[rs74648499](https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=74648499) *[Homo sapiens]*

AAGCTGAACATTATGGCAGCCAAAA[A/G]ACAACAGGTACAGTCATGATTTGGG

Chromosome: 5:138812295

Gene:CTNNA1

1. Allele specific primer design on wild type nucleotide of CTNNA1 gene

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|  | Forward Primer | Reverse Primer |
| Sequence | AACATTATGGCAGCCAAAAG | CAGCACAATACTGAAAAGACACC |
| Length | 20 bp | 23bp |
| Start | 529 | 711 |
| Tm | 57.8 °C | 58.8 °C |
| GC | 40.0 % | 43.5 % |
| Tm | 55.71 °C | 56.99 °C |
| GC% | 40.0 | 43.48 |
| Self-Dimer ( ΔG) | -8.16 kcal/mol |  |
| Hairpin ( ΔG) |  |  |
| Cross Dimer (ΔG) |  | |
| Product size | 183 bp | |

2. Allele specific primer design on mutant nucleotide of CTNNA1 gene

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|  | Forward Primer | Reverse Primer |
| Sequence | AACATTATGGCAGCCAAAAA | CAGCACAATACTGAAAAGACACC |
| Length | 20 bp | 23 bp |
| Start | 529 | 711 |
| Tm | 58.2 °C | 58.8 °C |
| GC | 35.0 % | 43.5 % |
| Tm | 56.15 °C | 56.99 °C |
| GC% | 35.0 | 43.48 |
| Self-Dimer ( ΔG) | -8.16  kcal/mol |  |
| Hairpin ( ΔG) |  |  |
| Cross Dimer (ΔG) |  | |
| Product size | 183 bp | |

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| Pair 5: |  |  |  |  |  |
| Left Primer 5: | | | | | |
| Sequence: |  | | | | |
| Start:   529 | Length:   20 bp | Tm:   57.8 °C | GC:   40.0 % | ANY:   9.0 | SELF:   7.0 |
|  | | | | | |
| Right Primer 5: | | | | | |
| Sequence: |  | | | | |
| Start:   711 | Length:   23 bp | Tm:   58.8 °C | GC:   43.5 % | ANY:   4.0 | SELF:   0.0 |
|  | | | | | |
| Product Size:   183 bp | | Pair Any: 4.0 | Pair End: 1.0 |  |  |

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| **Analysis Results #1: AACATTATGGCAGCCAAAAG** | |
| |  |  |  |  | | --- | --- | --- | --- | | Rating | : | 85.0 |  | | Molecular Wt | : | 6143.11 |  | | Tm | : | 55.71 | °C | | GC% | : | 40.0 |  | | GC Clamp | : | 1 |  | | nmol/A260 | : | 4.83 |  | | ug/A260 | : | 29.65 |  | | ΔG | : | -33.9 | kcal/mol | | |  |  |  |  | | --- | --- | --- | --- | | 3' end stability | : | -7.43 | kcal/mol | | ΔH | : | -158.2 | kcal/mol | | ΔS | : | -0.42 | kcal/°K/mol | | 5' end ΔG | : | -6.71 | kcal/mol | | Self Dimer ( ΔG) | : | [-8.16](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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| **Analysis Results #2: CAGCACAATACTGAAAAGACACC** | |
| |  |  |  |  | | --- | --- | --- | --- | | Rating | : | 100.0 |  | | Molecular Wt | : | 7003.69 |  | | Tm | : | 56.99 | °C | | GC% | : | 43.48 |  | | GC Clamp | : | 2 |  | | nmol/A260 | : | 4.28 |  | | ug/A260 | : | 29.94 |  | | ΔG | : | -35.0 | kcal/mol | | |  |  |  |  | | --- | --- | --- | --- | | 3' end stability | : | -7.71 | kcal/mol | | ΔH | : | -162.7 | kcal/mol | | ΔS | : | -0.43 | kcal/°K/mol | | 5' end ΔG | : | -8.65 | kcal/mol | | Self Dimer ( ΔG) | : |  | 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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| Pair 5: |  |  |  |  |  |
| Left Primer 5: | | | | | |
| Sequence: |  | | | | |
| Start:   529 | Length:   20 bp | Tm:   58.2 °C | GC:   35.0 % | ANY:   9.0 | SELF:   7.0 |
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| Right Primer 5: | | | | | |
| Sequence: |  | | | | |
| Start:   711 | Length:   23 bp | Tm:   58.8 °C | GC:   43.5 % | ANY:   4.0 | SELF:   0.0 |
|  | | | | | |
| Product Size:   183 bp | | Pair Any: 4.0 | Pair End: 0.0 |  |  |

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| **Analysis Results #1: AACATTATGGCAGCCAAAAA** | |
| |  |  |  |  | | --- | --- | --- | --- | | Rating | : | 85.0 |  | | Molecular Wt | : | 6127.11 |  | | Tm | : | 56.15 | °C | | GC% | : | 35.0 |  | | GC Clamp | : | 0 |  | | nmol/A260 | : | 4.77 |  | | ug/A260 | : | 29.23 |  | | ΔG | : | -34.25 | kcal/mol | | |  |  |  |  | | --- | --- | --- | --- | | 3' end stability | : | -7.78 | kcal/mol | | ΔH | : | -159.5 | kcal/mol | | ΔS | : | -0.42 | kcal/°K/mol | | 5' end ΔG | : | -6.71 | kcal/mol | | Self Dimer ( ΔG) | : | [-8.16](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | | Hairpin ( ΔG) | : |  | kcal/mol | | Repeats (# of pairs) | : |  | kcal/mol | | Run (# of bases) | : | [5](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | |

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| **Analysis Results #2: CAGCACAATACTGAAAAGACACC** | |
| |  |  |  |  | | --- | --- | --- | --- | | Rating | : | 100.0 |  | | Molecular Wt | : | 7003.69 |  | | Tm | : | 56.99 | °C | | GC% | : | 43.48 |  | | GC Clamp | : | 2 |  | | nmol/A260 | : | 4.28 |  | | ug/A260 | : | 29.94 |  | | ΔG | : | -35.0 | kcal/mol | | |  |  |  |  | | --- | --- | --- | --- | | 3' end stability | : | -7.71 | kcal/mol | | ΔH | : | -162.7 | kcal/mol | | ΔS | : | -0.43 | kcal/°K/mol | | 5' end ΔG | : | -8.65 | kcal/mol | | Self Dimer ( ΔG) | : |  | 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) | : |  | kcal/mol | |

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