

wnt11r^{fh224}**Nature of the mutation**

The *fh224* allele contains a single G-to-T point mutation that results in a premature stop codon at amino acid residue 94 (Moens C., personal communication).

Genotyping assay

Genotyping of the *fh224* 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 *fh224* mutation abolishes a site recognized by the *Ava*II restriction enzyme.

Primers:

fh224_05: 5' CCA TCA TCC AAG CAG CAC G 3'

fh224_06: 5' GCA CTC ATA AAG TTT TCC TCC AAC 3'

PCR program (55_30_30):

1. 94°C for 3 min
2. 94°C for 30 sec
3. 55°C for 30 sec
4. 72°C for 30 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: 355 bp**Digestion of the PCR product with the *Ava*II restriction enzyme:**

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	cleaved	271 bp and 84 bp
PCR product containing the mutation	unaffected	355 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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