sptb^{tb237}

Nature of the mutation

The *tb237* allele contains a single C-to-T point mutation that changes Gln into a premature stop codon at position 1044 of the Sptb protein (Liao et al., Development 127: 5123-5132, 2000).

Genotyping assay

Genotyping of the *tb237* 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 *tb237* allele, a mismatch (marked in red) has been introduced into the reverse primer. During PCR, this mismatch creates a HpyCH4III restriction enzyme site in the amplified product derived from the WT DNA template. The HpyCH4III site is not present in the PCR product containing the *tb237* mutation.

Primers:
SPT_03: 5' GAA ACA TAG GGT AGA TTC TGC TCT 3'
SPT_01d: 5' CAT GCT GCA TCC AAC TCT TAC T 3'

PCR program (55_30_30):

1. 94ºC for 3 min
2. 94ºC for 30 sec
3. **55**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: 291 bp

Digestion of the PCR product with the HpyCH4III restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	cleaved	270 bp and 21 bp
PCR product containing the mutation	unaffected	291 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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