[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) | -6.61kcal/mol | -4.16kcal/mol |
| Hairpin ( ΔG) |  | -0.24kcal/mol |
| Cross Dimer (ΔG) | -6.24kcal/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) | -6.61kcal/mol | -4.16kcal/mol |
| Hairpin ( ΔG) |  | -0.24kcal/mol |
| Cross Dimer (ΔG) | -6.24kcal/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) | -6.61kcal/mol | -4.16kcal/mol |
| Hairpin ( ΔG) |  | -0.24kcal/mol |
| Cross Dimer (ΔG) | -6.24kcal/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 |
|  |
|  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: 1.0 |  |  |

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

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| 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** |
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| 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 |

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| --- | --- | --- | --- |
| 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 |
|  |
|  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: 1.0 |  |  |

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

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

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

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

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