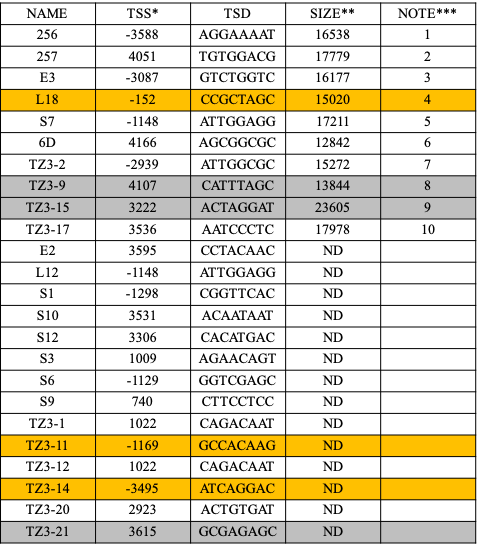
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| # | Name | Sector size | # | Name | Sector size |
| 1 | S1 | 1 kernel | 26 | TZ3-13 | 1/2 kernel |
| 2 | S3 | 1 kernel | 27 | TZ3-14 | 1/2 kernel |
| 3 | S6 | 1 kernel | 28 | TZ3-15 | 1/2 kernel |
| 4 | S7 | 1 kernel | 29 | TZ3-17 | 1/2 kernel |
| 5 | S9 | 1 kernel | 30 | TZ3-18 | 1/2 kernel |
| 6 | S10 | 1 kernel | 31 | TZ3-20 | 1/2 kernel |
| 7 | S11 | 1 kernel | 32 | TZ3-21 | 1/2 kernel |
| 8 | S12 | 1 kernel | 33 | TZ3-22 | 1/2 kernel |
| 9 | S30 | 1 kernel | 34 | TZ3-23 | 1/2 kernel |
| 10 | L12 | > 1/2 ear | 35 | TZ3-24 | 1/2 kernel |
| 11 | L18 | > 1/2 ear | 36 | TZ2-13 | 1 kernel |
| 12 | E2 | Whole ear | 37 | TZ2-19 | 1 kernel |
| 13 | E3 | Whole ear | 38 | TZ2-34 | 1 kernel |
| 14 | 251 | 1 kernel | 39 | 6D | 1 kernel |
| 15 | 256 | 1 kernel | 40 | SP1 | < 1/2 ear |
| 16 | 257 | 1 kernel | 41 | SP2 | < 1/2 ear |
| 17 | TZ3-1 | 1/2 kernel | 42 | SP3 | < 1/2 ear |
| 18 | TZ3-2 | 1/2 kernel | 43 | SP4 | < 1/2 ear |
| 19 | TZ3-3 | 1/2 kernel | 44 | SP5 | < 1/2 ear |
| 20 | TZ3-5 | 1/2 kernel | 45 | SP6 | < 1/2 ear |
| 21 | TZ3-8 | 1/2 kernel | 46 | SP7 | < 1/2 ear |
| 22 | TZ3-9 | 1/2 kernel | 47 | SP8 | < 1/2 ear |
| 23 | TZ3-10 | 1/2 kernel | 48 | SP9 | < 1/2 ear |
| 24 | TZ3-11 | 1/2 kernel | 49 | SP10 | < 1/2 ear |
| 25 | TZ3-12 | 1/2 kernel | 50 | SP11 | < 1/2 ear |

Supplemental file S1

Size of the red sectors of the 50 ears initially screened.

Supplemental file S2

Map of 24 alleles



\*Distance between CI insertion to the transcription start site of p2

\*\*CI sizes, ND: Not Determined

\*\*\*Lane number in Figure 4B and 5B

\*\*\*\*Yellow rows indicate the CIs with reversed orientation, grey rows indicate Ac excision events.

Supplemental file S3

Primers for detecting *Ac* and *fAc* junctions.

Primer for *Ac* side insertion

|  |  |
| --- | --- |
| *Ac* primer: CCCGTTTCCGTTCCGTTTTCGT | |
| 6D | GTGCAACAAACTAGCTCACTCATTG |
| L18 | GTGCAAATACGGAGTCTGCT |
| TZ3-2 | CCCTCGTCCTCCAAGATTCTCCCCCTG |
| E3 | CCCTCGTCCTCCAAGATTCTCCCCCTG |
| 256 | CCCTCGTCCTCCAAGATTCTCCCCCTG |
| S7 | GGAAGGATGAATAGTGTGCTCCGCTGTGC |
| 257 | GTGCAACAAACTAGCTCACTCATTG |
| TZ3-17 | GTGCAACAAACTAGCTCACTCATTG |
| TZ3-15 | CTCTGTAAGCTGTGAAGCATGAATTTGTG  GTGCAACAAACTAGCTCACTCATTG |
| TZ3-9 | CTCTGTAAGCTGTGAAGCATGAATTTGTG  GTGCAACAAACTAGCTCACTCATTG |

Primer for *fAc* side insertion

|  |  |
| --- | --- |
| *fAc* primer: GATTACCGTATTTATCCCGTTCGTTTTC | |
| 6D | CCCACAAGGCTAAATACACCCTCTCAA |
| TZ3-9 | CCCACAAGGCTAAATACACCCTCTCAA |
| L18 | CAAGTAACTGGTCCTCCTCC |
| TZ3-2 | TTATACTTGCGACGCTGTGG |
| E3 | TTATACTTGCGACGCTGTGG |
| 256 | TTATACTTGCGACGCTGTGG |
| S7 | ACCGTTGACACCACCAGTTT |
| 257 | ATCGTCTCACCACATGACCA |
| TZ3-17 | CCCACAAGGCTAAATACACCCTCTCAA |
| TZ3-15 | CCCACAAGGCTAAATACACCCTCTCAA |

Supplemental file S4

Primers for internal junctions

|  |  |  |
| --- | --- | --- |
| 6D | CCGATGCTCTTTTCCTTCTCTTCC | CTCTTCACAAGACTTTTGGTTAGTAGAAC |
| TZ3-9 | TGTTGGAGTGATGGCAGGAT | CCGCTGCTCACTCATTCGCTCT |
| L18 | CCGCTGCTCACTCATTCGCTCT | TGGTCTCTAATATCCGCCTTGT |
| TZ3-2 | TGGTCTCTAATATCCGCCTTGT | CCGCTGCTCACTCATTCGCTCT |
| E3 | GCCCTTATTTCAGCCGTATTCAA | CCGCTGCTCACTCATTCGCTCT |
| 256 | GCCCTTATTTCAGCCGTATTCAA | CTCTTCACAAGACTTTTGGTTAGTAGAAC |
| S7 | TAAAGGAGGTGCAAAGATGGTT | CTCTTCACAAGACTTTTGGTTAGTAGAAC |
| 257 | GCCCTTATTTCAGCCGTATTCAA | GGACTTCAACCAAACCCGATGATT |
| TZ3-17 | GCCCTTATTTCAGCCGTATTCAA | AGTCGTGCCTTGTTCCTTGTC |
| TZ3-15 | TGGGGTGACAGAATAAATAGGG | ACTACTAGCCATAGGGGCCTTC |

Supplemental file S5

File S1: Junction sequence of the ten alleles

Microhomology sequences (yellow), Filler DNA (green) and the original *p1* sequences that produced the filler DNA (blue).

A picture containing screenshot, monitor, green, screen

Description automatically generated

Supplemental file S6

Primers for RT-PCR: Purple sequences indicate the exon 1 and exon 2, red underlined sequences indicate the primers used for RT-PCR, and the white sequence in the middle indicates the intron 1 of *p2*.

AGCCAGCACAGCACACACACTGGAAAGTGCAAGCTGTAGTGAGACCTGTGCGACTGCCAGCGTGTGTCCGCGCGGTCGTCGGCCCGCACGGCCACCAACTCCCTTGGACGCACGCGCGCACGCGACCAGCTGCTAGCCGTGCGCAAGTAGTGCGAGCTCGCCGCCGGCCAGGGTCGCTAGCTCGATTGGAGGGACCAGCTGATACGACTCCGGTGTGGCGCGCGATGGGGAGGGCGCCGTGCTGCGAGAAGGTGGGGCTCAAGCGAGGGAGGTGGACGGCGGAGGAGGACCAGTTACTTGCCAACTACATTGCGGAGCACGGCGAGGGGTCCTGGAGGTCGCTGCCCAAGAATGCAGGTAACCAAAGCCGGCCGCGCGCCATGCATCGCCACGTAGCATCAATCTCCGATCCATGCATATATGAGCTAGCTTCTTCTTCGTCGCCGTCGTCGTTCTTAGCTAGTTAGGACGCGCATGCAGGCCTGCTCCGGTGCGGCAAGAGCTGCCGGCTCCGGTGGATCAACTACCTCCGGGCGGACGTCAAGAGGGGGAACATCTCCAAGGAGGAAGAAGACATCATCATCAAGCTCCACGCCACCCTCGGGAACAG

Supplemental file S7

RT-PCR sequence aligned to *p2* exon 1 and exon 2, the upper line indicates RT-PCR product from *S7*, and the lower line indicates the *p2* sequence.

**Exon 1**

**8 GCGA-CTCGCCGCCGGCCAAGGGTCGCTAGCTCGATTGGAGGGACCAGCTGATACGACTC**

**|||| ||||||||||||| |||||||||||||||||||||||||||||||||||||||||**

**4637 GCGAGCTCGCCGCCGGCC-AGGGTCGCTAGCTCGATTGGAGGGACCAGCTGATACGACTC**

**CGGTGTGGCGCGCGATGGGGAGGGCGCCGTGCTGCGAGAAGGTGGGGCTCAAGCGAGGGA**

**||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||**

**CGGTGTGGCGCGCGATGGGGAGGGCGCCGTGCTGCGAGAAGGTGGGGCTCAAGCGAGGGA**

**GGTGGACGGCGGAGGAGGACCAGTTACTTGCCAACTACATTGCGGAGCACGGCGAGGGGT**

**||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||**

**GGTGGACGGCGGAGGAGGACCAGTTACTTGCCAACTACATTGCGGAGCACGGCGAGGGGT**

**CCTGGAGGTCGCTGCCCAAGAATGCAG 213**

**|||||||||||||||||||||||||||**

**CCTGGAGGTCGCTGCCCAAGAATGCAG 4842**

**Exon 2**

**214 GCCTGCTCCGGTGCGGCAAGAGCTGCCGGCTCCGGTGGATCAACTACCTCCGGG**

**||||||||||||||||||||||||||||||||||||||||||||||||||||||**

**4960 GCCTGCTCCGGTGCGGCAAGAGCTGCCGGCTCCGGTGGATCAACTACCTCCGGG**

**CGGACGTCAAGAGGGGGAACATCTCCAAGGAGGAAGAAGACATCATCA 315**

**||||||||||||||||||||||||||||||||||||||||||||||||**

**CGGACGTCAAGAGGGGGAACATCTCCAAGGAGGAAGAAGACATCATCA 5067**

Supplemental file S8

Primers for Bisulfite Sequencing

|  |  |
| --- | --- |
|  |  |
| BSS17 | ATGATGAGTTTTAGGTAGGTTGA |
| BSS18\* | TTATCATAATAACATCGAACAACC |
| BSS8 | TCAAATAACRCCTACAAACA |
|  |  |
| fAc-C1 | CTTATATTAACCAAAAACCCAAAACT |
| p1f-C | TTTTAATTAAGGGGATAGAAGTCGAT |
| fAc-C | CCCAAAACTTATCACTTATATACTAC |
|  |  |
| AcR-C1 | TTTGGTCGTTTGGGAGAGAGGTAGA |
| P1-C | GTCGACACGAAAACCATATACCATTAC |
| Ac\_Rc | GTTAGGTATTTTTTATATGGGTTGGGT |
|  |  |
| p2-1287fc | GGGGTAGATTAAGTTGGGGCGGTTTT |
| p2-1461rc | CACCGAACCAAAACCATAAAATACAAC |

Supplemental file S9

DNA methylation status in *B54*, *E3* and *E3M*.

A close up of a traffic light

Description automatically generated

A. Genomic segments targeted for bisulfite sequencing to analyze cytosine methylation. Dotted boxes numbered 1 – 7 indicate sites analyzed. B. Summary of cytosine methylation data in the regions annotated in panel A. The first column indicates the genomic positions corresponding to A, the second column indicates the total number of cytosines in each segment; remaining columns indicate the number of methylated cytosines in *B54*, *E3* and *E3M*. “/” indicates absence of that sequence in the *B54* allele.

Supplemental file S10

Bisulfite sequencing alignments: Cytosines in the sequence are labeled in yellow, unmethylated cytosines (T) are labeled in red and methylated cytosines remain in yellow.

A picture containing drawing

Description automatically generated

A picture containing drawing

Description automatically generated

A screenshot of a cell phone

Description automatically generated