1. Primer design for selected nsSNP of CTNNB1 gene

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| Primer Criteria | Forward Primer | Reverse Primer |
| Sequence  | AAGCGGCTGTTAGTCACTGG | AAAATCCCTGTTCCCACTCA |
| Length | 20 bp | 20 bp |
| Start | 507 | 662 |
| Tm | 60.5 °C | 59.4 °C |
| GC | 55.0 % | 45.0 % |
| Tm | 57.91 °C | 56.74 °C |
| GC% | 55.0 | 45.0 |
| Self-Dimer ( ΔG) | -3.94 kcal/mol |  |
| Hairpin ( ΔG) |  |  |
| Cross Dimer (ΔG) | -6.02 kcal/mol |
| Product size | 156 bp |

2. Restriction enzyme for selected nsSNP of CTNNB1gene

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| Enzyme Name | Position | Recognition Site |
| TfiI | 200 255 397 552 | G/AWTC  |

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| **TfiI** |

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| Help |

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| Comments |

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| [[Back to main display](http://nc2.neb.com/NEBcutter2/cutshow.php?name=53b2da32-)]  |

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| **5'...** |  |  |  | **Ghttp://nc2.neb.com/NEBcutter2/cut5.gifA W Thttp://nc2.neb.com/NEBcutter2/pix8.gifC** |  |  |  | **... 3'** |
| **3'...** |  |  |  | **Chttp://nc2.neb.com/NEBcutter2/pix8.gifT W Ahttp://nc2.neb.com/NEBcutter2/cut3.gifG** |  |  |  | **... 5'** |

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Enzyme No. Positions Recognition
name cuts of sites sequence
TfiI 4 200 255 397 552 g/awtc

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| Pair 3: |  |  |  |  |  |
|  Left Primer 3:      |
| Sequence: |  |
| Start:   507 | Length:   20 bp | Tm:   60.5 °C  | GC:   55.0 % | ANY:   5.0 | SELF:   1.0 |
|  |
|  Right Primer 3:      |
| Sequence: |  |
| Start:   662 | Length:   20 bp | Tm:   59.4 °C  | GC:   45.0 % | ANY:   2.0 | SELF:   1.0 |
|  |
| Product Size:   156 bp | Pair Any: 3.0 | Pair End: 3.0 |  |  |
| **Analysis Results #1: AAGCGGCTGTTAGTCACTGG** |
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| --- | --- | --- | --- |
| Rating | :  | 92.0 |   |
| Molecular Wt | :  | 6173.09 |   |
| Tm | :  | 57.91 | °C |
| GC% | :  | 55.0 |   |
| GC Clamp | :  | 2 |   |
| nmol/A260 | :  | 5.19 |   |
| ug/A260 | :  | 32.03 |   |
| ΔG | :  | -34.23 | kcal/mol |

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| 3' end stability | :  | -7.96 | kcal/mol |
| ΔH | :  | -154.0 | kcal/mol |
| ΔS | :  | -0.4 | kcal/°K/mol |
| 5' end ΔG | :  | -10.29 | kcal/mol |
| Self Dimer ( ΔG) | :  | [-3.94](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: AAAATCCCTGTTCCCACTCA** |
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| Rating | :  | 100.0 |   |
| Molecular Wt | :  | 5981.01 |   |
| Tm | :  | 56.74 | °C |
| GC% | :  | 45.0 |   |
| GC Clamp | :  | 1 |   |
| nmol/A260 | :  | 5.38 |   |
| ug/A260 | :  | 32.16 |   |
| ΔG | :  | -33.49 | kcal/mol |

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| 3' end stability | :  | -6.47 | kcal/mol |
| ΔH | :  | -151.8 | kcal/mol |
| ΔS | :  | -0.4 | kcal/°K/mol |
| 5' end ΔG | :  | -7.31 | kcal/mol |
| Self Dimer ( ΔG) | :  |  | kcal/mol |
| Hairpin ( ΔG) | :  |  | 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) | :  | [-6.02](http://www.premierbiosoft.com/NetPrimer/www.premierbiosoft.com) | kcal/mol |

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