**mef2ca<sup>b1086</sup>**

### Nature of the mutation
The b1086 allele contains a single A-to-T point mutation that introduces an early stop codon upstream of the MEF2 domain (Miller et al., Developmental Biology 308: 144-157, 2007).

### Genotyping assay
Genotyping of the b1086 allele is based on the dCAPS assay (derived Cleaved Amplified Polymorphic Sequence; Neff et al., The Plant Journal 14(3): 387-392, 1998). In this assay, a restriction enzyme recognition site that includes the single nucleotide polymorphism (SNP) is introduced into the PCR product by a primer containing one or more mismatches to template DNA. The PCR product modified in this manner is then subjected to restriction enzyme digestion and the presence or absence of the SNP is determined by the resulting restriction pattern.

To genotype the b1086 allele, a mismatch (marked in red) has been introduced into the reverse primer. During PCR, this mismatch and the b1086 mutation create an SfcI restriction enzyme site in the amplified product. The SfcI site is not present in the PCR product derived from the WT DNA template.

#### Primers:
- **HOO_10:** 5’ ATT TCA TGT CAT GGA ACT AAA TCT GTT 3’
- **HOO_13d:** 5’ CGG CTC GTT GTA CTC GGT GTA CTC 3’

#### PCR program (55_30_30):
1. 94°C for 3 min
2. 94°C for 30 sec
3. 55°C for 30 sec
4. 72°C for 30 sec
5. Go to step 2 (above) for 39 cycles
6. 72°C for 5 min
7. 8.0°C hold
8. END

#### Product size: 243 bp

#### Digestion of the PCR product with the SfcI restriction enzyme:

<table>
<thead>
<tr>
<th>Product type</th>
<th>Product digestion</th>
<th>DNA fragments after digestion (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR product derived from the WT template</td>
<td>unaffected</td>
<td>243 bp</td>
</tr>
<tr>
<td>PCR product containing the mutation</td>
<td>cleaved</td>
<td>217 bp and 26 bp</td>
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</tbody>
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