piwil1^{fh219}

Nature of the mutation

The *fh219* allele contains a single C-to-T point mutation that substitutes Arg (561) by a stop codon in the Piwil1 protein (Moens C., personal communication).

Genotyping assay

Genotyping of the *fh219* allele is based on the RFLP assay (**R**estriction **F**ragment **L**ength **P**olymorphism; Botstein *et al.*, Am. J. Hum. Genet. 32: 314-331, 1980). This method is used to detect a mutation that either creates or abolishes a site recognized by a specific restriction enzyme. In the RFLP assay, a sequence of interest is first PCR-amplified and then the PCR product is subjected to restriction enzyme digestion. The presence or absence of the mutation is determined by the resulting restriction pattern. The *fh219* mutation creates a site recognized by the MfeI restriction enzyme.

Primers:
fh219_05: 5' ACG TGA AAC TCA GAT GGT AAG TCG AG 3'
fh219_06: 5' GGA ATT TCT ACA CTC CAC AGC TCT CC 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: 288 bp

Digestion of the PCR product with the MfeI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	288 bp
PCR product containing the mutation	cleaved	179 bp and 109 bp

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