

kctd12.1^{fh236}**Nature of the mutation**

The *fh236* allele contains a single T-to-A point mutation that introduces a premature stop codon at amino acid 101, resulting in truncation of the Kctd12.1 protein (Moens C., personal communication).

Genotyping assay

Genotyping of the *fh236* 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 *fh236* allele, a mismatch (marked in red) has been introduced into the forward primer. During PCR, this mismatch and the *fh236* mutation create a HincII restriction enzyme site in the amplified product. The HincII site is not present in the PCR product derived from the WT DNA template.

Primers:

fh236_01d: 5' TGC CGG ACT ATT TTC CAG AGA AAA GTA **GTT** 3'

fh236_02: 5' CAA CCG TGA TGT ACC CAG ACT TT 3'

PCR program (58_40_40):

1. 94°C for 3 min
2. 94°C for 30 sec
3. 58°C for 40 sec
4. 72°C for 40 sec
5. Go to step 2 (above) for 39 cycles
6. 72°C for 5 min
7. 8.0°C hold
8. END

Product size: 258 bp**Digestion of the PCR product with the HincII restriction enzyme:**

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	258 bp
PCR product containing the mutation	cleaved	228 bp and 30 bp

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