

The bHLH transcription factor VfTT8 underlies *zt2*, the locus determining zero tannin content in faba bean (*Vicia faba* L.)

Natalia Gutierrez^{1*}, CM Avila¹, Ana M. Torres¹

¹ Área de Genómica y Biotecnología, IFAPA-Centro Alameda del Obispo, Apdo 3092, E-14080 Córdoba, Spain.

*Corresponding author e-mail: natalia.gutierrez.leiva@juntadeandalucia.es

Supplementary Table S1. Molecular markers surveyed in faba bean, derived from the syntenic *zt2* region (Medtr1g040675-Medtr1g087900). The table shows the name given to each marker, the corresponding Medicago locus in NCBI, the gene description, the orthologous locus in faba bean (after BLASTing against the in-house faba bean transcriptome), the genotyping technique used and the result after sequence analysis and genotyping in three segregating populations.

| Name | Medicago locus NCBI | Gene description | BLASTn_VF_Transcriptome (Ocaña et al. 2016) | Genotyping technique | Sequence analysis | Markers mapped in M x D* | Markers mapped in W x D* | Markers mapped in 6 x ZT2* |
|----------------------------------|---------------------|--|---|----------------------|--------------------------------|--------------------------|--------------------------|----------------------------|
| PISGEN_8_1_1* | MTR_1g040675 | Auxin response factor | - | ER digestion | Monomorphic | - | - | - |
| Vf_Mt1g043080^b | MTR_1g043080 | MYB relation transcription (MYB) | Contig15498 | ER digestion | ER not available | - | - | - |
| Vf_Mt1g044570 ^c | MTR_1g044570 | CAX-interacting protein | - | KASPAR assay | Polymorphic | - | - | X |
| Vf_Mt1g045610^b | MTR_1g045610 | R2R3-MYB Transcription factor | No hits found | ALP | Polymorphic | X | X | X |
| Vf_Mt1g045800 ^c | MTR_1g045800 | G3BP-like protein | - | KASPAR assay | Monomorphic | X | - | - |
| mtml_GEN_01090_01_1 | MTR_1g050520 | Protein kinase and PP2C-like domain-containing protein | - | ER digestion | Monomorphic | - | - | - |
| Vf_Mt1g050730 ^c | MTR_1g050730 | Pre-mRNA-processing ATP-dependent RNA helicase prp5 | - | KASPAR assay | Polymorphic | - | X | - |
| Vf_Mt1g056180 ^c | MTR_1g056180 | uncharacterized LOC11431185 | - | KASPAR assay | Polymorphic | X | - | - |
| cgP137FSNP ^c | MTR_1g056180 | uncharacterized LOC11431185 | - | KASPAR assay | Polymorphic | - | - | X |
| Vf_Mt1g061800 ^c | MTR_1g061800 | Threonine dehydratase biosynthetic, chloroplastic | - | KASPAR assay | Monomorphic | - | - | - |
| Vf_Mt1g064060 ^c | MTR_1g064060 | Adenosine kinase 2 | - | KASPAR assay | Monomorphic | - | - | - |
| PTSS4 ^c | MTR_1g069105 | 26S proteasome regulatory subunit 4 homolog A | - | ER digestion | Polymorphic | - | - | X (MaI) |
| Vf_Mt1g070180^b | MTR_1g070180 | bHLH transcription factor | Lucus_34012_Transcript_1/1_Confidence_1_000_Length_809 | ER digestion | Monomorphic | - | - | - |
| Vf_Mt1g070455^b | MTR_1g070455 | WRKY family transcription factor | Lucus_48067_Transcript_1/1_Confidence_1_000_Length_887 | ER digestion | Monomorphic | - | - | - |
| Vf_Mt1g070870^b | MTR_1g070870 | bHLH transcription factor | Contig4759 | ER digestion | Monomorphic | - | - | - |
| Vf_Mt1g071110 ^c | MTR_1g071110 | 2-methyl-6-phytyl-1,4-hydroquinone methyltransferase, | - | KASPAR assay | Polymorphic | - | - | X |
| GLIP245SNP ^c | MTR_1g071430 | Protein LIFEGUARD 4 | - | KASPAR assay | Polymorphic | X | X | X |
| PISGEN_25-2-3-1* | MTR_1g071610 | Acetyl-CoA carboxylase, putative | - | ER digestion | Monomorphic | - | - | - |
| Vf_Mt1g072130^b | MTR_1g072130 | PHDfingerprotein, putative | Lucus_31961 | ER digestion | Monomorphic | - | - | - |
| Vf_Mt1g072140^b | MTR_1g072140 | Transducin/WD40 repeat protein | No hits found | ER digestion | Polymorphic | X (SgrAI) | - | X (PfeI) |
| Vf_Mt1g072320^b | MTR_1g072320 | Transcription factor bHLH (TT18) | No hits found | ER digestion | Polymorphic | X (Eco130I/TasI) | X (DraI) | X (DraI/RsaI) |
| Vf_Mt1g072530^b | MTR_1g072530 | Transcription factor bHLH66 | Lucus_21144 | ER digestion | Polymorphic | X (NdeI) | X (SmaI) | X (Tetra-arm) |
| Vf_Mt1g072640 ^c | MTR_1g072640 | Protein PALECRESS, chloroplastic | - | KASPAR assay | Monomorphic | - | - | - |
| Vf_Mt1g072740 ^c | MTR_1g072740 | Pre-mRNA-processing factor 17 | - | KASPAR assay | Polymorphic | X | - | - |
| Vf_Mt1g073000 ^c | MTR_1g073000 | Polyadenylate-binding protein 1 | - | KASPAR assay | Monomorphic | - | - | - |
| Vf_Mt1g073170^b | MTR_1g073170 | MYB related transcription factor | Lucus_29372 | ER digestion | Monomorphic | - | - | - |
| Vf_Mt1g073920^b | MTR_1g073920 | Actin related rprotein ARP4 | Contig21805 | ER digestion | ER not available | - | - | - |
| Vf_Mt1g074370^b | MTR_1g074370 | Ethylene-responsive transcription factor | Contig5707 | ER digestion | Unreliable data for genotyping | - | - | - |
| Vf_Mt1g075140 ^c | MTR_1g075140 | DNA-directed RNA polymerase II subunit RPB7 | - | KASPAR assay | Monomorphic | - | - | - |
| Vf_Mt1g075320^b | MTR_1g075320 | N-carbamyl-L-amino acid amidohydrolase | Lucus_2084_Transcript 2180 | ER digestion | Monomorphic | - | - | - |
| Vf_Mt1g075610 ^c | MTR_1g075610 | Cell division control protein 2 homolog D | - | KASPAR assay | Monomorphic | - | - | - |
| Vf_Mt1g075740^b | MTR_1g075740 | COPI-interacting-like protein | Contig16561 | ER digestion | ER not available | - | - | - |
| Vf_Mt1g076150^b | MTR_1g076150 | MYB related transcription factor | No hits found | ER digestion | Monomorphic | - | - | - |
| Vf_Mt1g076600^b | MTR_1g076600 | Cyclin-D-binding Myb-like transcription factor | Lucus_12353_Transcript_2/3_Confidence_0_600_Length_1242 | ER digestion | Monomorphic | - | - | - |
| Vf_Mt1g076780^b | MTR_1g076780 | WD40 repeat protein | Contig15218 | ER digestion | Unreliable data for genotyping | - | - | - |
| Vf_Mt1g077640^b | MTR_1g077640 | bHLH transcription factor | No hits found | ER digestion | Polymorphic | - | - | X (BamHI) |
| GLP5NP ^c | MTR_1g079490 | Germine-like protein subfamily 1 member 16 | - | KASPAR assay | Polymorphic | X | X | X |
| Vf_Mt1g079520 ^c | MTR_1g079520 | LRR receptor-like kinase | - | KASPAR assay | Monomorphic | - | - | - |
| Vf_Mt1g079870 ^c | MTR_1g079870 | Hypothetical protein | - | KASPAR assay | Polymorphic | X | - | - |
| Vf_Mt1g079930 ^c | MTR_1g079930 | Activating signal cointegrator | - | KASPAR assay | Monomorphic | - | - | - |
| Vf_Mt1g080150 ^c | MTR_1g080150 | RNA-binding protein 42 | - | KASPAR assay | Monomorphic | - | - | - |
| Vf_Mt1g081290 ^c | MTR_1g081290 | Zeta-carotene desaturase | - | KASPAR assay | Monomorphic | - | - | - |
| Vf_Mt1g082210 ^c | MTR_1g082210 | Gibberellin receptor GID1c-like protein | - | KASPAR assay | Polymorphic | - | X | X |
| Vf_Mt1g082420 ^c | MTR_1g082420 | Thylakoid lumen 18.3 kDa protein | - | KASPAR assay | Polymorphic | X | - | - |
| Vf_Mt1g082480^b | MTR_1g082480 | bHLH transcription factor | No hits found | ER digestion | Unreliable data for genotyping | - | - | - |
| Vf_Mt1g082580 ^c | MTR_1g082580 | Serine/Threonine kinase family protein | - | KASPAR assay | Polymorphic | - | - | X |
| Vf_Mt1g083230 ^c | MTR_1g083230 | WD repeat phosphoinositide-interacting-like protein | - | KASPAR assay | Monomorphic | - | - | - |
| Vf_Mt1g083450 ^c | MTR_1g083450 | Hypothetical protein | - | KASPAR assay | Monomorphic | - | - | - |
| Vf_Mt1g083460 ^c | MTR_1g083460 | 60S ribosomal protein L18-3 | - | KASPAR assay | Polymorphic | X | X | - |
| Vf_Mt1g083960 ^c | MTR_1g083960 | Calreticulin | - | KASPAR assay | Monomorphic | - | - | - |
| Vf_Mt1g084160^b | MTR_1g084160 | bHLH transcription factor | Contig23306 | ER digestion | Unreliable data for genotyping | - | - | - |
| Vf_Mt1g085040 ^c | MTR_1g085040 | MYB transcription factor | - | KASPAR assay | Polymorphic | X | X | - |
| Vf_Mt1g086180^b | MTR_1g086180 | MYB related transcription factor | Contig1005 | ER digestion | Unreliable data for genotyping | - | - | - |
| GLIP133SNP ^c | MTR_1g086340 | Polyketide cyclase/dehydrase and lipid transporter | - | KASPAR assay | Monomorphic | - | - | - |
| Vf_Mt1g086790^b | MTR_1g086790 | WRKY family transcription factor | Lucus_9490_Transcript_1/1_Confidence_1_000_Length_898 | ER digestion | ER not available | - | - | - |
| Vf_Mt1g086810 ^c | MTR_1g086810 | Heme-binding protein | - | KASPAR assay | Monomorphic | - | - | - |
| Vf_Mt1g087120^b | MTR_1g087120 | MYB related transcription factor | Lucus_40110_Transcript_1/1_Confidence_1_000_Length_274 | ER digestion | Unreliable data for genotyping | - | - | - |
| LG031SNP ^c | MTR_1g087760 | Kynurenine-oxoglutarate transaminase-like protein | - | KASPAR assay | Monomorphic | - | - | - |
| LG038SNP ^c | MTR_1g087900 | Fumarate hydratase | - | KASPAR assay | Polymorphic | X | - | - |
| Vf_Mt1g088190 ^c | MTR_1g088190 | Endoglucanase 17 | - | KASPAR assay | Polymorphic | X | X | - |
| Vf_Mt1g089980 ^c | MTR_1g089980 | No hit found | - | KASPAR assay | Polymorphic | X | - | - |
| Vf_Mt1g114560 ^c | MTR_1g114560 | Riboflavin kinase | - | KASPAR assay | Monomorphic | - | - | - |
| Vf_Mt1g116110 ^c | MTR_1g116110 | Prototheme IX farnesyltransferase | - | KASPAR assay | Polymorphic | - | X | - |
| Vf_Mt1g116230 ^c | MTR_1g116230 | Leukotriene A4 hydrolase-like protein | - | KASPAR assay | Monomorphic | - | - | - |
| Vf_Mt1g116810 ^c | MTR_1g116810 | Prolyl 4-hydroxylase alpha-like protein | - | KASPAR assay | Monomorphic | - | - | - |
| GLIP065 ^c | MTR_4g021350 | Medicago truncatula clone mth2-14c14 | - | ER digestion | Polymorphic | - | - | X (AclI) |
| Vf_Mt4g080370 ^c | MTR_4g080370 | No results NCBI | - | KASPAR assay | Polymorphic | X | X | - |
| Vf_Mt5g042440 ^c | MTR_5g042440 | LysM-domain receptor-like kinase | - | KASPAR assay | Polymorphic | X | - | - |
| GLIP651 ^c | MTR_5g085850 | NRT1/ PTR FAMILY | - | ER digestion | Monomorphic | - | - | - |
| Vf_Mt7g035110 ^c | MTR_7g035110 | RXT3-like protein | - | KASPAR assay | Polymorphic | X | - | - |
| Vf_Mt7g100500 ^c | MTR_7g100500 | Serine/threonine protein kinase PBS1 | - | KASPAR assay | Polymorphic | X | X | X |
| REP5NP ^c | MTR_8g068875 | Medicago truncatula protein RAE1 | - | KASPAR assay | Monomorphic | - | - | - |

*Restriction Enzymes used for genotyping are between brackets

^a EST (Ellwood et al. 2008; Satovic et al. 2013)

^b New gene-based markers developed in this study (in bold)

^c KASPar markers (Webb et al. 2016)

The bHLH transcription factor VfTT8 underlies *zt2*, the locus determining zero tannin content in faba bean (*Vicia faba* L.)

Natalia Gutierrez^{1*}, CM Avila¹, Ana M. Torres¹

¹ Área de Genómica y Biotecnología, IFAPA-Centro Alameda del Obispo, Apdo 3092, E-14080 Córdoba, Spain.

*Corresponding author e-mail: natalia.gutierrez.leiva@juntadeandalucia.es

Supplementary Table S2. Characteristics of the 23 *Medicago truncatula* markers associated with anthocyanin expression and designed in this study. The information about marker name and type, gene description, orthologous sequence in faba bean, primer sequence, annealing temperature, locus name and product size of the amplicon is shown.

| Marker name | Marker type | Medicago locus | Gene description | BLASTn_VF_Transcriptome (Ocaña et al. 2016) | Primer Sequence (5'-3') | Ta | PCR product size (bp) |
|---------------|-------------|----------------|--|---|---|------|-----------------------|
| Vf_Mt1g043080 | SNP | MTR_1g043080 | MYB relation transcription (MYB) | Contig15498 | TAGGGAACAGGTGGTCAGCA TGCACCCCTTCACTTGTGTA | 55°C | 667 |
| Vf_Mt1g045610 | ALP | MTR_1g045610 | R2R3-MYB Transcription factor | No hits found | TGGACTGCTGAAGAGGATAGGTT ATTATGCTTTCTCTTGTGGTGTTA | 57°C | 719 (Vf6)/ 574 (zt2) |
| Vf_Mt1g070180 | SNP | MTR_1g070180 | bHLH transcription factor | Locus_34012_Transcript_1/1_Confidence_1.000_Length_809 | CCAAAGCATAACTAAGCATGCTGAT TCCATTGCCCACTTGAACCC | 60°C | 201 |
| Vf_Mt1g070455 | SNP | MTR_1g070455 | WRKY family transcription factor | Locus_48067_Transcript_1/1_Confidence_1.000_Length_887 | CGTGGAGGAAGTACGGGCA CAAGCACCTCTCGACATGCTTC | 58°C | 550 |
| Vf_Mt1g070870 | SNP | MTR_1g070870 | bHLH transcription factor | Contig4759 | GAGCTGCCATGTTGGATGAATTG GGATATCCAGTTACCAGTGGGGC | 60°C | 336 |
| Vf_Mt1g072130 | SNP | MTR_1g072130 | PHDfingerprotein,putative | Locus_31961 | TTCTAGATCCAATGGCTGATGTT CCTTGTGTCATCGTCTCATTGTG | 54°C | 639 |
| Vf_Mt1g072140 | SNP | MTR_1g072140 | transducin/WD40 repeatprotein | No hits found | TGCGTCAAAATCTCCAACGA AGAGTAGCAACAGTATAAATTTCCC | 54°C | 554 |
| Vf_Mt1g072320 | SNP | MTR_1g072320 | Transcription factor TT8 | No hits found | GTTGCCAGGAAGGCATATGC CTTATCAGTTGTGCCAATCTCAACG | 57°C | 690 ⁽¹⁾ |
| Vf_Mt1g072530 | SNP | MTR_1g072530 | Transcription factor bHLH66 | Locus_21144 | AAGAAGACACACTACTCTCAAAC TCAATCTCCAACAAGCATCACTC | 57°C | 670 ⁽²⁾ |
| Vf_Mt1g072530 | SNP | MTR_1g072530 | Transcription factor bHLH66 | Locus_21144 | TGCAACATGGCAACTGATTCA ACTAGTGGTATAGCATTGAGTCA | 60°C | 615 |
| Vf_Mt1g073170 | SNP | MTR_1g073170 | MYB related transcription factor | Locus_29372 | CAGGACTGAGAAGAAATGGGAAAG CATCAAGCAACAGTCTATGATCCC | 58°C | 493 |
| Vf_Mt1g073920 | SNP | MTR_1g073920 | Actin related rprotein ARP4 | Contig21805 | GAGGGGCAACTTGTATGCTGT TCTTCAACTGCAGAACTAAACCA | 56°C | 800 |
| Vf_Mt1g074370 | SNP | MTR_1g074370 | Ethylene-responsive transcription factor | Contig5707 | GAATTCAACTATCTCCCCTTCAACG CGGAAGACATCAACAATTGTTCCA | 60°C | 568 |
| Vf_Mt1g075320 | SNP | MTR_1g075320 | N-carbamyl-L-amino acid amidohydrolase | Locus_2084_Transcript_2180 | CGCTTATTGGCTGGGAGTGA GCTAGGGATACTATTGATTGCTCCA | 60°C | 820 |
| Vf_Mt1g075740 | SNP | MTR_1g075740 | COP1-interacting-like protein | Contig16561 | GAGGTTGGATTCGGCTGTTTTTC CCACCC TTGTCATCTGATCTT | 56°C | 668 |
| Vf_Mt1g076150 | SNP | MTR_1g076150 | MYB related transcription factor | No hits found | GAGCTCCTTGTGTGAAAAGATGG CTGCAGCTTTTCCACACCTTAAC | 54°C | 320 |
| Vf_Mt1g076600 | SNP | MTR_1g076600 | cyclin-D-binding Myb-like transcription factor | Locus_12353_Transcript_2/3_Confidence_0.600_Length_1242 | GATGACTTCGCTTGATAGGTGC GCATCGGCATCCCACC | 60°C | 352 |
| Vf_Mt1g076780 | SNP | MTR_1g076780 | WD-40 repeat protein | Contig15218 | GGGGAACTTGCATTTGACATTG CCACATCTGTAGCCAGTATCCGAG | 64°C | 720 |
| Vf_Mt1g077640 | SNP | MTR_1g077640 | bHLH transcription factor | No hits found | GTGTAGCATCGATTCTTTGTAGAC AATTATGTCGACTATCTCAAGGAAGAA | 56°C | 477 |
| Vf_Mt1g082480 | SNP | MTR_1g082480 | bHLH transcription factor | No hits found | GAAATTGAGAATCAGAGAATGACTCAC GAATACTGTGAAATGTAAGAAGCTCA | 56°C | 503 |
| Vf_Mt1g084160 | SNP | MTR_1g084160 | bHLH transcription factor | Contig23306 | CTCTCTCTCTTCTTCTCTCTCA CGGATTAGATCTCCACCCG | 56°C | 584 |
| Vf_Mt1g086180 | SNP | MTR_1g086180 | MYB related transcription factor | Contig1005 | TCTCATGATTCATGTGGCCCTTCT CAAGCATAAGGATTACGCAAGGTCA | 56°C | 452 |
| Vf_Mt1g086790 | SNP | MTR_1g086790 | WRKY family transcription factor | Locus_9490_Transcript_1/1_Confidence_1.000_Length_898 | CAGAGAGAACCAGATTTCGCGTT CCCTAAGAAAGGAGTAGTTGTCCC | 56°C | 333 |
| Vf_Mt1g087120 | SNP | MTR_1g087120 | MYB related transcription factor | Locus_40110_Transcript_1/1_Confidence_1.000_Length_274 | GGCAATCTTGAAGTAGACCGAG GTTGGTTAAAGGGTGTCTTCATT | 58°C | 393 |

(1) Product size obtained with primer pair 1

(2) Product size obtained with primer pair 2

The bHLH transcription factor VfTT8 underlies *zt2*, the locus determining zero tannin content in faba bean (*Vicia faba* L.)

Natalia Gutierrez^{1*}, CM Avila¹, Ana M. Torres¹

¹ Área de Genómica y Biotecnología, IFAPA-Centro Alameda del Obispo, Apdo 3092, E-14080 Córdoba, Spain.

*Corresponding author e-mail: natalia.gutierrez.leiva@juntadeandalucia.es

Supplementary Table S3 Haplotype analysis of the markers flanking the gene *zt2* in three faba bean populations. The allele for each phenotype (flower color) and for each gene maker is specified. The grey color indicates the misclassified individuals.

| Population | Phenotype F3 family | Vf Mt1g072320 ⁽²⁾ | | GLIP245SNP | | Vf Mt1g072140 | | Vf Mt7g100500 | |
|----------------------------------|------------------------|------------------------------|----------|------------|-----------|---------------|-----|---------------|-----|
| | | | | | | | | | |
| MAYA_P1 | wild type (A) | T:T | T:T | T:T | T:T | C:C | C:C | C:C | C:C |
| DISCO_P2 | <i>zt2</i> (B) | C:C | C:C | C:C | C:C | A:A | A:A | T:T | T:T |
| 1 | A | T:T | T:T | T:T | T:T | C:C | C:C | C:C | C:C |
| 2 | A | T:C | T:C | T:C | T:C | C:A | C:A | T:C | T:C |
| 3 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:C | T:C |
| 4 | A | T:T | T:T | T:T | T:T | C:C | C:C | C:C | C:C |
| 5 | B | C:C | C:C | C:C | C:C | A:A | A:A | T:T | T:T |
| 6 | A | T:C | T:C | T:C | T:C | C:A | C:A | T:C | T:C |
| 7 | A | T:C | T:C | T:C | T:C | C:A | C:A | T:C | T:C |
| 8 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:T | T:T |
| 9 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:T | T:T |
| 10 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:C | T:C |
| 11 | A | T:T | T:T | T:T | T:T | C:C | C:C | C:C | C:C |
| 12 | B | C:C | C:C | C:C | C:C | A:A | A:A | T:T | T:T |
| 13 | B | C:C | C:C | C:C | C:C | A:A | A:A | T:T | T:T |
| 14 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:C | T:C |
| 15 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:C | T:C |
| 16 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:C | T:C |
| 17 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:C | T:C |
| 18 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:C | T:C |
| 19 | A | T:T | T:T | T:T | T:T | C:C | C:C | C:C | C:C |
| 20 | A | T:T | T:T | T:T | T:T | C:C | C:C | T:C | T:C |
| 21 | H | T:C | T:C | T:C | T:C | A:A | A:A | T:C | T:C |
| 22 | B | C:C | C:C | C:C | C:C | A:A | A:A | T:T | T:T |
| 23 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:C | T:C |
| 24 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:C | T:C |
| 25 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:C | T:C |
| 26 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:T | T:T |
| 27 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:C | T:C |
| 28 | H | T:C | T:C | T:C | T:C | C:A | C:A | C:C | C:C |
| 29 | B | C:C | C:C | C:C | C:C | C:A | C:A | T:T | T:T |
| 30 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:T | T:T |
| 31 | B | C:C | C:C | C:C | C:C | A:A | A:A | T:T | T:T |
| 32 | A | T:T | T:T | T:T | T:T | C:A | C:A | C:C | C:C |
| 33 | A | T:T | T:T | T:T | T:T | C:A | C:A | T:C | T:C |
| 34 | A | T:T | T:T | T:T | T:T | C:A | C:A | C:C | C:C |
| 35 | B | C:C | C:C | C:C | C:C | A:A | A:A | T:C | T:C |
| 36 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:C | T:C |
| 37 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:C | T:C |
| 38 | H | T:C | T:C | T:C | T:C | C:A | C:A | C:C | C:C |
| 39 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:T | T:T |
| 40 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:C | T:C |
| 41 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:T | T:T |
| 42 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:C | T:C |
| 43 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:T | T:T |
| 44 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:C | T:C |
| 45 | H | T:C | T:C | T:C | T:C | A:A | A:A | T:C | T:C |
| 46 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:C | T:C |
| 47 | A | T:T | T:T | T:T | T:T | C:C | C:C | T:T | T:T |
| 48 | H | T:C | T:C | T:C | T:C | C:A | C:A | T:C | T:C |
| 49 | B | C:C | C:C | C:C | C:C | A:A | A:A | T:T | T:T |
| 50 | H | T:C | T:C | T:C | T:C | C:A | C:A | C:C | C:C |
| Misclassified individual: | | 3 | 5 | 9 | 17 | | | | |

| Population | Phenotype F3 family | Vf Mt1g072320 ⁽¹⁾ | Vf Mt1g072530 | GLPSNP | Vf Mt7g100500 |
|----------------------------------|------------------------|------------------------------|---------------|----------|---------------|
| WIZARD_P1 | wild type (A) | A:A | A:A | G:G | C:C |
| DISCO_P2 | z12 (B) | G:G | C:C | C:C | T:T |
| 1 | A | A:A | A:A | G:G | C:C |
| 2 | H | G:A | C:A | G:C | T:C |
| 3 | H | G:A | C:A | G:C | T:C |
| 4 | H | G:A | C:A | G:C | T:C |
| 5 | A | A:A | A:A | G:G | C:C |
| 6 | H | G:A | C:A | G:C | T:C |
| 7 | B | G:G | C:A | C:C | T:T |
| 8 | A | A:A | C:A | G:G | T:C |
| 9 | A | A:A | A:A | G:G | C:C |
| 10 | A | A:A | A:A | G:C | T:C |
| 11 | B | G:G | C:C | G:C | T:C |
| 12 | H | G:A | C:A | G:C | T:C |
| 13 | H | G:A | C:A | C:C | T:C |
| 14 | H | G:A | C:A | G:C | T:C |
| 15 | A | A:A | A:A | G:G | C:C |
| 16 | B | G:G | C:C | C:C | T:T |
| 17 | H | G:A | C:A | G:C | C:C |
| 18 | H | G:A | C:A | G:C | T:C |
| 19 | A | G:A | C:A | C:C | C:C |
| 20 | A | A:A | A:A | G:C | C:C |
| 21 | B | G:G | C:C | G:C | T:T |
| 22 | B | G:G | C:C | C:C | T:T |
| 23 | B | G:G | C:C | C:C | T:T |
| 24 | H | G:A | C:A | G:C | T:C |
| 25 | A | A:A | A:A | G:G | C:C |
| 26 | A | A:A | A:A | G:G | C:C |
| 27 | H | G:A | C:A | G:C | T:C |
| 28 | B | G:G | C:C | C:C | T:T |
| 29 | H | G:A | C:A | G:C | T:C |
| 30 | H | G:A | C:A | G:C | T:T |
| 31 | H | G:A | C:A | G:C | T:C |
| 32 | H | G:A | C:A | G:C | T:C |
| 33 | A | A:A | A:A | G:C | C:C |
| 34 | H | G:A | C:A | G:C | T:C |
| 35 | A | G:A | C:A | G:C | T:T |
| 36 | H | G:A | C:A | G:C | T:C |
| 37 | B | G:G | C:C | C:C | T:T |
| 38 | A | A:A | A:A | G:G | C:C |
| 39 | H | G:A | C:A | G:C | T:C |
| 40 | A | A:A | A:A | G:C | T:C |
| 41 | H | G:A | C:A | G:C | T:C |
| 42 | H | G:A | C:A | G:C | T:C |
| 43 | B | G:G | C:C | C:C | T:T |
| 44 | B | G:G | C:C | C:C | T:T |
| 45 | H | G:A | C:A | G:C | T:C |
| 46 | A | A:A | A:A | G:G | T:C |
| 47 | H | G:A | ? | G:C | T:T |
| 48 | A | A:A | A:A | G:G | C:C |
| 49 | A | A:A | A:A | G:G | C:C |
| 50 | A | A:A | A:A | G:G | T:T |
| 51 | B | G:G | C:C | C:C | T:T |
| 52 | B | G:G | C:C | C:C | T:T |
| 53 | H | G:A | C:A | G:C | T:C |
| 54 | B | G:G | C:C | C:C | T:T |
| 55 | H | G:A | C:A | G:C | T:C |
| 56 | B | G:G | C:A | C:C | T:T |
| Misclassified individuals | | 2 | 6 | 9 | 10 |

| | Phenotype | Vf Mt1g072320 ⁽¹⁾ | Vf Mt1g072530 | Vf Mt1g077640 | Vf Mt7g100500 |
|----------------------------------|------------------------------|------------------------------|---------------|---------------|---------------|
| VF6_P1 | RIL individual wild type (A) | A:A | C:C | A:A | C:C |
| ZT2_P2 | zt2 (B) | G:G | G:G | G:G | T:T |
| 1 | A | A:A | C:C | A:A | C:C |
| 2 | B | G:G | G:G | G:G | T:T |
| 3 | A | A:A | C:C | A:A | C:C |
| 4 | A | A:A | C:C | A:A | T:T |
| 5 | B | G:G | G:G | G:G | T:T |
| 6 | A | A:A | C:C | A:A | C:C |
| 7 | B | G:G | G:G | A:A | T:T |
| 8 | B | G:G | G:G | G:G | T:T |
| 9 | A | A:A | C:C | A:A | C:C |
| 10 | A | A:A | C:C | G:G | C:C |
| 11 | A | A:A | C:C | A:A | C:C |
| 12 | A | A:A | C:C | A:A | C:C |
| 13 | B | G:G | G:G | G:G | C:C |
| 14 | A | A:A | G:C | G:G | T:T |
| 15 | A | A:A | C:C | A:A | T:T |
| 16 | A | A:A | C:C | A:A | C:C |
| 17 | B | G:G | G:G | G:G | T:T |
| 18 | A | A:A | C:C | A:A | C:C |
| 19 | A | A:A | C:C | A:A | C:C |
| 20 | B | G:G | G:G | A:A | T:T |
| 21 | A | A:A | C:C | G:G | C:C |
| 22 | B | G:G | G:G | G:G | C:C |
| 23 | B | G:G | ? | G:G | ? |
| 24 | A | A:A | C:C | A:A | T:T |
| 25 | A | G:A | G:C | A:A | C:C |
| 26 | A | G:A | C:C | G:G | C:C |
| 27 | B | G:G | G:G | G:G | T:T |
| 28 | B | G:G | G:G | A:A | T:T |
| 29 | A | A:A | C:C | A:A | C:C |
| 30 | A | A:A | C:C | A:A | C:C |
| 31 | A | A:A | C:C | A:A | T:C |
| 32 | A | A:A | C:C | A:A | T:T |
| 33 | B | G:G | G:G | G:G | T:T |
| 34 | A | A:A | C:C | A:A | C:C |
| 35 | A | A:A | C:C | A:A | C:C |
| 36 | A | A:A | C:C | A:A | C:C |
| 37 | A | A:A | C:C | A:A | C:C |
| 38 | A | A:A | C:C | A:A | T:C |
| 39 | A | A:A | G:C | A:A | T:C |
| 40 | B | G:G | G:G | G:G | T:C |
| 41 | B | G:G | G:G | G:G | C:C |
| 42 | B | G:G | G:G | G:G | T:T |
| 43 | A | A:A | C:C | A:A | C:C |
| 44 | A | A:A | G:C | A:A | T:C |
| 45 | A | A:A | C:C | A:A | C:C |
| 46 | A | A:A | C:C | A:A | C:C |
| 47 | A | A:A | C:C | A:A | C:C |
| 48 | B | G:G | G:G | G:G | C:C |
| 49 | B | G:G | G:G | G:G | C:C |
| 50 | B | G:G | G:G | A:A | C:C |
| 51 | A | A:A | C:C | A:A | C:C |
| 52 | B | G:G | G:G | G:G | T:T |
| 53 | A | A:A | C:C | G:G | T:C |
| 54 | B | G:G | G:G | G:G | T:T |
| 55 | A | A:A | C:C | A:A | C:C |
| 56 | B | G:G | G:G | G:G | T:T |
| 57 | B | G:G | G:G | G:G | T:T |
| 58 | B | G:G | G:G | G:G | T:C |
| 59 | B | G:G | G:G | G:G | T:T |
| 60 | B | G:G | G:G | G:G | T:T |
| 61 | A | A:A | C:C | A:A | T:C |
| 62 | B | G:G | G:G | G:G | T:T |
| Misclassified individuals | | 2 | 5 | 8 | 20 |