

DIRECT HYBRIDIZATION ASSAY FOR BDNF GENE

Prepared By



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Content

1. Sequence Retrieval for BDNF Gene	2
2. Design Parameters	3
3. Advanced Search Parameters	4
3. Direct Hybridization Assay Design Results	5

Sequence Retrieved from NCBI Reference Assembly

rs6265

Available Information				
Organism	Molecule type	Allele Position	Total length	Alleles
Homo Sapiens	Genomic	318	1458	A/G

Total Sequence considered for designing the assay: 511 bp

* Sequence highlighted in green is considered for the design

CTGCAGAAAG GCCTGGAATT ACAATCAGAT GGGCCACATG GCATCCCGGT GAAAGAAAGC
CC
TAACCAGTTT TCTGTCTTGT TTCTGCTTTC TCCCTACAGT TCCACCAGGT GAGAAGAGTG
ATGACCATCC TTTTCCTTAC TATGGTTATT TCATACTTTG GTTGCATGAA GGCTGCCCCC
ATGAAAGAAG CAAACATCCG AGGACAAGGT GGCTTGGCCT ACCCAGGTGT GCGGACCCAT
GGGACTCTGG AGAGCGTGAA TGGGCCAAG GCAGGTTCAA GAGCCTTGAC ATCATTGGCT
GACACTTTCG AACAC
R
TGATAGAAGA GCTGTTGGAT GAGGACCAGA AAGTTCGGCC CAATGAAGAA AACAATAAGG
ACGCAGACTT GTACACGTCC AGGGTGATGC TCAGTAGTCA AGTGCCTTTG GAGCCTCCTC
TTCTCTTTCT GCTGGAGGAA TACAAAAATT ACCTAGATGC TGCAAACATG TCCATGAGGG
TCCGGCGCCA CTCTGACCCT GCCCGCCGAG GGGAGCTGAG CGTGTGTGAC AGTATTAGTG
AGTGGGTAAC GGCGG
CAGACAAAAA GACTGCAGTG GACATGTCGG GCGGGACGGT CACAGTCCTT GAAAAGGTCC
CTGTATCAAA AGGCCAACTG AAGCAATACT TCTACGAGAC CAAGTGCAAT CCCATGGGTT
ACACAAAAGA AGGCTGCAGG GGCATAGACA AAAGGCATTG GAACTCCCAG TGCCGAACATA
CCCAGTCGTA CGTGCGGGCC CTTACCATGG ATAGCAAAAA GAGAATTGGC TGGCGATTCA
TAAGGATAGA CACTTCTTGT GTATGTACAT TGACCATTAA AAGGGGAAGA TAGTGGATTT
ATGTTGTATA GATTAGATTA TATTGAGACA AAAATTATCT ATTTGTATAT ATACATAACA
GGGTAAATTA TTCAGTTAAG AAAAAAATAA TTTTATGAAC TGCATGTATA AATGAAGTTT
ATACAGTACA GTGGTTCTAC AATCTATTTA TTGGACATGT CCATGACCAG AAGGGAAACA
GTCATTTGCG CACAACCTAA AAAGTCTGCA TTACATTCCT TGATAATGTT GTGGTTTTGT
GCCGTTGCCA AGAACTGAAA ACATAAAAAG TTAATAAAAA TAATAAATTG CATGCTGCTT
TAATTGTGAA TTGATAATAA ACTGTCCTCT TTCAGAAAAC AGAAAAAAC ACACACACAC
ACAACAAAAA TTTGAACCAA AACATTCCGT TTACATTTTA GACAGTAAGT ATCTTCGTTT
TTGTTAGTAC TATATCTGTT TTAAGTCTTT TAACTTCTGA TAGCGTTGGA ATTAACAA
TGCAAGGTG CTGTTGTCAT TGCACCCCA AGGGGAACTA ACCGCCTCCC ACACACTATA
TTCTGCCAC CCCC GCCCA CCCTACACCG GCCCGCACC GCCC

Design Parameters

Direct Hybridization Assay(Capture Probe) Search [X]

Resultant multiplex set

Multiplex Set Name:

Design options

Allele Specific Capture Probes

Capture probe parameters

Tm: +/- °C

Length: to: bp

Avoid Cross Homology

Primers to Amplify Template

Primer parameters

Tm: +/- °C

Ta: +/- °C

Length: to: bp

Amplicon Length: to: bp

Avoid Cross Homology

Output options

Alternate Results: Design Sense Probe Design Anti-sense Probe

ADVANCED SEARCH PARAMETERS

Advanced Capture Probe Parameters [X]

— Advance design parameters —

Hairpin Maximum ΔG (3' End):	<input type="text" value="2.0"/>	-kcal/mol
Self Dimer Maximum ΔG (3' End):	<input type="text" value="5.0"/>	-kcal/mol
Run/Repeat (dinucleotide) Maximum Length:	<input type="text" value="4"/>	bp
Maximum ΔG for Multiplex Reactions:	<input type="text" value="5.0"/>	-kcal/mol

Default OK Cancel Help

Advanced Search Parameters [X]

— Advanced primer parameters —

Hairpin Maximum ΔG (3' End):	<input type="text" value="2.0"/>	-kcal/mol
Hairpin Maximum ΔG (Internal):	<input type="text" value="3.0"/>	-kcal/mol
3' End Maximum ΔG :	<input type="text" value="10.0"/>	-kcal/mol
Self Dimer Maximum ΔG (3' End):	<input type="text" value="5.0"/>	-kcal/mol
Self Dimer Maximum ΔG (Internal):	<input type="text" value="6.0"/>	-kcal/mol
Run/Repeat (dinucleotide) Maximum Length:	<input type="text" value="4"/>	bp
G/C clamp - Target Consecutive G/Cs at 3' End:	<input type="text" value="1"/>	

— Advanced primer pair parameters —

Maximum Ambiguous Bases in Amplicon:	<input type="text" value="0"/>	
Maximum Primer Pair T_m Mismatch:	<input type="text" value="3.0"/>	°C
Cross Dimer Maximum ΔG (3' End):	<input type="text" value="5.0"/>	-kcal/mol
Cross Dimer Maximum ΔG (Internal):	<input type="text" value="6.0"/>	-kcal/mol

Default OK Cancel Help

Direct Hybridization Assay Design Results

Capture Probe Results

Rating	T_m	Sequence	<i>Base Composition</i>	GC%
68.4	54.8	Sense Wild	TGGAGAGCGTGAATGGG	61.1
59.3	52.1	Sense Mutant	TGGAGAGCGTAAATGGGC	55.6
Position on the sequence	186	Length of the probe	18 bp	
Hairpin G kcal/mol	0	Self Dimer kcal/mol	0	

Primer Pair Results

Rating	T_m	Sequence	<i>Base Composition</i>	GC%
85.4	55.2	Sense Primer	ACAGTTCCACCAGGTAGAAGAG	50
Run Length bp	2	GC Clamp	1	
Position on the sequence	36	Length of the primers	22 bp	
Hairpin G kcal/mol	0	Self Dimer kcal/mol	0	
TaOpt (Product)	56			

Rating	T_m	Sequence	<i>Base Composition</i>	GC%
85.9	55	Sense Primer	AGTGTCAGCCAATGATGTCAAG	45.5
Run Length bp	2	GC Clamp	1	
Position on the sequence	244	Length of the primers	22 bp	
Hairpin G kcal/mol	-0.8	Self Dimer kcal/mol	-0.8	
TaOpt (Product)	56			