wnt16 fh235

Nature of the mutation

The *fh235* allele contains a single T-to-A point mutation that substitutes Cys by Ser at residue 339 of the Wnt16 protein (Moens C., personal communication).

Genotyping assay

Genotyping of the *fh235* 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 *fh235* mutation creates a site recognized by the BsgI restriction enzyme.

Primers:
fh235_05: 5' GGA CCG TTC CAA ACG TAA GG 3'
fh235_06: 5' AGG CTG ACA ACA CAA AGA AGG TC 3'

PCR program (60_30_30):

1. 94ºC for 3 min
2. 94ºC for 30 sec
3. **60**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: 387 bp

Digestion of the PCR product with the BsgI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	387 bp
PCR product containing the mutation	cleaved	280 bp and 107 bp

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