

Ejemplo comparación de resultados predictores in silico

Cambio de estudio CLDN12 c.*581T>C (chr7:90413992 T/C, NM_001185072.3: c.*581T>C)

Exón 3 e intrones adyacentes:

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ttcctgctctgtttctgctattgtccctcatgattttgtcctctttgtgtgtcaccccctag
TCTGACTGACAGTACTCCACAAGCTTGCCTGCCATGGGCTGTCCGGATGTCCACGCAGCC
ACAGTCCTTTCCTTCCTGTGTGGAATGCCCTCAGTAGCAGGCCCTTTGCAGGGACTCTG
CTTCCCAACTGGAGAAAATTACGATTGATCACATTCAACAGAAACGAGAAGAACCTGACT
GTTTACACAGGCCTGTGGGTGAATGTGCCGGTATGACGGGAGCAGTGACTGCCTGATG
TACGACACTACTTGGTACTCATCAGTTGACCAGCTGGACCTCCGTGTCTCCAGTTTGC
CTACCCCTCAGCATGCTGATCGCCATGGGTGCCCTGCTCTCTGCCTGATTGGAATGTGC
AACACTGCCCTCAGGTCCTCGGTGCCCAACATCAAACCTGGCCAGTGTCTGGTCAATAGT
GCAGGTTGCCACCTGGTGGCTGGCTGCTATTTTTCTGGCAGGTAAGTGAGCCTCTCC
CCATCTATCTGGGTCACTTTTTATAACATCCATCTGAACAAGAGTTTGAGCCAGTCTTT
TCATTGACTATGTCAGTGTATGTCACTATTGCTAGTGCTGGGGGCCGTGTTTATGACTTCC
CTTATACTATTATTTGGTATTGTACATGCCAATCTTTGCCTTCTCCTTTCTGCAACCA
TTGTACTCCATCCACCAGTATGCATACCTACTCACAGCCCTATTAGCACGCTCTCGC
CTCTCTGCCATTGAATTGACATTCCAGTAGTTTCACACACCACCTAATGGGGAAATAGT
TAATTCTTAAAGAAAACCTCTTGAGCCTCACATTCCCCTTGTGCAAAAGAGCTCTTTTGG
ACCTACATACATTTTTCCTTTGTTTTTGAACCAATCAATGAAGCCAAATTTATATGTCCTAG
TAGAATGAAGTGCTGCTAGTTTTTATGAGAAGTATATTATATTAAATGTGAATTTTTTAA
ATTTTGCTTCTTATACTGGAAGGAATTTTAGCCTTCATATTGATATCTAATTAATTATTT
AAGTGAAGAGGCCTGCATCACAATTGAGGTAATGTAGAGCAACATGTTAAAGAATGATG
GTTAGCAGAAGCTGTTGTATACATCTTCATGAAAATTTCAAGTGTGTTTTTTCTTTTC
TATAATACCTTTAACTGCAAGAAAAGGCAGTTTTCAAATATAAGAAATTTATTTTCAGGTA
AGGGTAATATTTTAATAGTAGTCAATAATCTAGCTTAAGGCTGTAACTCTTCTATCGGGG
CTAATTGTATGAATAGGTGTCAGTATGTTGAAGATTACTTTCTTTTGTGACTTTCTTCTA
CCTCATGCCACTGTTTAAAAAGTAAACCGTATTTTAAAGATGTTAGAATAAGACTACCAT
CTAAATATCACCTACTTATGAATAACATGTAATAAATTTTAAACATTAAATGATTCCATAAA
TTGTTATTATTGGGATTAGAATGTGCTTTATGACAGGTTAGTGTTTCTCTGAGGCAGAAA
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El cambio se encuentra en la antepenúltima fila del 3'UTR después del exón 3 (la **t** en color gris).

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'
273		2	+	0.37	AATGTGCCCG	^GTATGACGGG		
524		1	+	0.69	TTCCTGGCAG	^GTACTGTGAG		
1258		0	+	0.54	TTTATTTT	CAG^GTAAGGGTAA		
1536		-	+	0.00	TTTATGACAG	^GTTAGTGTTT		

Donor splice sites, complement strand

pos 3'→5'	pos 5'→3'	phase	strand	confidence	5'	exon	intron	3'
753	808	1	-	0.86	AGGGCTGTGA	^GTAAGTATGC		

Acceptor splice sites, direct strand

pos	5'→3'	phase	strand	confidence	5'	intron	exon	3'
172		1	+	0.19	CTCTTTGCAG	^GGACTCTGCT		
194		2	+	0.32	CCAACTGGAG	^AAAATTACGA		
221		2	+	0.19	CATTCAACAG	^AAACGAGAAG		
228		0	+	0.18	CAGAAACGAG	^AAGAACCTGA		
231		0	+	0.17	AAACGAGAAG	^AACCTGACTG		
371		2	+	0.25	TACCCCTCAG	^CATGCTGATC		

Acceptor splice sites, complement strand

pos 3'→5'	pos 5'→3'	phase	strand	confidence	5'	intron	exon	3'
1391	170	2	-	0.25	TTTTAAACAG	^TGGCATGAGG		
1215	346	1	-	0.33	TTCTTTGCAG	^TTAAAGGTAT		
493	1068	0	-	0.07	CAGCCACCAG	^GTGGCAACCT		
469	1092	0	-	0.18	TATTGACCAG	^ACACTTGGCC		
457	1104	2	-	0.18	ACTTGGCCAG	^TTTGATGTTG		
438	1123	0	-	0.07	GGGCACCGAG	^GACCTGAAGG		
253	1308	2	-	0.27	TCACCCACAG	^GCCTGTGTAA		

Donor splice sites, direct strand

pos	5'→3'	phase	strand	confidence	5'	exon	intron	3'
273		2	+	0.37	AATGTGCCCG	^GTATGACGGG		
524		1	+	0.69	TTCCTGGCAG	^GTACTGTGAG		
1258		0	+	0.56	TTTATTTT	CAG^GTAAGGGTAA		
1536		-	+	0.00	TTTATGACAG	^GTTAGTGTTT		

Donor splice sites, complement strand

pos 3'→5'	pos 5'→3'	phase	strand	confidence	5'	exon	intron	3'
753	808	1	-	0.86	AGGGCTGTGA	^GTAAGTATGC		

Acceptor splice sites, direct strand

pos	5'→3'	phase	strand	confidence	5'	intron	exon	3'
172		1	+	0.19	CTCTTTGCAG	^GGACTCTGCT		
194		2	+	0.32	CCAACTGGAG	^AAAATTACGA		
221		2	+	0.19	CATTCAACAG	^AAACGAGAAG		
228		0	+	0.18	CAGAAACGAG	^AAGAACCTGA		
231		0	+	0.17	AAACGAGAAG	^AACCTGACTG		
371		2	+	0.25	TACCCCTCAG	^CATGCTGATC		

Acceptor splice sites, complement strand

pos 3'→5'	pos 5'→3'	phase	strand	confidence	5'	intron	exon	3'
1391	170	2	-	0.24	TTTTAAACAG	^TGGCATGAGG		
1215	346	1	-	0.33	TTCTTTGCAG	^TTAAAGGTAT		
493	1068	0	-	0.07	CAGCCACCAG	^GTGGCAACCT		
469	1092	0	-	0.18	TATTGACCAG	^ACACTTGGCC		
457	1104	2	-	0.18	ACTTGGCCAG	^TTTGATGTTG		
438	1123	0	-	0.07	GGGCACCGAG	^GACCTGAAGG		
253	1308	2	-	0.27	TCACCCACAG	^GCCTGTGTAA		

Splice Site Prediction by Neural Network (NNSplice)

Donor site predictions for 10.42.0.139.572497.0 :

Start	End	Score	Exon	Intron
266	280	0.78	gtgccc	gt atgacg
517	531	0.83	ctggcag	gt actgtg
1103	1117	0.88	aattgag	gt aatgta
1134	1148	0.71	aatgatg	gt tagcag
1251	1265	1.00	atttcag	gt aagggt
1529	1543	0.98	atgacag	gt tagtgt

Donor site predictions for 10.42.1.119.572509.0 :

Start	End	Score	Exon	Intron
266	280	0.78	gtgccc	gt atgacg
517	531	0.83	ctggcag	gt actgtg
1103	1117	0.88	aattgag	gt aatgta
1134	1148	0.71	aatgatg	gt tagcag
1251	1265	1.00	atttcag	gt aagggt
1529	1543	0.98	atgacag	gt tagtgt

Acceptor site predictions for 10.42.0.139.572497.0 :

Start	End	Score	Intron	Exon
40	80	0.41	ctcttgtgtgtcacc	ag tctgactgacagtactccac
152	192	0.78	cagtagcaggcctctttgc	ag ggactctgcttcccaactgg
230	270	0.49	agaacctgactgtttacac	ag gcctgtgggtgaaatgtgcc
334	374	0.75	ctggacctgcgtgtcctcc	ag tttgcctaccctcagcat
351	391	0.64	ccagtttgccctaccctc	ag catgctgatcgccatgggtg
414	454	0.83	aatgtgcaacactgccttc	ag gtcctcggtgcccaacatca
503	543	0.98	ggctgctatTTTTTctggc	ag gtactgtgagcctctcccca
1515	1555	0.73	ttagaatgtgctttatgac	ag gttagtgtttcctctgaggc

Acceptor site predictions for 10.42.1.119.572509.0 :

Start	End	Score	Intron	Exon
40	80	0.41	ctcttgtgtgtcacc	ag tctgactgacagtactccac
152	192	0.78	cagtagcaggcctctttgc	ag ggactctgcttcccaactgg
230	270	0.49	agaacctgactgtttacac	ag gcctgtgggtgaaatgtgcc
334	374	0.75	ctggacctgcgtgtcctcc	ag tttgcctaccctcagcat
351	391	0.64	ccagtttgccctaccctc	ag catgctgatcgccatgggtg
414	454	0.83	aatgtgcaacactgccttc	ag gtcctcggtgcccaacatca
503	543	0.98	ggctgctatTTTTTctggc	ag gtactgtgagcctctcccca
1515	1555	0.73	ttagaatgtgctttatgac	ag gttagtgtttcctctgaggc

Spliceman

Point mutation	Wildtype (wt)	Mutation (mt)	L1 distance	Ranking (L1)
aaacg(t/c)atatt	tatttt	catttt	31133	81%

CRYP-SKIP

Human Splicing Finder

Este tipo de nomenclatura no la permite.

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*.

SVM-BPfinder

seq_id	agez	ss_dist	bp_seq	bp_scr	y_cont	ppt_off	ppt_len	ppt_scr	svm_scr	
wt	52	133	tgttgaaga		-1.00956740914	0.5625	4	14	30	0.2144376
wt	52	127	agattactt		-1.88101073987	0.573770491803	1	11	26	0.029497937
wt	52	114	ttgtgactt		1.10624650428	0.550458715596	1	16	32	1.2475164
wt	52	99	acctcatgc		1.00201220116	0.5	3	9	14	0.89612524
wt	52	87	tgtttaaaa		-3.9786620974	0.475609756098	12	6	13	-1.6409222
wt	52	86	gtttaaaag		-1.44518207278	0.481481481481	11	6	13	-0.58375036
wt	52	80	aagtaaaac		-0.527492388703	0.506666666667	5	6	13	0.16349253
wt	52	69	attttaacg		-3.64777439711	0.5	18	10	16	-1.8553292
wt	52	68	ttttaacga		0.714929736122	0.507936507937	17	10	16	-0.081262664
wt	52	60	atgtagaa		-4.72411342716	0.509090909091	9	10	16	-1.7041458
wt	52	54	gaataagac		-0.842110751682	0.551020408163	3	10	16	0.2091737
wt	52	40	ttctaaata		0.24633338382	0.542857142857	4	12	17	0.57873205
wt	52	34	atatcacct		-0.938217719104	0.551724137931	1	9	14	0.27973553
wt	52	26	tacttatga		-1.01486748302	0.47619047619	21	0	0	-1.1710599
wt	52	23	ttatgaata		-2.01321219191	0.5	18	0	0	-1.3643743
wt	52	19	gaataacat		0.970303282875	0.571428571429	14	0	0	0.080075666
mut	52	133	tgttgaaga		-1.00956740914	0.5625	4	14	30	0.2144376
mut	52	127	agattactt		-1.88101073987	0.573770491803	1	11	26	0.029497937
mut	52	114	ttgtgactt		1.10624650428	0.550458715596	1	16	32	1.2475164
mut	52	99	acctcatgc		1.00201220116	0.5	3	9	14	0.89612524
mut	52	87	tgtttaaaa		-3.9786620974	0.475609756098	36	10	16	-3.1321344
mut	52	86	gtttaaaag		-1.44518207278	0.481481481481	35	10	16	-2.0749625
mut	52	80	aagtaaaac		-0.527492388703	0.506666666667	29	10	16	-1.3277197
mut	52	69	attttaacg		-3.64777439711	0.5	18	10	16	-1.8553292
mut	52	68	ttttaacga		0.714929736122	0.507936507937	17	10	16	-0.081262664
mut	52	60	atgtagaa		-4.72411342716	0.509090909091	9	10	16	-1.7041458
mut	52	54	gaataagac		-0.842110751682	0.551020408163	3	10	16	0.2091737
mut	52	40	ttctaaata		0.24633338382	0.542857142857	4	12	17	0.57873205
mut	52	34	atatcacct		-0.938217719104	0.551724137931	1	9	14	0.27973553
mut	52	26	tacttatga		-1.01486748302	0.47619047619	21	0	0	-1.1710599
mut	52	23	ttatgaata		-2.01321219191	0.5	18	0	0	-1.3643743
mut	52	19	gaataacat		0.970303282875	0.571428571429	14	0	0	0.080075666

Variant Effect Predictor tool

Se trata de una variante que está afectando al sitio de *splicing*, por lo que va a provocar que se altere el *splicing* normal.

ENST00000394605.2:c.*581T>C	7-90413992-90413992	C	3_prime_UTR_variant	CLDN12	ENSG00000157224	Transcript	ENST00000287916.8	protein_coding	3/3	1603	-	-	-	-	-
ENST00000394605.2:c.*581T>C	7-90413992-90413992	C	downstream_gene_variant	CLDN12	ENSG00000157224	Transcript	ENST00000394604.5	protein_coding	-	-	-	-	-	-	-
ENST00000394605.2:c.*581T>C	7-90413992-90413992	C	3_prime_UTR_variant	CLDN12	ENSG00000157224	Transcript	ENST00000394605.2	protein_coding	3/3	1526	-	-	-	-	-
ENST00000394605.2:c.*581T>C	7-90413992-90413992	C	downstream_gene_variant	CLDN12	ENSG00000157224	Transcript	ENST00000416322.5	protein_coding	-	-	-	-	-	-	-
ENST00000394605.2:c.*581T>C	7-90413992-90413992	C	downstream_gene_variant	CLDN12	ENSG00000157224	Transcript	ENST00000427904.1	protein_coding	-	-	-	-	-	-	-
ENST00000394605.2:c.*581T>C	7-90413992-90413992	C	intron_variant non_coding_transcript_variant	CLDN12	ENSG00000157224	Transcript	ENST00000451941.6	processed_transcript	-	-	-	-	-	-	-
ENST00000394605.2:c.*581T>C	7-90413992-90413992	C	downstream_gene_variant	CLDN12	ENSG00000157224	Transcript	ENST00000462636.5	retained_intron	-	-	-	-	-	-	-
ENST00000394605.2:c.*581T>C	7-90413992-90413992	C	intron_variant non_coding_transcript_variant	AC006153.1	ENSG00000273299	Transcript	ENST00000480135.1	lncRNA	-	-	-	-	-	-	-
ENST00000394605.2:c.*581T>C	7-90413992-90413992	C	intron_variant non_coding_transcript_variant	CLDN12	ENSG00000157224	Transcript	ENST00000483862.5	processed_transcript	-	-	-	-	-	-	-
ENST00000394605.2:c.*581T>C	7-90413992-90413992	C	3_prime_UTR_variant	CLDN12	ENSG00000157224	Transcript	ENST00000496677.6	protein_coding	4/4	1571	-	-	-	-	-
ENST00000394605.2:c.*581T>C	7-90413992-90413992	C	intron_variant non_coding_transcript_variant	CLDN12	ENSG00000157224	Transcript	ENST00000498033.5	processed_transcript	-	-	-	-	-	-	-
ENST00000394605.2:c.*581T>C	7-90413992-90413992	C	downstream_gene_variant	CLDN12	ENSG00000157224	Transcript	ENST00000498326.5	processed_transcript	-	-	-	-	-	-	-

ESEfinder

Se tienen dos resultados con sus acciones positivas sobre las materias 5':

¹³⁸⁶ (-175)	TGCCACTGTTTAAAGTAAACGTAATTTTA	3.40630	¹³⁸⁶ (-175)	TGCCACTGTTTAAAGTAAACGTAATTTTA	-11.32190	¹³⁸⁶ (-175)	TGCCACTGTTTAAAGTAAACGTAATTTTA	2.79880	¹³⁸⁶ (-175)	TGCCACTGTTTAAAGTAAACGTAATTTTA	-14.57930
¹⁴⁰⁶ (-155)	ACGTATTTTAACGATGTTAGAATAAGACTA	0.97340	¹⁴⁰⁶ (-155)	ACGTATTTTAACGATGTTAGAATAAGACTA	-16.22440	¹⁴⁰⁶ (-155)	ACGTATTTTAACGATGTTAGAATAAGACTA	0.57910	¹⁴⁰⁶ (-155)	ACGTATTTTAACGATGTTAGAATAAGACTA	-17.94610

Si se comparan con los resultados para la secuencia mutante se observa que las puntuaciones han aumentado ligeramente:

¹³⁸⁶ (-175)	TGCCACTGTTTAAAGTAAACGCAATTTTA	3.73950	¹³⁸⁶ (-175)	TGCCACTGTTTAAAGTAAACGCAATTTTA	-10.85980	¹³⁸⁶ (-175)	TGCCACTGTTTAAAGTAAACGCAATTTTA	3.28730	¹³⁸⁶ (-175)	TGCCACTGTTTAAAGTAAACGCAATTTTA	-14.15910	¹³⁸⁶ (-175)	TGCCACT	-4.00960
¹⁴⁰⁶ (-155)	ACGCATTTTAACGATGTTAGAATAAGACTA	1.74920	¹⁴⁰⁶ (-155)	ACGCATTTTAACGATGTTAGAATAAGACTA	-16.62950	¹⁴⁰⁶ (-155)	ACGCATTTTAACGATGTTAGAATAAGACTA	1.32640	¹⁴⁰⁶ (-155)	ACGCATTTTAACGATGTTAGAATAAGACTA	-18.33060	¹⁴⁰⁶ (-155)	ACGCATT	-6.01670

Por lo tanto podría estar activándose o haciéndose más fuerte un sitio *donor*.

En cuento a los sitios ESE, parece que algunos de lo sitios se están modificando, por lo que podrían estar afectando al *splicing*:

1403 (-158)	AAAACGT	1.42065	1403 (-158)	AAAACGT	0.27244	1403 (-158)	AAAACGTA	-0.73529	1403 (-158)	AAAACGT	-2.67967
1404 (-157)	AAACGTA	-1.94380	1404 (-157)	AAACGTA	-1.65690	1404 (-157)	AAACGTAT	-2.58192	1404 (-157)	AAACGTA	-3.95077
1405 (-156)	AACGTAT	-4.27938	1405 (-156)	AACGTAT	-3.27373	1405 (-156)	AACGTATT	-2.65561	1405 (-156)	AACGTAT	-4.30710
1406 (-155)	ACGTATT	-5.33565	1406 (-155)	ACGTATT	-4.14202	1406 (-155)	ACGTATTT	-4.33651	1406 (-155)	ACGTATT	-4.12293
1407 (-154)	CGTATTT	-1.16515	1407 (-154)	CGTATTT	0.04748	1407 (-154)	CGTATTTT	-3.59235	1407 (-154)	CGTATTT	-2.12015
1408 (-153)	GTATTTT	-5.86862	1408 (-153)	GTATTTT	-4.93691	1408 (-153)	GTATTTTA	1.62430	1408 (-153)	GTATTTT	-3.52663
1409 (-152)	TATTTTA	-5.76604	1409 (-152)	TATTTTA	-4.78866	1409 (-152)	TATTTTAA	-0.71987	1409 (-152)	TATTTTA	-4.02423

1403 (-158)	AAAACGC	-0.43275	1403 (-158)	AAAACGC	-0.92425	1403 (-158)	AAAACGCA	-1.37973	1403 (-158)	AAAACGC	-1.14057
1404 (-157)	AAACGCA	-2.40151	1404 (-157)	AAACGCA	-1.76090	1404 (-157)	AAACGCAT	-0.85291	1404 (-157)	AAACGCA	-2.23385
1405 (-156)	AACGCAT	-1.75565	1405 (-156)	AACGCAT	-1.46194	1405 (-156)	AACGCATT	-1.89637	1405 (-156)	AACGCAT	-5.79835
1406 (-155)	ACGCATT	-3.87833	1406 (-155)	ACGCATT	-2.88726	1406 (-155)	ACGCATTT	-4.10567	1406 (-155)	ACGCATT	-1.73830
1407 (-154)	CGCATTT	1.14487	1407 (-154)	CGCATTT	2.11634	1407 (-154)	CGCATTTT	-3.02342	1407 (-154)	CGCATTT	-3.46842
1408 (-153)	GCATTTT	-6.47155	1408 (-153)	GCATTTT	-5.18569	1408 (-153)	GCATTTTA	0.23590	1408 (-153)	GCATTTT	-3.21458
1409 (-152)	CATTTTA	-2.81555	1409 (-152)	CATTTTA	-1.68361	1409 (-152)	CATTTTAA	-0.69874	1409 (-152)	CATTTTA	-4.38092

EX-SKIP

No se puede analizar porque las secuencias son demasiado largas.

HOT-SKIP

ttcctgctctgttctgctattgtccccctcatgatttgcctcttgtgtgtcacccttagTCTGACTGACAGTACTCCAC
AAGCTTGCCTGCCATGGGCTGTGCGGATGTCCACGCAGCCACAGTCTTTCTTCTGTGTGGAATCGCCTCAGTAGCAG
GCCTCTTTGAGGGACTCTGCTTCCCAACTGGAGAAAATTACGATTGATCACATTCAACAGAAACGAGAAGAACCTGACT
GTTTACACAGGCCTGTGGGTGAAATGTGCCCGGTATGACGGGAGCAGTGACTGCCTGATGTACGACACTACTTGGTACTC
ATCAGTTGACCAGCTGGACCTGCGTGTCTCCAGTTTGCCTACCCCTCAGCATGCTGATCGCCATGGGTGCCCTGCTGC
TCTGCCTGATTGGAATGTGCAACACTGCCTTCAGGTCCTCGGTGCCCAACATCAAACCTGGCCAAGTGTCTGGTCAATAGT
GCAGGTTGCCACCTGGTGGCTGGGCTGCTATTTTCTTCTGGCAGGTACTGTGAGCCTCTCCCCATCTATCTGGGTCACTTT
TTATAACATCCATCTGAACAAGAAGTTTGAGCCAGTCTTTTCAATTTGACTATGCAGTGTATGTCACTATTGCTAGTGCTG
GGGGCCTGTTTATGACTTCCCTTATACCTATTTATTTGGTATTGTACATGCAAACTTTTGCCTTCTCCTTTCTGGCAACCA
TTGTACTCCCATCCACCCAGTATGCATACTTACTCACAGCCCTATTGAGCAGCTCTCGCCTCTCTGCCATTGAAATTGA
CATTCCAGTAGTTTACACACCACTTAATGGGGAATAGTTAATTGTTAAAGAAAACCTTTGTAGCCTCACATTCCTCT
TGTGCAAGAGCTCTTTTGGACCTACATACATTTTCTTTGTGTTTGGACCAATCAATGAAGCCAAATTTATATGCTCTAG
TAGAATGAAGTGCTGCTAGTTTATGAGAAGTATATTATTAATGTGAATTTTTAAATTTTGCTTCTTACTGGA
AGGAATTTTAGCCTTCATATTGATATCTAATTAATTATTTAGTGGAAGAGGCCTGCATCACAATTGAGGTAATGTAGAG
CAACATGTTAAAGAATGATGGTTAGCAGAAGCTGTTGTATACAATCTTCATGAAAATTTAGTGTGATTTTTCTTTTTCT
TATAATACCTTTAACTGCAAGAAAAGGAGTTTCAAATATAAGAAATTTATTTAGGTAAGGGTAATTTTTAATAGTA
GTCAATAATCTAGCTTAAGGCTGTAACCTCTCTATCGGGCTAATTGTATGAATAGGTGTAGTATGTTGAAGATTACTT
TCTTTTGTGACTTTCTTCTACCTCATGCCACTGTTTAAAGTAAACGTATTTAACGATGTTAGAATAAGACTACCATT
CTAAATATCACCTACTTATGAATAACATGAATAATTTTAAACATTAATGATTCATAAATTGTATTATTGGGATTAGAA
TGTGCTTTATGACAGGTAGTGTTTCTCTGAGGCAGAAAACCTTTTTTGGAGATATCTCCATCAAGCAGTACTCGTG
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