**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
 | * ∞
 |