

## *mecp2<sup>fh232</sup>*

### Nature of the mutation

The *fh232* allele contains a single C-to-T point mutation that introduces a premature stop codon at amino acid 63, resulting in truncation of the Mecp2 protein (Moens C., personal communication).

### Genotyping assay

Genotyping of the *fh232* 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 *fh232* allele, a mismatch (marked in red) has been introduced into the reverse primer. During PCR, this mismatch creates a PstI restriction enzyme site in the amplified product derived from the WT DNA template. The PstI site is not present in the PCR product containing the *fh232* mutation.

### **Primers:**

**fh232\_05:** 5' ACA GCT GAC TAT AAT ACA GAC CTG TCA AA 3'

**fh232\_06d:** 5' TGG GTT CAG ACT TCC CTG CCT CTG CCT GCA 3'

### **PCR program (60\_30\_30):**

1. 94°C for 3 min
2. 94°C for 30 sec
3. 60°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: 250 bp**

### **Digestion of the PCR product with the PstI restriction enzyme:**

| Product type                             | Product digestion | DNA fragments after digestion (bp) |
|--|-------------------|------------------------------------|
| PCR product derived from the WT template | cleaved           | 224 bp and 26 bp                   |
| PCR product containing the mutation      | unaffected        | 250 bp                             |

**IMPORTANT NOTE:** It is highly recommended to use WT positive controls to monitor whether enzyme digestion has been carried out to completion. Without this control, partially digested WT samples can be mistakenly regarded as heterozygous samples.

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