smad5^{tc227a}

Nature of the mutation

The *tc227a* allele contains a stop codon at amino acid position 21 in the MH1 domain (Kramer et al., Developmental Biology 250: 263-279, 2002).

Genotyping assay

Genotyping of the *tc227a* 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 *tc227a* allele, a mismatch (marked in red) has been introduced into the forward primer. During PCR, this mismatch creates a BsrI restriction enzyme site in the amplified product derived from the WT DNA template. The BsrI site is not present in the PCR product containing the *tc227a* mutation.

Primers:
SMA_20d: 5' CAG TGA AGC GGT TGC TGG ACT 3'
SMA_21: 5' CCC TTC CTG TGG GAC ACC TG 3'

PCR program (60_30_30):

1. 94ºC for 3 min
2. 94ºC for 30 sec
3. **60**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: 208 bp

Digestion of the PCR product with the BsrI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	cleaved	184 bp and 24 bp
PCR product containing the mutation	unaffected	208 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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