[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

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| 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

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| 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 | -4.17  kcal/mol |
| Hairpin ( ΔG) | -0.42 kcal/mol |  |
| Cross Dimer (ΔG) | -7.47 kcal/mol | |
| Product size | 152 bp | |

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

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| **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 | |

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| **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 | |

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

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

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| **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 | |

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| **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 | |

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

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

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| 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 |  |  |

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| **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 | |

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| **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 | |

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