[rs115283528](https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=115283528) *[Homo sapiens]*

TTGCTGTTATTGTGGTTGTGGTGAT[A/G]GCAGTTGTTGCTGGAATTGTTGTGC

Chromosome: 2:47379942

Gene: EPCAM

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

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|  | Forward Primer | Reverse Primer |
| Sequence | GTTATTGTGGTTGTGGTGATA | GTCTCAGCCTCCCAAAGTGT |
| Length | 21 bp | 20 bp |
| Start | 567 | 731 |
| Tm | 53.4 °C | 59.3 °C |
| GC | 38.1 % | 55.0 % |
| Tm | 50.65 °C | 56.32 °C |
| GC% | 38.1 | 55.0 |
| Self-Dimer ( ΔG) | -3.44 kcal/mol |  |
| Hairpin ( ΔG) |  |  |
| Cross Dimer (ΔG) | -4.53 kcal/mol | |
| Product size | 165 bp | |

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

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|  | Forward Primer | Reverse Primer |
| Sequence | GTTATTGTGGTTGTGGTGATG | GTCTCAGCCTCCCAAAGTGT |
| Length | 21 bp | 20bp |
| Start | 567 |  |
| Tm | 56.2 °C | 59.3 °C |
| GC | 42.9 % | 55.0 % |
| Tm | 53.32 °C | 56.32 °C |
| GC% | 42.86 | 55.0 |
| Self-Dimer ( ΔG) |  |  |
| Hairpin ( ΔG) |  |  |
| Cross Dimer (ΔG) | -4.53 kcal/mol | |
| Product size | 165 bp | |

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| Pair 4: |  |  |  |  |  |
| Left Primer 4: | | | | | |
| Sequence: |  | | | | |
| Start:   567 | Length:   21 bp | Tm:   53.4 °C | GC:   38.1 % | ANY:   3.0 | SELF:   2.0 |
|  | | | | | |
| Right Primer 4: | | | | | |
| Sequence: |  | | | | |
| Start:   731 | Length:   20 bp | Tm:   59.3 °C | GC:   55.0 % | ANY:   3.0 | SELF:   1.0 |
|  | | | | | |
| Product Size:   165 bp | | Pair Any: 3.0 | Pair End: 1.0 |  |  |

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| **Analysis Results #1: GTTATTGTGGTTGTGGTGATA** | |
| |  |  |  |  | | --- | --- | --- | --- | | Rating | : | 93.0 |  | | Molecular Wt | : | 6553.33 |  | | Tm | : | 50.65 | °C | | GC% | : | 38.1 |  | | GC Clamp | : | 1 |  | | nmol/A260 | : | 4.81 |  | | ug/A260 | : | 31.52 |  | | ΔG | : | -30.39 | kcal/mol | | |  |  |  |  | | --- | --- | --- | --- | | 3' end stability | : | -5.96 | kcal/mol | | ΔH | : | -145.6 | kcal/mol | | ΔS | : | -0.39 | kcal/°K/mol | | 5' end ΔG | : | -5.72 | kcal/mol | | Self Dimer ( ΔG) | : | [-3.44](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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| **Analysis Results #2: GTCTCAGCCTCCCAAAGTGT** | |
| |  |  |  |  | | --- | --- | --- | --- | | Rating | : | 100.0 |  | | Molecular Wt | : | 6053.03 |  | | Tm | : | 56.32 | °C | | GC% | : | 55.0 |  | | GC Clamp | : | 1 |  | | nmol/A260 | : | 5.44 |  | | ug/A260 | : | 32.91 |  | | ΔG | : | -32.74 | kcal/mol | | |  |  |  |  | | --- | --- | --- | --- | | 3' end stability | : | -6.24 | kcal/mol | | ΔH | : | -147.2 | kcal/mol | | ΔS | : | -0.38 | kcal/°K/mol | | 5' end ΔG | : | -6.09 | 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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| |  |  |  |  | | --- | --- | --- | --- | | Cross Dimer (ΔG) | : | [-4.53](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | |

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| Pair 4: |  |  |  |  |  |
| Left Primer 4: | | | | | |
| Sequence: |  | | | | |
| Start:   567 | Length:   21 bp | Tm:   56.2 °C | GC:   42.9 % | ANY:   2.0 | SELF:   0.0 |
|  | | | | | |
| Right Primer 4: | | | | | |
| Sequence: |  | | | | |
| Start:   731 | Length:   20 bp | Tm:   59.3 °C | GC:   55.0 % | ANY:   3.0 | SELF:   1.0 |
|  | | | | | |
| Product Size:   165 bp | |  |  |  |  |

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| **Analysis Results #1: GTTATTGTGGTTGTGGTGATG** | |
| |  |  |  |  | | --- | --- | --- | --- | | Rating | : | 100.0 |  | | Molecular Wt | : | 6569.33 |  | | Tm | : | 53.32 | °C | | GC% | : | 42.86 |  | | GC Clamp | : | 1 |  | | nmol/A260 | : | 4.91 |  | | ug/A260 | : | 32.28 |  | | ΔG | : | -31.39 | kcal/mol | | |  |  |  |  | | --- | --- | --- | --- | | 3' end stability | : | -6.96 | kcal/mol | | ΔH | : | -145.4 | kcal/mol | | ΔS | : | -0.38 | kcal/°K/mol | | 5' end ΔG | : | -5.72 | kcal/mol | | Self Dimer ( ΔG) | : |  | kcal/mol | | Hairpin ( ΔG) | : |  | kcal/mol | | Repeats (# of pairs) | : |  | kcal/mol | | Run (# of bases) | : |  | kcal/mol | |

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| **Analysis Results #2: GTCTCAGCCTCCCAAAGTGT** | |
| |  |  |  |  | | --- | --- | --- | --- | | Rating | : | 100.0 |  | | Molecular Wt | : | 6053.03 |  | | Tm | : | 56.32 | °C | | GC% | : | 55.0 |  | | GC Clamp | : | 1 |  | | nmol/A260 | : | 5.44 |  | | ug/A260 | : | 32.91 |  | | ΔG | : | -32.74 | kcal/mol | | |  |  |  |  | | --- | --- | --- | --- | | 3' end stability | : | -6.24 | kcal/mol | | ΔH | : | -147.2 | kcal/mol | | ΔS | : | -0.38 | kcal/°K/mol | | 5' end ΔG | : | -6.09 | 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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| |  |  |  |  | | --- | --- | --- | --- | | Cross Dimer (ΔG) | : | [-4.53](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | |