[rs116498325](https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=116498325) *[Homo sapiens]*

AATACAACACAGCCTGGGTCCCTTC[A/T]GCTCACTGTGGGAAACCTGAAGCCA

Chromosome: 18:53179000

Gene: DCC

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

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| Primer Criteria | Forward Primer | Reverse Primer |
| Sequence  | ACACAGCCTGGGTCCCTTCA | TCTTCGCTTTTGTCACTTCG |
| Length | 20 bp | 20 bp |
| Start | 448 | 686 |
| Tm | 65.3 °C | 59.2 °C |
| GC | 60.0 % | 45.0 % |
| Tm | 62.32 °C | 56.49 °C |
| GC% | 60.0 | 45.0 |
| Self-Dimer ( ΔG) |  |  |
| Hairpin ( ΔG) |  |  |
| Cross Dimer (ΔG) |  |
| Product size | 239 bp |

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

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| Primer Criteria | Forward Primer | Reverse Primer |
| Sequence  | ACACAGCCTGGGTCCCTTCT | TCTTCGCTTTTGTCACTTCG |
| Length | 20 bp | 20 bp |
| Start | 448 | 686 |
| Tm | 63.3 °C | 59.2 °C |
| GC | 60.0 % | 45.0 % |
| Tm | 60.65 °C | 56.49 °C |
| GC% | 60.0 | 45.0 |
| Self-Dimer ( ΔG) |  |  |
| Hairpin ( ΔG) |  |  |
| Cross Dimer (ΔG) |  |
| Product size | 239 bp |

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| Pair 4: |  |  |  |  |  |
|  Left Primer 4:      |
| Sequence: |  |
| Start:   448 | Length:   20 bp | Tm:   65.3 °C  | GC:   60.0 % | ANY:   7.0 | SELF:   1.0 |
|  |
|  Right Primer 4:      |
| Sequence: |  |
| Start:   686 | Length:   20 bp | Tm:   59.2 °C  | GC:   45.0 % | ANY:   3.0 | SELF:   2.0 |
|  |
| Product Size:   239 bp | Pair Any: 3.0 | Pair End: 0.0 |  |  |

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| **Analysis Results #1: ACACAGCCTGGGTCCCTTCA** |
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| Rating | :  | 100.0 |   |
| Molecular Wt | :  | 6038.02 |   |
| Tm | :  | 62.32 | °C |
| GC% | :  | 60.0 |   |
| GC Clamp | :  | 1 |   |
| nmol/A260 | :  | 5.45 |   |
| ug/A260 | :  | 32.92 |   |
| ΔG | :  | -35.71 | kcal/mol |

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| 3' end stability | :  | -7.07 | kcal/mol |
| ΔH | :  | -152.5 | kcal/mol |
| ΔS | :  | -0.39 | kcal/°K/mol |
| 5' end ΔG | :  | -6.59 | kcal/mol |
| Self Dimer ( ΔG) | :  |  | kcal/mol |
| Hairpin ( ΔG) | :  |  | kcal/mol |
| Repeats (# of pairs) | :  |  | kcal/mol |
| Run (# of bases) | :  | [3](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol |

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| **Analysis Results #2: TCTTCGCTTTTGTCACTTCG** |
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| Rating | :  | 100.0 |   |
| Molecular Wt | :  | 6016.0 |   |
| Tm | :  | 56.49 | °C |
| GC% | :  | 45.0 |   |
| GC Clamp | :  | 2 |   |
| nmol/A260 | :  | 5.94 |   |
| ug/A260 | :  | 35.75 |   |
| ΔG | :  | -33.27 | kcal/mol |

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| 3' end stability | :  | -8.73 | kcal/mol |
| ΔH | :  | -150.8 | kcal/mol |
| ΔS | :  | -0.39 | kcal/°K/mol |
| 5' end ΔG | :  | -6.69 | 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 4: |  |  |  |  |  |
|  Left Primer 4:      |
| Sequence: |  |
| Start:   448 | Length:   20 bp | Tm:   63.3 °C  | GC:   60.0 % | ANY:   7.0 | SELF:   1.0 |
|  |
|  Right Primer 4:      |
| Sequence: |  |
| Start:   686 | Length:   20 bp | Tm:   59.2 °C  | GC:   45.0 % | ANY:   3.0 | SELF:   2.0 |
|  |
| Product Size:   239 bp | Pair Any: 3.0 | Pair End: 0.0 |  |  |

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| **Analysis Results #1: ACACAGCCTGGGTCCCTTCT** |
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| --- | --- | --- | --- |
| Rating | :  | 100.0 |   |
| Molecular Wt | :  | 6029.01 |   |
| Tm | :  | 60.65 | °C |
| GC% | :  | 60.0 |   |
| GC Clamp | :  | 1 |   |
| nmol/A260 | :  | 5.64 |   |
| ug/A260 | :  | 33.99 |   |
| ΔG | :  | -35.36 | kcal/mol |

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| 3' end stability | :  | -6.72 | kcal/mol |
| ΔH | :  | -154.5 | kcal/mol |
| ΔS | :  | -0.4 | kcal/°K/mol |
| 5' end ΔG | :  | -6.59 | kcal/mol |
| Self Dimer ( ΔG) | :  |  | kcal/mol |
| Hairpin ( ΔG) | :  |  | kcal/mol |
| Repeats (# of pairs) | :  |  | kcal/mol |
| Run (# of bases) | :  | [3](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol |

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| **Analysis Results #2: TCTTCGCTTTTGTCACTTCG** |
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| --- | --- | --- | --- |
| Rating | :  | 100.0 |   |
| Molecular Wt | :  | 6016.0 |   |
| Tm | :  | 56.49 | °C |
| GC% | :  | 45.0 |   |
| GC Clamp | :  | 2 |   |
| nmol/A260 | :  | 5.94 |   |
| ug/A260 | :  | 35.75 |   |
| ΔG | :  | -33.27 | kcal/mol |

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|  |  |  |  |
| --- | --- | --- | --- |
| 3' end stability | :  | -8.73 | kcal/mol |
| ΔH | :  | -150.8 | kcal/mol |
| ΔS | :  | -0.39 | kcal/°K/mol |
| 5' end ΔG | :  | -6.69 | 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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