furina^{td204e}

Nature of the mutation

The *td204e* allele contains a single T-to-A point mutation that changes a conserved GT splice donor site to GA (Walker et al., Developmental Biology 295: 194-205, 2006).

Genotyping assay

Genotyping of the *td204e* 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 *td204e* allele, a mismatch (marked in red) has been introduced into the forward primer. During PCR, this mismatch and the *td204e* mutation create a SmlI restriction enzyme site in the amplified product. The SmlI site is not present in the PCR product derived from the WT DNA template.

Primers:
STU_01d: 5' GCG TCA ATG AGA AAC AGC TTG 3'
STU_02: 5' AAA AAC ATT TCC AGT ATC TTC CAC 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: 257 bp

Digestion of the PCR product with the SmlI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	257 bp
PCR product containing the mutation	cleaved	239 bp and 18 bp

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