

Supplemental Table 1. Newly designed SSR markers.

| SSR name    | Contig number<br>(Apple Genome V1.0 contigs<br>by Velasco et al. (2010)) | Forward primer        | Reverse primer       | Motif    | Predicted<br>product size<br>(bp) |
|-------------|--|-----------------------|----------------------|----------|-----------------------------------|
| Mdo.chr1.01 | MDC029632.22   | TTCTAAACCAATCCAACCGC  | ACGCGCGTATTCTCTCATTT | (AT)15   | 303                               |
| Mdo.chr1.05 | MDC013694.193  | ACCCAAGTCTCATCTGCACC  | CATGAGTGAACAGGGGGACT | (TC)20.5 | 347                               |
| Mdo.chr1.06 | MDC010753.200  | TGCAGTTCCCTCGACTCATTG | ATGGTGCTTTGGGTTGAAG  | (AT)11   | 350                               |
| Mdo.chr1.08 | MDC021770.295  | CGTCCTAGATAGATGCCCA   | CAGGACTCTAAGGACTGCCG | (TA)14.5 | 223                               |
| Mdo.chr1.09 | MDC009959.204  | TGACTGAACGTAGCGTCCG   | TTTACAGCTACCGACACCCC | (AG)11   | 232                               |
| Mdo.chr1.10 | MDC006308.247  | AAAAATCGGGATCTCTCCC   | TTGCTATCAATTCCCAAGCC | (GT)10   | 221                               |
| Mdo.chr1.11 | MDC016506.307  | TGACTAAATCGGCAGTTCAC  | CTTTGTCAACCAAAAGAGGG | (GA)24   | 321                               |
| Mdo.chr1.15 | MDC004709.213  | GGCCTGTAAAAGAGGGAACC  | GGCTTTTCTCCGTCTTCT   | (CT)12   | 204                               |
| Mdo.chr1.18 | MDC012246.295  | CGTGTCCACAAGCTGAGAAA  | AGGGATCCCGCTCTACTGTT | (TC)13   | 283                               |
| Mdo.chr1.19 | MDC013459.136  | TTATTGACGCCCTTGGTGT   | CGAGCGAGCTTCTTGTCT   | (TA)10.5 | 332                               |
| Mdo.chr1.21 | MDC022241.434  | TGCTGGCTATCTGCTACCCT  | TGTCTCTCCTCTCCTCCCA  | (GA)15.5 | 323                               |
| BCA141      | -  | ACCAAACCCAGACCAAA     | TGTCTTCTCATCATCTCTC  | (GA)5    | 121                               |

Supplemental Table 2. Overview of the genetic maps constructed for ‘Orin’ and ‘Akane’ (Fig. 1).

| Linkage Group | 1                                    | 2                   | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11     | 12   | 13   | 14   | 15   | 16     | 17   | Total    |        |
|---------------|--------------------------------------|---------------------|------|------|------|------|------|------|------|------|--------|------|------|------|------|--------|------|----------|--------|
| ‘Orin’        | Genetic Distance (cM)                | 71.5                | 70.6 | 72.6 | 67.0 | 65.5 | 47.1 | 67.2 | 57.5 | 69.6 | 73.2   | 74.4 | 56.1 | 14.2 | 56.3 | 92.5   | 69.2 | 70.8     | 1095.3 |
|               | Number of loci (apple <sup>a</sup> ) | 15 (1) <sup>b</sup> | 9    | 5    | 10   | 10   | 6    | 9    | 11   | 11   | 16 (1) | 10   | 17   | 2    | 10   | 21 (2) | 6    | 14 (1)   | 182    |
|               | Number of loci (pear <sup>a</sup> )  | 5                   | 1    | 4    | 9    | 1    | 2    | 3    | 5    | 3    | 4      | 5    | 3    | 0    | 5    | 7      | 7    | 5        | 69     |
| ‘Akane’       | Marker Density (cM/marker)           | 3.6                 | 7.1  | 8.1  | 3.5  | 6.0  | 5.9  | 5.6  | 3.6  | 5.0  | 3.7    | 5.0  | 2.8  | 7.1  | 3.8  | 3.3    | 5.3  | 3.7      | 4.4    |
|               | Genetic Distance (cM)                | 64.1                | 69.8 | 81.8 | 56.6 | 80.8 | 63.4 | 73.0 | 54.3 | 69.4 | 73.1   | 77.2 | 56.9 | 60.4 | 33   | 99.1   | 41.3 | 42.5+1.5 | 1098.2 |
|               | Number of loci (apple <sup>a</sup> ) | 7                   | 8    | 5    | 10   | 17   | 13   | 13   | 11   | 9    | 21     | 15   | 20   | 10   | 15   | 19 (1) | 10   | 14+2     | 219    |
| ‘Akane’       | Number of loci (pear <sup>a</sup> )  | 4                   | 3    | 3    | 7    | 4    | 0    | 4    | 4    | 2    | 8      | 8    | 0    | 5    | 4    | 7      | 3    | 6+0      | 72     |
|               | Marker Density (cM/marker)           | 5.8                 | 6.3  | 10.2 | 3.3  | 3.8  | 4.9  | 4.3  | 3.6  | 6.3  | 2.5    | 3.4  | 2.8  | 4.0  | 1.7  | 3.8    | 3.2  | 2.0      | 3.8    |

For ‘Akane’, LG17 is divided into LG17-1 and LG17-2.

<sup>a</sup>Number of loci is presented separately for loci detected using primer pairs designed from apple and pear sequences.

<sup>b</sup>Numbers in parentheses represent the number of loci detected using a gene-specific marker.

Marker density was calculated by dividing the genetic distance by the number of loci.

Supplemental Table 3. Significant QTLs detected in the ‘Orin’ × ‘Akane’ F1 population in each year .

| Map                              | Orin   |      |    |               |      |                           | Akane  |                  |      |    |               |      |                           |             |                |                |
|----------------------------------|--------|------|----|---------------|------|---------------------------|--------|------------------|------|----|---------------|------|---------------------------|-------------|----------------|----------------|
|                                  | Traits | Year | LG | Position (cM) | LOD  | Significance <sup>a</sup> | % var. | Nearest marker   | Year | LG | Position (cM) | LOD  | Significance <sup>a</sup> | % var.      | Nearest marker |                |
| Harvest                          |        | 2011 | 6  | 3.5           | 3.70 | ***                       | 9.3    | <i>Hi08g03</i>   | 2010 | 3  | 59.5          | 3.52 | **                        | 17.8        | NB109a         |                |
|                                  |        |      |    |               |      |                           |        |                  | 2011 | 3  | 60.5          | 5.13 | ****                      | 17.1        | NB109a         |                |
|                                  |        |      |    |               |      |                           |        |                  | 2012 | 3  | 69.2          | 6.49 | *****                     | 19.8        | NH030a-2       |                |
|                                  |        |      |    |               |      |                           |        |                  | 2010 | 10 | 31.9          | 2.66 | *                         | 11.4        | CH02c11        |                |
|                                  |        |      |    |               |      |                           |        |                  | 2012 | 10 | 30.9          | 3.05 | **                        | 8.7         | CH02c11        |                |
|                                  |        |      |    |               |      |                           |        |                  |      |    |               |      |                           |             |                |                |
|                                  |        |      |    |               |      |                           |        |                  |      |    |               |      |                           |             |                |                |
|                                  |        |      |    |               |      |                           |        |                  |      |    |               |      |                           |             |                |                |
|                                  |        |      |    |               |      |                           |        |                  |      |    |               |      |                           |             |                |                |
|                                  |        |      |    |               |      |                           |        |                  |      |    |               |      |                           |             |                |                |
| Russet-Calyx <sup>b</sup>        |        | 2010 | 2  | 3.0           | 3.83 | *** (KW: *****)           | 19.1   | <i>Hi22d06</i>   | 2010 | 2  | 59.8          | 2.45 | *                         | (KW: ***)   | 12.7           | TsuENH087      |
|                                  |        |      |    |               |      |                           |        |                  | 2011 | 2  | 50.4          | 5.80 | ***** (KW: *****)         | 19.1        | MEST142        |                |
|                                  |        |      |    |               |      |                           |        |                  | 2012 | 10 | 8.8           | 2.34 | *                         | (KW: ***)   | 8.8            | <i>MEST111</i> |
|                                  |        |      |    |               |      |                           |        |                  |      |    |               |      |                           |             |                |                |
| Russet-Pedicel <sup>b</sup>      |        | 2011 | 4  | 55.9          | 2.78 | ** (KW: *****)            | 9.7    | NB141b           |      |    |               |      |                           |             |                |                |
|                                  |        |      |    |               |      |                           |        |                  |      |    |               |      |                           |             |                |                |
|                                  |        |      |    |               |      |                           |        |                  |      |    |               |      |                           |             |                |                |
|                                  |        |      |    |               |      |                           |        |                  |      |    |               |      |                           |             |                |                |
| Depth of Skin Color <sup>b</sup> |        | 2011 | 6  | 39.4          | 3.50 | *** (KW: *****)           | 12.0   | <i>TsuENH126</i> | 2010 | 6  | 39.0          | 2.64 | *                         | (KW: *****) | 11.4           | CH03d12        |
|                                  |        |      |    |               |      |                           |        |                  | 2012 | 6  | 38.6          | 2.28 | *                         | (KW: *****) | 8.6            | CH03d12        |
|                                  |        |      |    |               |      |                           |        |                  | 2010 | 15 | 87.0          | 3.22 | **                        | (KW: *****) | 16.4           | MEST128        |
|                                  |        |      |    |               |      |                           |        |                  | 2011 | 15 | 86.3          | 3.22 | **                        | (KW: *****) | 11.1           | <i>Hi11a01</i> |
| Juiciness                        |        | 2011 | 16 | 4.0           | 2.72 | * (KW: ***)               | 9.5    | <i>Hi22f06</i>   |      |    |               |      |                           |             |                |                |
|                                  |        |      |    |               |      |                           |        |                  |      |    |               |      |                           |             |                |                |
|                                  |        |      |    |               |      |                           |        |                  |      |    |               |      |                           |             |                |                |
|                                  |        |      |    |               |      |                           |        |                  |      |    |               |      |                           |             |                |                |

The peak with the highest LOD is presented as the QTL, followed by its position and the percentage of the phenotypic variance that it explained (% var.).

<sup>a</sup> Asterisks (\*, \*\*, \*\*\*, \*\*\*\*, \*\*\*\*\*) represent significance level of p < 0.10, <0.05, <0.01, <0.001, <0.0001 respectively. <sup>b</sup> The significance of QTLs for traits with a non-normal distribution was confirmed using the Kruskal-Wallis (KW) single-locus analysis, using the nearest marker. Major QTLs that explained >20% variance are indicated in bold. QTLs undetected in the analyses using average value of data during 2 or 3 years (Table 3) are shown by italic.

Supplemental Table 3. (Continued)

| Map                                | Orin              |      |      |               |                   |                           |        | Akane          |      |      |               |      |                           |                   |                |           |      |                   |      |         |
|------------------------------------|-------------------|------|------|---------------|-------------------|---------------------------|--------|----------------|------|------|---------------|------|---------------------------|-------------------|----------------|-----------|------|-------------------|------|---------|
|                                    | Traits            | Year | LG   | Position (cM) | LOD               | Significance <sup>a</sup> | % var. | Nearest marker | Year | LG   | Position (cM) | LOD  | Significance <sup>a</sup> | % var.            | Nearest marker |           |      |                   |      |         |
| Firmness                           | 2011              | 10   | 44.0 | 3.83          | ***               |                           | 13.2   | CH02c11        | 2012 | 3    | 74.2          | 2.60 | *                         | 9.7               | NH030a-2       |           |      |                   |      |         |
|                                    | 2012              | 11   | 6.9  | 2.63          | *                 |                           | 9.8    | IPPN02         |      |      |               |      |                           |                   |                |           |      |                   |      |         |
| Weight                             | 2011              | 11   | 74.4 | 2.87          | *                 |                           | 10.0   | CH04d07        |      |      |               |      |                           |                   |                |           |      |                   |      |         |
|                                    | 2012              | 17   | 23.8 | 2.84          | **                |                           | 10.8   | Hi03c05        |      |      |               |      |                           |                   |                |           |      |                   |      |         |
| Preharvest Fruit Drop <sup>b</sup> | 2012              | 3    | 66.0 | 2.69          | *                 | (KW: *****)               | 7.2    | NB109a         |      | 2011 | 5             | 14.1 | 3.71                      | *** (KW: *****)   | 12.7           | CH03a09   |      |                   |      |         |
|                                    | 2011              | 15   | 62.5 | 7.68          | ***** (KW: *****) | 24.5                      | MdACS1 |                |      |      |               |      |                           |                   |                |           |      |                   |      |         |
|                                    | 2012              | 15   | 62.5 | 8.43          | ***** (KW: *****) | 28.2                      | MdACS1 |                |      |      |               |      |                           |                   |                |           |      |                   |      |         |
| Acidity                            |                   |      |      |               |                   |                           |        |                |      |      |               |      |                           |                   |                |           |      |                   |      |         |
|                                    | 2011              | 16   | 9.1  | 6.83          | *****             |                           | 22.4   | CH05c06        | 2011 | 8    | 26.8          | 3.97 | ***                       | 10.0              | CH02g09        |           |      |                   |      |         |
|                                    | 2012              | 16   | 8.1  | 6.38          | *****             |                           | 22.2   | CH05c06        | 2012 | 8    | 27.8          | 4.38 | ***                       | 12.6              | CH02g09        |           |      |                   |      |         |
|                                    | 2011              | 16   | 20.1 | 6.78          | *****             |                           | 19.8   | TsuENH182-2    | 2012 | 15   | 42.7          | 2.50 | *                         | 6.3               | MdACS3         |           |      |                   |      |         |
|                                    | 2012              | 16   | 12.6 | 3.86          | *** (KW: *****)   |                           | 14.1   | CH05c06        | 2011 | 16   | 6.2           | 9.51 | *****                     | 26.6              | Hi12a02        |           |      |                   |      |         |
| °Brix                              | 2011              | 15   | 63.2 | 3.72          | ***               |                           | 10.7   | MdACS1         |      | 2012 | 16            | 22.5 | 3.50                      | ** (KW: *****)    | 12.9           | Hi01a08   |      |                   |      |         |
|                                    | 2012 <sup>b</sup> | 15   | 62.5 | 2.65          | *                 | (KW: **)                  | 8.6    | MdACS1         |      |      |               |      |                           |                   |                |           |      |                   |      |         |
|                                    | 2011              | 16   | 20.1 | 6.78          | *****             |                           | 19.8   | TsuENH182-2    |      |      |               |      |                           |                   |                |           |      |                   |      |         |
|                                    | 2012 <sup>b</sup> | 16   | 12.6 | 3.86          | *** (KW: *****)   |                           | 14.1   | CH05c06        |      |      |               |      |                           |                   |                |           |      |                   |      |         |
| Sucrose                            |                   |      |      |               |                   |                           |        |                |      |      |               |      |                           |                   |                |           |      |                   |      |         |
|                                    | 2012              | 10   | 26.3 | 4.21          | ***               |                           |        |                | 2012 | 10   | 26.3          | 4.21 | ***                       | 14.3              | TsuENH109      |           |      |                   |      |         |
| Glucose                            |                   |      |      |               |                   |                           |        |                |      |      |               |      |                           |                   |                |           |      |                   |      |         |
|                                    | 2012              | 15   | 92.5 | 3.06          | **                |                           |        |                | 2012 | 15   | 92.5          | 3.06 | **                        | 10.1              | MEST147        |           |      |                   |      |         |
| Fructose                           |                   |      |      |               |                   |                           |        |                |      |      |               |      |                           |                   |                |           |      |                   |      |         |
|                                    | 2012              | 5    | 0.0  | 3.34          | **                |                           |        |                | 2012 | 5    | 0.0           | 3.34 | **                        | 12.4              | Hi09b04        |           |      |                   |      |         |
| Sorbitol <sup>b</sup>              |                   |      |      |               |                   |                           |        |                |      |      |               |      |                           |                   |                |           |      |                   |      |         |
|                                    | 2012              | 6    | 8.6  | 3.27          | **                |                           |        |                | 2012 | 6    | 8.6           | 3.27 | **                        | 10.9              | CH03d07        |           |      |                   |      |         |
| Juice browning <sup>b</sup>        |                   |      |      |               |                   |                           |        |                |      |      |               |      |                           |                   |                |           |      |                   |      |         |
|                                    | 2012              | 16   | 18.5 | 3.00          | **                |                           |        |                | 2012 | 16   | 18.5          | 3.00 | **                        | 10.0              | Hi15a13        |           |      |                   |      |         |
| Flowering date <sup>b</sup>        | 2012              | 12   | 23.6 | 2.79          | *                 | (KW: *****)               | 10.5   | CH05d11        |      | 2012 | 16            | 15.5 | 3.74                      | *** (KW: *****)   | 13.8           | Hi15a13   |      |                   |      |         |
|                                    | 2012              | 10   | 54.6 | 2.72          | *                 | (KW: ***)                 | 10.2   | Hi22f04        |      | 2012 | 10            | 19.3 | 4.89                      | **** (KW: *****)  | 11.5           | NH039a    |      |                   |      |         |
|                                    | 2012              | 16   | 9.3  | 10.55         | ***** (KW: *****) |                           |        |                |      | 2012 | 16            | 9.3  | 10.55                     | ***** (KW: *****) | 28.2           | TsuENH022 |      |                   |      |         |
|                                    |                   |      |      |               |                   |                           |        |                |      |      |               |      |                           |                   |                |           |      |                   |      |         |
|                                    |                   |      |      |               |                   |                           |        |                |      |      |               |      |                           | 2012              | 15             | 86.3      | 9.63 | ***** (KW: *****) | 31.8 | Hi09f01 |