dharma^{m168}

Nature of the mutation

The *m168* allele contains a single G-to-A point mutation that introduces a premature stop codon and truncates the *dharma* open reading frame at codon 70, 45 amino acids upstream of the homeodomain (Fekany et al., Development 126: 1427-1438, 1999).

Genotyping assay

Genotyping of the *m168* 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 *m168* mutation abolishes a site recognized by the MscI restriction enzyme.

Primers:
DHA_03: 5' CCA TTG ATT ACA TCT TAG GAG ACA CC 3'
DHA_04: 5' CCT GAC TTA AAA CTG CTT GCA TAA C 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: 422 bp

Digestion of the PCR product with the MscI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	cleaved	240 bp and 182 bp
PCR product containing the mutation	unaffected	422 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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