fgf20a^{zp3}

Nature of the mutation

The *zp3* allele contains a single A-to-C point mutation that converts tyrosine 148 to serine (Whitehead et al., Science 310: 1957-1960, 2005).

Genotyping assay

Genotyping of the *zp3* 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 *zp3* allele, a mismatch (marked in red) has been introduced into the forward primer. During PCR, this mismatch and the *zp3* mutation create an AloI restriction enzyme site in the amplified product. The AloI site is not present in the PCR product derived from the WT DNA template.

Primers:
FGT_01d: 5' TTT GAG GAG AAT TGG AAC AAC ACT T 3'
FGT_02: 5' TTT TTG GGG TGG TTT TGA GTT T 3'

PCR program (58_40_40):

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

Product size: 246 bp

Digestion of the PCR product with the AloI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	246 bp
PCR product containing the mutation	cleaved	207 bp and 39 bp

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