[rs121913507](https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=121913507) *[Homo sapiens]*

ATTTGTGATTTTGGTCTAGCCAGAG[A/T]CATCAAGAATGATTCTAATTATGTG

Chromosome: 4:54733155

Gene: KIT

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

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|  | Forward Primer | Reverse Primer |
| Sequence  | GATTTTGGTCTAGCCAGAGA | TGTTCAGCATACCATGCAAAT |
| Length | 20 bp | 21 bp |
| Start | 535 | 759 |
| Tm | 54.6 °C | 59.1 °C |
| GC | 45.0 % | 38.1 % |
| Tm | 51.86 °C | 56.57 °C |
| GC% | 45.0 | 38.1 |
| Self-Dimer ( ΔG) | -4.17 kcal/mol | -8.05 kcal/mol |
| Hairpin ( ΔG) | -0.62 kcal/mol | -1.37 kcal/mol |
| Cross Dimer (ΔG) | -6.84 kcal/mol |
| Product size | 225 bp |

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| 2. Allele specific primer design on mutant nucleotide of KIT gene |  |  |  |  |  |

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|  | Forward Primer | Reverse Primer |
| Sequence  | GATTTTGGTCTAGCCAGAGT | TGTTCAGCATACCATGCAAAT |
| Length | 20 bp | 21bp |
| Start | 535 | 759 |
| Tm | 53.6 °C | 59.1 °C |
| GC | 45.0 % | 38.1 % |
| Tm | 50.99 °C | 56.57 °C |
| GC% | 45.0 | 38.1 |
| Self-Dimer ( ΔG) | -4.17kcal/mol | -8.05kcal/mol |
| Hairpin ( ΔG) | -0.62kcal/mol | -1.37kcal/mol |
| Cross Dimer (ΔG) | -6.84kcal/mol |
| Product size | 225 bp |

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|  Left Primer 3:      |
| Sequence: |  |
| Start:   535 | Length:   20 bp | Tm:   54.6 °C  | GC:   45.0 % | ANY:   6.0 | SELF:   4.0 |
|  |
|  Right Primer 3:      |
| Sequence: |  |
| Start:   759 | Length:   21 bp | Tm:   59.1 °C  | GC:   38.1 % | ANY:   5.0 | SELF:   2.0 |
|  |
| Product Size:   225 bp | Pair Any: 4.0 | Pair End: 1.0 |  |  |

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| **Analysis Results #1: GATTTTGGTCTAGCCAGAGA** |
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| Rating | :  | 91.0 |   |
| Molecular Wt | :  | 6172.1 |   |
| Tm | :  | 51.86 | °C |
| GC% | :  | 45.0 |   |
| GC Clamp | :  | 1 |   |
| nmol/A260 | :  | 5.02 |   |
| ug/A260 | :  | 31.0 |   |
| ΔG | :  | -30.99 | kcal/mol |

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| 3' end stability | :  | -6.35 | kcal/mol |
| ΔH | :  | -146.7 | kcal/mol |
| ΔS | :  | -0.39 | kcal/°K/mol |
| 5' end ΔG | :  | -6.94 | kcal/mol |
| Self Dimer ( ΔG) | :  | [-4.17](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol |
| Hairpin ( ΔG) | :  | [-0.62](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | 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: TGTTCAGCATACCATGCAAAT** |
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| Rating | :  | 83.0 |   |
| Molecular Wt | :  | 6389.27 |   |
| Tm | :  | 56.57 | °C |
| GC% | :  | 38.1 |   |
| GC Clamp | :  | 1 |   |
| nmol/A260 | :  | 4.89 |   |
| ug/A260 | :  | 31.27 |   |
| ΔG | :  | -33.64 | kcal/mol |

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| 3' end stability | :  | -7.32 | kcal/mol |
| ΔH | :  | -153.5 | kcal/mol |
| ΔS | :  | -0.4 | kcal/°K/mol |
| 5' end ΔG | :  | -6.82 | kcal/mol |
| Self Dimer ( ΔG) | :  | [-8.05](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol |
| Hairpin ( ΔG) | :  | [-1.37](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | 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) | :  | [-6.84](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol |

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| Pair 2: |  |  |  |  |  |
|  Left Primer 2:      |
| Sequence: |  |
| Start:   535 | Length:   20 bp | Tm:   53.6 °C  | GC:   45.0 % | ANY:   6.0 | SELF:   6.0 |
|  |
|  Right Primer 2:      |
| Sequence: |  |
| Start:   759 | Length:   21 bp | Tm:   59.1 °C  | GC:   38.1 % | ANY:   5.0 | SELF:   2.0 |
|  |
| Product Size:   225 bp | Pair Any: 4.0 | Pair End: 1.0 |  |  |

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| **Analysis Results #1: GATTTTGGTCTAGCCAGAGT** |
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| Rating | :  | 91.0 |   |
| Molecular Wt | :  | 6163.09 |   |
| Tm | :  | 50.99 | °C |
| GC% | :  | 45.0 |   |
| GC Clamp | :  | 1 |   |
| nmol/A260 | :  | 5.16 |   |
| ug/A260 | :  | 31.78 |   |
| ΔG | :  | -30.76 | kcal/mol |

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| 3' end stability | :  | -6.11 | kcal/mol |
| ΔH | :  | -147.6 | kcal/mol |
| ΔS | :  | -0.39 | kcal/°K/mol |
| 5' end ΔG | :  | -6.94 | kcal/mol |
| Self Dimer ( ΔG) | :  | [-4.17](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol |
| Hairpin ( ΔG) | :  | [-0.62](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | 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: TGTTCAGCATACCATGCAAAT** |
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| --- | --- | --- | --- |
| Rating | :  | 83.0 |   |
| Molecular Wt | :  | 6389.27 |   |
| Tm | :  | 56.57 | °C |
| GC% | :  | 38.1 |   |
| GC Clamp | :  | 1 |   |
| nmol/A260 | :  | 4.89 |   |
| ug/A260 | :  | 31.27 |   |
| ΔG | :  | -33.64 | kcal/mol |

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| --- | --- | --- | --- |
| 3' end stability | :  | -7.32 | kcal/mol |
| ΔH | :  | -153.5 | kcal/mol |
| ΔS | :  | -0.4 | kcal/°K/mol |
| 5' end ΔG | :  | -6.82 | kcal/mol |
| Self Dimer ( ΔG) | :  | [-8.05](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol |
| Hairpin ( ΔG) | :  | [-1.37](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | 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) | :  | [-6.84](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol |

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