[rs28931589](https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=28931589) *[Homo sapiens]*

CAGCAACAGTCTTACCTGGACTCTG[A/G/T]AATCCATTCTGGTGCCACTACCACA

Chromosome: 3:41224613

Gene: CTNNB1

1. Allele specific primer design on wild type nucleotide of CTNNB1 gene

|  |  |  |
| --- | --- | --- |
| Primer Criteria | Forward Primer | Reverse Primer |
| Sequence  | CAGTCTTACCTGGACTCTGG | TCAAAACTGCATTCTGACTTTCA |
| Length | 20 bp | 23 bp |
| Start | 533 | 743 |
| Tm | 54.8 °C | 59.9 °C |
| GC | 55.0 % | 34.8 % |
| Tm | 51.63 °C | 57.97 °C |
| GC% | 55.0 | 34.78 |
| Self-Dimer ( ΔG) | -4.55 kcal/mol | -7.05 kcal/mol |
| Hairpin ( ΔG) | -0.42 kcal/mol |  |
| Cross Dimer (ΔG) | -4.89 kcal/mol |
| Product size | 211 bp |

1. Allele specific primer design on mutant nucleotide of CTNNB1 gene

|  |  |  |
| --- | --- | --- |
| Primer Criteria | Forward Primer | Reverse Primer |
| Sequence  | CAGTCTTACCTGGACTCTGA | TGTTCTTGAGTGAAGGACTGAGA |
| Length | 20 bp | 23 bp |
| Start | 533 | 684 |
| Tm | 52.7 °C | 59.1 °C |
| GC | 50.0 % | 43.5 % |
| Tm | 49.2 °C | 56.64 °C |
| GC% | 50.0 | 43.48 |
| Self-Dimer ( ΔG) | -4.55 kcal/mol | -4.17kcal/mol |
| Hairpin ( ΔG) | -0.42 kcal/mol |  |
| Cross Dimer (ΔG) | -7.47 kcal/mol |
| Product size | 152 bp |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Pair 1: |  |  |  |  |  |
|  Left Primer 1:      |
| Sequence: |  |
| Start:   533 | Length:   20 bp | Tm:   54.8 °C  | GC:   55.0 % | ANY:   4.0 | SELF:   1.0 |
|  |
|  Right Primer 1:      |
| Sequence: |  |
| Start:   743 | Length:   23 bp | Tm:   59.9 °C  | GC:   34.8 % | ANY:   8.0 | SELF:   3.0 |
|  |
| Product Size:   211 bp | Pair Any: 5.0 | Pair End: 1.0 |  |  |

|  |
| --- |
| **Analysis Results #1: CAGTCTTACCTGGACTCTGG** |
|

|  |  |  |  |
| --- | --- | --- | --- |
| Rating | :  | 91.0 |   |
| Molecular Wt | :  | 6084.04 |   |
| Tm | :  | 51.63 | °C |
| GC% | :  | 55.0 |   |
| GC Clamp | :  | 2 |   |
| nmol/A260 | :  | 5.51 |   |
| ug/A260 | :  | 33.5 |   |
| ΔG | :  | -30.22 | kcal/mol |

 |

|  |  |  |  |
| --- | --- | --- | --- |
| 3' end stability | :  | -8.2 | kcal/mol |
| ΔH | :  | -140.8 | kcal/mol |
| ΔS | :  | -0.37 | kcal/°K/mol |
| 5' end ΔG | :  | -6.47 | kcal/mol |
| Self Dimer ( ΔG) | :  | [-4.55](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol |
| Hairpin ( ΔG) | :  | [-0.42](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol |
| Repeats (# of pairs) | :  |  | kcal/mol |
| Run (# of bases) | :  |  | kcal/mol |

 |

|  |
| --- |
| **Analysis Results #2: TCAAAACTGCATTCTGACTTTCA** |
|

|  |  |  |  |
| --- | --- | --- | --- |
| Rating | :  | 87.0 |   |
| Molecular Wt | :  | 6957.65 |   |
| Tm | :  | 57.97 | °C |
| GC% | :  | 34.78 |   |
| GC Clamp | :  | 1 |   |
| nmol/A260 | :  | 4.64 |   |
| ug/A260 | :  | 32.26 |   |
| ΔG | :  | -35.33 | kcal/mol |

 |

|  |  |  |  |
| --- | --- | --- | --- |
| 3' end stability | :  | -7.42 | kcal/mol |
| ΔH | :  | -162.1 | kcal/mol |
| ΔS | :  | -0.43 | kcal/°K/mol |
| 5' end ΔG | :  | -7.42 | kcal/mol |
| Self Dimer ( ΔG) | :  | [-7.05](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol |
| Hairpin ( ΔG) | :  |  | kcal/mol |
| Repeats (# of pairs) | :  |  | kcal/mol |
| Run (# of bases) | :  | [4](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol |

 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|

|  |  |  |  |
| --- | --- | --- | --- |
| Cross Dimer (ΔG) | :  | [-4.89](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol |

 |

|  |
| --- |
|  |

00000000000000000000000000000000000000000

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Pair 2: |  |  |  |  |  |
|  Left Primer 2:      |
| Sequence: |  |
| Start:   533 | Length:   20 bp | Tm:   52.7 °C  | GC:   50.0 % | ANY:   4.0 | SELF:   3.0 |
|  |
|  Right Primer 2:      |
| Sequence: |  |
| Start:   684 | Length:   23 bp | Tm:   59.1 °C  | GC:   43.5 % | ANY:   7.0 | SELF:   1.0 |
|  |
| Product Size:   152 bp | Pair Any: 5.0 | Pair End: 3.0 |  |  |

|  |
| --- |
| **Analysis Results #1: CAGTCTTACCTGGACTCTGA** |
|

|  |  |  |  |
| --- | --- | --- | --- |
| Rating | :  | 91.0 |   |
| Molecular Wt | :  | 6068.04 |   |
| Tm | :  | 49.2 | °C |
| GC% | :  | 50.0 |   |
| GC Clamp | :  | 1 |   |
| nmol/A260 | :  | 5.4 |   |
| ug/A260 | :  | 32.76 |   |
| ΔG | :  | -28.72 | kcal/mol |

 |

|  |  |  |  |
| --- | --- | --- | --- |
| 3' end stability | :  | -6.7 | kcal/mol |
| ΔH | :  | -135.4 | kcal/mol |
| ΔS | :  | -0.36 | kcal/°K/mol |
| 5' end ΔG | :  | -6.47 | kcal/mol |
| Self Dimer ( ΔG) | :  | [-4.55](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol |
| Hairpin ( ΔG) | :  | [-0.42](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol |
| Repeats (# of pairs) | :  |  | kcal/mol |
| Run (# of bases) | :  |  | kcal/mol |

 |

|  |
| --- |
| **Analysis Results #2: TGTTCTTGAGTGAAGGACTGAGA** |
|

|  |  |  |  |
| --- | --- | --- | --- |
| Rating | :  | 92.0 |   |
| Molecular Wt | :  | 7158.74 |   |
| Tm | :  | 56.64 | °C |
| GC% | :  | 43.48 |   |
| GC Clamp | :  | 1 |   |
| nmol/A260 | :  | 4.32 |   |
| ug/A260 | :  | 30.94 |   |
| ΔG | :  | -33.68 | kcal/mol |

 |

|  |  |  |  |
| --- | --- | --- | --- |
| 3' end stability | :  | -6.7 | kcal/mol |
| ΔH | :  | -153.6 | kcal/mol |
| ΔS | :  | -0.4 | kcal/°K/mol |
| 5' end ΔG | :  | -6.82 | kcal/mol |
| Self Dimer ( ΔG) | :  | [-4.17](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol |
| Hairpin ( ΔG) | :  |  | kcal/mol |
| Repeats (# of pairs) | :  |  | kcal/mol |
| Run (# of bases) | :  |  | kcal/mol |

 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|

|  |  |  |  |
| --- | --- | --- | --- |
| Cross Dimer (ΔG) | :  | [-7.47](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol |

 |

|  |
| --- |
|  |

000000000000000000000000000000000000000

|  |  |  |
| --- | --- | --- |
|  | Forward Primer | Reverse Primer |
| Sequence  |  |  |
| Length |  |  |
| Start |  |  |
| Tm |  |  |
| GC |  |  |
| Tm |  |  |
| GC% |  |  |
| Self-Dimer ( ΔG) |  |  |
| Hairpin ( ΔG) |  |  |
| Cross Dimer (ΔG) |  |
| Product size |  |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Pair 2: |  |  |  |  |  |
|  Left Primer 2:      |
| Sequence: |  |
| Start:   533 | Length:   20 bp | Tm:   51.7 °C  | GC:   50.0 % | ANY:   4.0 | SELF:   1.0 |
|  |
|  Right Primer 2:      |
| Sequence: |  |
| Start:   684 | Length:   23 bp | Tm:   59.1 °C  | GC:   43.5 % | ANY:   7.0 | SELF:   1.0 |
|  |
| Product Size:   152 bp | Pair Any: 5.0 | Pair End: 3.0 |  |  |

|  |
| --- |
| **Analysis Results #1: CAGTCTTACCTGGACTCTGT** |
|

|  |  |  |  |
| --- | --- | --- | --- |
| Rating | :  | 91.0 |   |
| Molecular Wt | :  | 6059.03 |   |
| Tm | :  | 48.29 | °C |
| GC% | :  | 50.0 |   |
| GC Clamp | :  | 1 |   |
| nmol/A260 | :  | 5.56 |   |
| ug/A260 | :  | 33.66 |   |
| ΔG | :  | -28.49 | kcal/mol |

 |

|  |  |  |  |
| --- | --- | --- | --- |
| 3' end stability | :  | -6.47 | kcal/mol |
| ΔH | :  | -136.3 | kcal/mol |
| ΔS | :  | -0.36 | kcal/°K/mol |
| 5' end ΔG | :  | -6.47 | kcal/mol |
| Self Dimer ( ΔG) | :  | [-4.55](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol |
| Hairpin ( ΔG) | :  | [-0.42](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol |
| Repeats (# of pairs) | :  |  | kcal/mol |
| Run (# of bases) | :  |  | kcal/mol |

 |

|  |
| --- |
| **Analysis Results #2: TGTTCTTGAGTGAAGGACTGAGA** |
|

|  |  |  |  |
| --- | --- | --- | --- |
| Rating | :  | 92.0 |   |
| Molecular Wt | :  | 7158.74 |   |
| Tm | :  | 56.64 | °C |
| GC% | :  | 43.48 |   |
| GC Clamp | :  | 1 |   |
| nmol/A260 | :  | 4.32 |   |
| ug/A260 | :  | 30.94 |   |
| ΔG | :  | -33.68 | kcal/mol |

 |

|  |  |  |  |
| --- | --- | --- | --- |
| 3' end stability | :  | -6.7 | kcal/mol |
| ΔH | :  | -153.6 | kcal/mol |
| ΔS | :  | -0.4 | kcal/°K/mol |
| 5' end ΔG | :  | -6.82 | kcal/mol |
| Self Dimer ( ΔG) | :  | [-4.17](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol |
| Hairpin ( ΔG) | :  |  | kcal/mol |
| Repeats (# of pairs) | :  |  | kcal/mol |
| Run (# of bases) | :  |  | kcal/mol |

 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|

|  |  |  |  |
| --- | --- | --- | --- |
| Cross Dimer (ΔG) | :  | [-7.47](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol |

 |