[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) | -8.16kcal/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** |
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|  |  |  |  |
| --- | --- | --- | --- |
| 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 |

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| 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** |
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| 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 |

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| --- | --- | --- | --- |
| 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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| Pair 5: |  |  |  |  |  |
|  Left Primer 5:      |
| Sequence: |  |
| Start:   529 | Length:   20 bp | Tm:   58.2 °C  | GC:   35.0 % | 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: 0.0 |  |  |

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

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| 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** |
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| --- | --- | --- | --- |
| 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 |

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