egr2b ^{fh227}

Nature of the mutation

The *fh227* allele contains a single C-to-A point mutation that results in a stop codon at residue 334 of the Egr2b protein (Moens C., personal communication).

Genotyping assay

Genotyping of the *fh227* 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 *fh227* mutation abolishes a site recognized by the NsiI restriction enzyme.

Primers:
KRO_05: 5' CAA GGA AAT ACC CCA ACA GAC C 3'
KRO_06: 5' TGG TTT GAA CTG GAC GAG CAG 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: 391 bp

Digestion of the PCR product with the NsiI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	cleaved	232 bp and 159 bp
PCR product containing the mutation	unaffected	391 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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