado del buscador, pero, cuando se buscan todas las secuencias, se obtiene una puntuación positiva para las matrices de 5'SS en dos resultados (1033 y 1037, aunque de este solo en una de las dos matrices y con un valor muy bajo) por lo que esta deberá ser la región que produzca este sitio de *splicing*. Si se hace la búsqueda para la secuencia mutada, el resultado equivalente aparece con valores más bajos (3.61380 y 3.19890 en cada matriz). Por lo tanto, se está perdiendo el sitio *donor*, afectando el *splicing*.

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	3	1	0	16	316.1988	14	-18.4369	2	7	20	233.6784	20	26.7130	34	49	0.69
mut	3	1	0	16	316.1988	14	-18.4369	2	7	20	233.6784	20	26.7130	34	49	0.69

Both alleles have a comparable chance of exon skipping.

HOT-SKIP

>wt
cataaaacgggtctgaggtacaaaaaggggactgctgctgtagagaatttcaaacaataagaaaactaaaagtgggtct
gcttttcattatcatcatgcaaaggcaagagcaatgtcagtgacaaaaatgctttccaccacaaaagggttcctactgtgtc
ccccacctgctatgtcactgcattttggtaaaggagcctaagttcttttgggaatggagaagatgtggacctctgtt
accatgatactcttagaaatctaatagccttttctttcttttgggtgtgtgtgtgtgttttagGCAAGGAAACAGTTACTATT
CTTCAGGAGCCTCTTTTCTCCAGCGATGAATCATTTCGAATGATTAAGGgtatgtaataacagagccctcatgtt
tcagacagtatggaatgagctgagtcacgtctgagtggttagcaaggctctgaaacacagccacatttcccagccaga
aaatatcaggagtttgctcggtgcagggtgtgaaattattcacatcgaatgttgggcattgaatatccagcccactgt
taggaaaagaacccccaaaatggaatccatgcctttgtgccaccatactgaaataaacatttctaagggtgctttctgag
gattctaacattttcttcaactgtgttcattttctagctagtgtttgtgttcaagtagtcacattctgcctcactgaag
gaaacagtcttctgtgtacacctgttcatagtttgattgaaacaatgtatactgtaactttttttcaaaaagaatt
tttagtgattattttctgtcagcatggagctgatctattaggaatcagttgtgtgtgtgggggaagcaagtatatatttg
gtggtatgaagttatttctaattgtctggcaagttcgaagcaaaggggacaaaagcttaatgaggttcactttattcttctct
tcttctctcagTGGACACGTGCATGCACATGCTAGGAGCGATGCAGGTTTCCAAATATGGTGACCTGGCTACCTGGAT
GATACCTgtaagttagacattttttattctgaattatgttggatcagaataaattatattttaataactaagaaaaagggc
catgagatcacgattctttaaaaatgaaatttttaatatatttcttactagaatcaaattttaattaagtaagcact
gagaagtattcagctttgccaatctactgtgcatttgggactaaaagtaggcaagggtcagagccgaaacctcttgcttag
aagaaccaaggttagagaagactttaaaaaacagaaaaagggctaattatactctgaaaaataagaaat

No da resultados para mutaciones intrónicas.