[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** |
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| 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 |

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| 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** |
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| 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 |

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

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

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