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** | |  | | --- | | [Help](javascript:void(0)) | |  | |  | | --- | | [Comments](javascript:void(0)) | |

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

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