# *mcm5*$^{m850}$

## Nature of the mutation

The *m850* allele contains a single T-to-C point mutation that takes place within the highly conserved splice donor site at the end of the second exon. The mutation results in a shorter *mcm5* transcript that lacks the entire second exon (Ryu et al., Proc. Natl. Acad. Sci. USA 102(51):18467-18472, 2005).

## Genotyping assay

Genotyping of the *m850* allele is based on the dCAPS assay (**d**erived **C**leaved **A**mplified **P**olymorphic **S**equence; 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 *m850* allele, a mismatch (marked in red) has been introduced into the reverse primer. During PCR, this mismatch and the *m850* mutation create a NlaIII restriction enzyme site in the amplified product. The NlaIII site is not present in the PCR product derived from the WT DNA template.

**Primers:**
**MCM_02:** 5' ATT TCG AGT CGG TTC AGT GAG 3'
**MCM_04d:** 5' GCA TTA AAC ACG AGT CTA TCC AT 3'

**PCR program (53_30_30):**

1. 94ºC for 3 min
2. 94ºC for 30 sec
3. **53**ºC for **30** sec
4. 72ºC for **30** sec
5. Go to step 2 (above) for 39 cycles
6. 72ºC for 5 min
7. 8.0ºC hold
8. END

**Product size: 275 bp**

**Digestion of the PCR product with the NlaIII restriction enzyme:**

| Product type | Product digestion | DNA fragments after digestion (bp) |
|---|---|---|
| PCR product derived from the WT template | unaffected | 275 bp |
| PCR product containing the mutation | cleaved | 255 bp and 20 bp |

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