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

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| **Analysis Results #1: ACACAGCCTGGGTCCCTTCA** | |
| |  |  |  |  | | --- | --- | --- | --- | | 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 | | |  |  |  |  | | --- | --- | --- | --- | | 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** | |
| |  |  |  |  | | --- | --- | --- | --- | | 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 | | |  |  |  |  | | --- | --- | --- | --- | | 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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| 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 |
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| Product Size:   239 bp | | Pair Any: 3.0 | Pair End: 0.0 |  |  |

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