**Supplementary File S1 for**

**Genomic insights into apple aroma diversity**

**Authors**

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**Methods for genotyping SNP in CMS and MYB retrotransposon**

Kompetitive Allele Specific PCR (KASP) genotyping assay method for citramalate synthase SNP

**Methods:** KASP assyas were run on genomic DNA samples normalized in 384-well plates. Primer sequences for the assay can be found in **Table 1**.

Primers used in the KASP assay.

| **Primer Name** | **Primer Sequence (5’ – 3’)** |
| --- | --- |
| MD\_CMS\_KASP\_G2 | GAAGGTGACCAAGTTCATGCTatgccagtggaattcacg |
| MD\_CMS\_KASP\_C2 | GAAGGTCGGAGTCAACGGATTatgccagtggaattcacc |
| MD\_CMS\_387\_R3 | AACTGCAAAATAAAAGTTAATATGGAAA |

Each assay (5ul reaction) was run using the following reagents:

0.07ul of 100uM primers

2.5ul KASP mastermix

2.5 ul of 3ng/ul DNA

We used the following program on a CFX cycler:

Stage 1: 94°C for 15 minutes (Hot-start activation)

Stage 2 (Touchdown):

94°C for 20 seconds

61°C for 1 minute

(decrease by 0.6°C per cycle)

[REPEAT 10x]

Stage 3 (amplification):

 94°C for 20 seconds

55°C for 1 minute

[REPEAT 26x]

The plates were then read in BioRad CFX for 1 minute at 37°C.

High-resolution DNA melting (HRM)-based assay method to detect redTE

**Methods:**

High-Resolution DNA melting(HRM)-based genotyping assays were run on genomic DNA samples normalized in 384-well plates. Primer sequences for the assay were taken from Zhang et al. (2019) and can be found in **Table 1**.

**Table 1.** Primers used in the HRM assay.

| **Primer Name** | **Primer Sequence (5’ – 3’)** |
| --- | --- |
| redTE\_F | GGTCACCCAACCCACACTGGGCCTTG |
| redTE\_R | CGGCCGCAATCGCAAGACGCAGA |

Each assay was run using the following reagents:

0.1ul of 100uM primers

2ul of 5ng/ul DNA

2.025 ul sterile water

0.5ul of 10x PCR buffer

0.05ul of 5U/ul Taq

0.125ul 10x Evagreen Dye

0.2 ul of 10mM dNTPs

We used the following program on a CFX cycler:

95°C for 3:30 minutes

95°C for 30 seconds

60°C for 30 seconds

72°C for 30 seconds

[REPEAT 34x]

72°C for 5 minutes

The plates were denatured for 3-5 minutes before they were read using a Lightscanner HR384 (Biofire) and LightScanner Software with Call-IT 2.5 in ‘amplicon genotyping’ mode.

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