**Supplementary Material 3.**

The sequence of primers used for TILLING screening of the *HvDMC1* gene fragment:

HvDMC1.F: 5’- AGGTCAGGGAAGACCCAGTT-3’

HvDMC1.R: 5’- TGCTGCAGTCACAGAACACA-3’

Optimized PCR mix:

|  |  |
| --- | --- |
| * ddH2O | * 13,5 μl |
| * Buffer B for ColorTaq polymerase (Eurx) | * 2,0 μl |
| * dNTPs (5 mM) (Promega) | * 1,0 μl |
| * Primer HvDMC1.F\* (10 pmol/μl) | * 0,5 μl |
| * Primer HvDMC1.R\*\* (10 pmol/μl) | * 0,5 μl |
| * ColorTaq polymerase (Eurx) | * 0,5 μl |
| * DNA (100ng/µl) | * 2,0 μl |

\*mixture of IRDye-700 labeled and unlabeled primers in 3:2 ratio

\*\* mixture of IRDye-800 labeled and unlabeled primers in 4:1 ratio

Temperature profile of PCR reaction:

|  |  |  |
| --- | --- | --- |
| * 1. Initial denaturation | * 94ºC | * 5 min |
| * 2. Denaturation | * 94ºC | * 45 sec   x2 |
| * 3. Annealing | * 71ºC | * 45 sec |
| * 4. Elongation | * 72ºC | * 1 min |
| * 5. Denaturation | * 94ºC | * 45 sec   x2 |
| * 6. Annealing | * 70ºC | * 45 sec |
| * 7. Elongation | * 72ºC | * 1 min |
| * 8. Denaturation | * 94ºC | * 45 sec |
| * 9. Annealing | * 69ºC | * 45 sec |
| * 10. Elongation | * 72ºC | * 1 min |
| * 11. Denaturation | * 94ºC | * 45 sec |
| * 12. Annealing | * 68ºC | * 45 sec |
| * 13. Elongation | * 72ºC | * 1 min |
| * 14. Denaturation | * 94ºC | * 45 sec |
| * 15. Annealing | * 67ºC | * 45 sec |
| * 16. Elongation | * 72ºC | * 1 min |
| * 17. Denaturation | * 94ºC | * 45 sec |
| * 18. Annealing | * 66ºC | * 45 sec |
| * 19. Elongation | * 72ºC | * 1 min |
| * 20. Denaturation | * 94ºC | * 45 sec   x29 |
| * 21. Annealing | * 65ºC | * 45 sec |
| * 22. Elongation | * 72ºC | * 1 min |
| * 23. Final elongation | * 72ºC | * 3 min |
| * 24. Pause | * 8ºC | * ∞ |