[rs41416044](https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=41416044) *[Homo sapiens]*

GCCACTACAAAGTCCTTGAAGGCAT[A/C/T]AATGGATCTATTAAAAATTCTAAAG

Chromosome: 12:88516409

Gene: KITLG

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

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|  | Forward Primer | Reverse Primer |
| Sequence | ACAAAGTCCTTGAAGGCATC | CAGCCATCTAAAAGTGCTAGTTGT |
| Length | 20 bp | 24 bp |
| Start | 497 | 729 |
| Tm | 56.8 °C | 59.1 °C |
| GC | 45.0 % | 41.7 % |
| Tm | 54.12 °C | 58.12 °C |
| GC% | 45.0 | 41.67 |
| Self-Dimer ( ΔG) | -6.61  kcal/mol | -4.16  kcal/mol |
| Hairpin ( ΔG) |  | -0.24  kcal/mol |
| Cross Dimer (ΔG) | -6.24  kcal/mol | |
| Product size | 233 bp | |

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

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|  | Forward Primer | Reverse Primer |
| Sequence | ACAAAGTCCTTGAAGGCATA | CAGCCATCTAAAAGTGCTAGTTGT |
| Length | 20 bp | 24bp |
| Start | 497 | 729 |
| Tm | 55.0 °C | 59.1 °C |
| GC | 40.0 % | 41.7 % |
| Tm | 52.41 °C | 58.12 °C |
| GC% | 40.0 | 41.67 |
| Self-Dimer ( ΔG) | -6.61  kcal/mol | -4.16  kcal/mol |
| Hairpin ( ΔG) |  | -0.24  kcal/mol |
| Cross Dimer (ΔG) | -6.24  kcal/mol | |
| Product size | 233 bp | |

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|  | Forward Primer | Reverse Primer |
| Sequence | ACAAAGTCCTTGAAGGCATT | CAGCCATCTAAAAGTGCTAGTTGT |
| Length | 20 bp | 24 bp |
| Start | 497 | 729 |
| Tm | 56.4 °C | 59.1 °C |
| GC | 40.0 % | 41.7 % |
| Tm | 53.97 °C | 58.12 °C |
| GC% | 40.0 | 41.67 |
| Self-Dimer ( ΔG) | -6.61  kcal/mol | -4.16  kcal/mol |
| Hairpin ( ΔG) |  | -0.24  kcal/mol |
| Cross Dimer (ΔG) | -6.24  kcal/mol | |
| Product size | 233 bp | |

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| Pair 2: |  |  |  |  |  |
| Left Primer 2: | | | | | |
| Sequence: |  | | | | |
| Start:   497 | Length:   20 bp | Tm:   56.8 °C | GC:   45.0 % | ANY:   7.0 | SELF:   3.0 |
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| Right Primer 2: | | | | | |
| Sequence: |  | | | | |
| Start:   729 | Length:   24 bp | Tm:   59.1 °C | GC:   41.7 % | ANY:   5.0 | SELF:   3.0 |
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| Product Size:   233 bp | | Pair Any: 4.0 | Pair End: 1.0 |  |  |

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| **Analysis Results #1: ACAAAGTCCTTGAAGGCATC** | |
| |  |  |  |  | | --- | --- | --- | --- | | Rating | : | 88.0 |  | | Molecular Wt | : | 6110.08 |  | | Tm | : | 54.12 | °C | | GC% | : | 45.0 |  | | GC Clamp | : | 2 |  | | nmol/A260 | : | 5.05 |  | | ug/A260 | : | 30.83 |  | | ΔG | : | -32.09 | kcal/mol | | |  |  |  |  | | --- | --- | --- | --- | | 3' end stability | : | -8.14 | kcal/mol | | ΔH | : | -148.7 | kcal/mol | | ΔS | : | -0.39 | kcal/°K/mol | | 5' end ΔG | : | -7.18 | kcal/mol | | Self Dimer ( ΔG) | : | [-6.61](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | 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: CAGCCATCTAAAAGTGCTAGTTGT** | |
| |  |  |  |  | | --- | --- | --- | --- | | Rating | : | 92.0 |  | | Molecular Wt | : | 7351.89 |  | | Tm | : | 58.12 | °C | | GC% | : | 41.67 |  | | GC Clamp | : | 1 |  | | nmol/A260 | : | 4.29 |  | | ug/A260 | : | 31.51 |  | | ΔG | : | -37.43 | kcal/mol | | |  |  |  |  | | --- | --- | --- | --- | | 3' end stability | : | -6.58 | kcal/mol | | ΔH | : | -177.5 | kcal/mol | | ΔS | : | -0.47 | kcal/°K/mol | | 5' end ΔG | : | -9.76 | kcal/mol | | Self Dimer ( ΔG) | : | [-4.16](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | | Hairpin ( ΔG) | : | [-0.24](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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| |  |  |  |  | | --- | --- | --- | --- | | Cross Dimer (ΔG) | : | [-6.24](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | |

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| Pair 2: |  |  |  |  |  |
| Left Primer 2: | | | | | |
| Sequence: |  | | | | |
| Start:   497 | Length:   20 bp | Tm:   55.0 °C | GC:   40.0 % | ANY:   7.0 | SELF:   3.0 |
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| Right Primer 2: | | | | | |
| Sequence: |  | | | | |
| Start:   729 | Length:   24 bp | Tm:   59.1 °C | GC:   41.7 % | ANY:   5.0 | SELF:   3.0 |
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| Product Size:   233 bp | | Pair Any: 4.0 | Pair End: 1.0 |  |  |

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| **Analysis Results #1: ACAAAGTCCTTGAAGGCATA** | |
| |  |  |  |  | | --- | --- | --- | --- | | Rating | : | 88.0 |  | | Molecular Wt | : | 6134.1 |  | | Tm | : | 52.41 | °C | | GC% | : | 40.0 |  | | GC Clamp | : | 2 |  | | nmol/A260 | : | 4.87 |  | | ug/A260 | : | 29.86 |  | | ΔG | : | -31.48 | kcal/mol | | |  |  |  |  | | --- | --- | --- | --- | | 3' end stability | : | -7.53 | kcal/mol | | ΔH | : | -149.1 | kcal/mol | | ΔS | : | -0.39 | kcal/°K/mol | | 5' end ΔG | : | -7.18 | kcal/mol | | Self Dimer ( ΔG) | : | [-6.61](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | 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: CAGCCATCTAAAAGTGCTAGTTGT** | |
| |  |  |  |  | | --- | --- | --- | --- | | Rating | : | 92.0 |  | | Molecular Wt | : | 7351.89 |  | | Tm | : | 58.12 | °C | | GC% | : | 41.67 |  | | GC Clamp | : | 1 |  | | nmol/A260 | : | 4.29 |  | | ug/A260 | : | 31.51 |  | | ΔG | : | -37.43 | kcal/mol | | |  |  |  |  | | --- | --- | --- | --- | | 3' end stability | : | -6.58 | kcal/mol | | ΔH | : | -177.5 | kcal/mol | | ΔS | : | -0.47 | kcal/°K/mol | | 5' end ΔG | : | -9.76 | kcal/mol | | Self Dimer ( ΔG) | : | [-4.16](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | | Hairpin ( ΔG) | : | [-0.24](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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| |  |  |  |  | | --- | --- | --- | --- | | Cross Dimer (ΔG) | : | [-6.24](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | |

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| Pair 2: |  |  |  | |  |  |
| Left Primer 2: | | | | | | |
| Sequence: |  | | | | | |
| Start:   497 | Length:   20 bp | Tm:   56.4 °C | GC:   40.0 % | | ANY:   7.0 | SELF:   7.0 |
|  | | | | | | |
| Right Primer 2: | | | | | | |
| Sequence: |  | | | | | |
| Start:   729 | Length:   24 bp | Tm:   59.1 °C | GC:   41.7 % | | ANY:   5.0 | SELF:   3.0 |
|  | | | | | | |
| Product Size:   233 bp | | Pair Any: 4.0 | Pair End: 0.0 | |  |  |
| **Analysis Results #1: ACAAAGTCCTTGAAGGCATT** | | | | | | | |
| |  |  |  |  | | --- | --- | --- | --- | | Rating | : | 88.0 |  | | Molecular Wt | : | 6125.09 |  | | Tm | : | 53.97 | °C | | GC% | : | 40.0 |  | | GC Clamp | : | 2 |  | | nmol/A260 | : | 5.03 |  | | ug/A260 | : | 30.81 |  | | ΔG | : | -32.46 | kcal/mol | | | | | |  |  |  |  | | --- | --- | --- | --- | | 3' end stability | : | -8.51 | kcal/mol | | ΔH | : | -152.2 | kcal/mol | | ΔS | : | -0.4 | kcal/°K/mol | | 5' end ΔG | : | -7.18 | kcal/mol | | Self Dimer ( ΔG) | : | [-6.61](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | 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: CAGCCATCTAAAAGTGCTAGTTGT** | |
| |  |  |  |  | | --- | --- | --- | --- | | Rating | : | 92.0 |  | | Molecular Wt | : | 7351.89 |  | | Tm | : | 58.12 | °C | | GC% | : | 41.67 |  | | GC Clamp | : | 1 |  | | nmol/A260 | : | 4.29 |  | | ug/A260 | : | 31.51 |  | | ΔG | : | -37.43 | kcal/mol | | |  |  |  |  | | --- | --- | --- | --- | | 3' end stability | : | -6.58 | kcal/mol | | ΔH | : | -177.5 | kcal/mol | | ΔS | : | -0.47 | kcal/°K/mol | | 5' end ΔG | : | -9.76 | kcal/mol | | Self Dimer ( ΔG) | : | [-4.16](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | | Hairpin ( ΔG) | : | [-0.24](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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| |  |  |  |  | | --- | --- | --- | --- | | Cross Dimer (ΔG) | : | [-6.24](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol | |