

gfpt1^{j23e1}

Nature of the mutation

The *j23e1* allele contains a single C-to-T point mutation that introduces a stop codon in the third exon of the *gfpt1* cDNA (Chao-Tsung Yang, personal communication).

Genotyping assay

Genotyping of the *j23e1* 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 *j23e1* allele, a mismatch (marked in red) has been introduced into the forward primer. During PCR, this mismatch creates an MmeI restriction enzyme site in the amplified product derived from the WT DNA template. The MmeI site is not present in the PCR product containing the *j23e1* mutation.

Primers:

JGF_01d: 5' CAA GTG CAT CCA CCT GAT CCA A 3'

JGF_02: 5' GTC GGG TTC GGG CTA AAA TCT G 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: 285 bp

Digestion of the PCR product with the MmeI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	cleaved	242 bp and 43 bp
PCR product containing the mutation	unaffected	285 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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