

# *ta72a*

## CZ43: *bmp2b*

### Nature of the mutation

The *ta72a* allele contains a single A-to-G point mutation that converts the stop codon into a Trp residue. As a result, the mutation extends the *bmp2b* open reading frame by six amino acids (Kishimoto et al., Development 124: 4457-4466, 1997; Nguyen et al., Developmental Biology 199: 93-110, 1998).

### Genotyping assay

Genotyping of the *ta72a* 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 *ta72a* allele, a mismatch (marked in red) has been introduced into the forward primer. During PCR, this mismatch and the *ta72a* mutation create a Styl restriction enzyme site in the amplified product. The Styl site is not present in the PCR product derived from the WT DNA template.

### Primers:

F: 5' GGATGAGGACTCGTGGGC 3'

R: 5' GTTTTGGAATGGGGCAAG3'

### **PCR program:**

1. 94°C for 3 min
2. 94°C for 30 sec
3. 55°C for 30 sec
4. 72°C for 50 sec
5. Go to step 2 (above) for 29 cycles
6. 72°C for 5 min
7. 12.0°C hold

**Product size:** 648bp

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

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	227bp
PCR product containing the mutation	cleaved	454 bp and 194bp