foxd3^{zdf10}

Nature of the mutation

The *zdf10* allele contains a single nucleotide deletion that induces a frame shift and results in a premature stop codon within the DNA-binding domain of the Foxd3 protein (Stewart et al., Developmental Biology 292: 174-188, 2006).

Genotyping assay

Genotyping of the *zdf10* 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 *zdf10* mutation abolishes a site recognized by the AloI restriction enzyme.

Primers:
FOXD_01: 5' CCG CAG AAG AAG TTG ACG C 3'
FOXD_02: 5' GAT TCC CAA TGC CGT ATG C 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: 319 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	cleaved	184 bp, 103 bp and 32 bp
PCR product containing the mutation	unaffected	319 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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