1. Primer design for selected nsSNP of ABCB1 gene

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| --- | --- | --- |
| Primer Criteria | Forward Primer | Reverse Primer |
| Sequence | CCCAGTTCAGACACAAGCAC | TCCAGTTTCCTTTTGGAGGA |
| Length | 20 bp | 20 bp |
| Start | 440 | 655 |
| Tm | 59.3 °C | 59.6 °C |
| GC | 55.0 % | 45.0 % |
| Tm | 55.79 °C | 57.11 °C |
| GC% | 55.0 | 45.0 |
| Self-Dimer ( ΔG) |  | -7.6 kcal/mol |
| Hairpin ( ΔG) |  | -3.4 kcal/mol |
| Cross Dimer (ΔG) |  | |
| Product size | 216 bp | |

2. Restriction enzyme for selected nsSNP of ABCB1 gene

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| Enzyme Name | Position | Recognition Site |
| MseI | 85 649 732 836 912 1052 1079 1088 | T/TAA |