tbx1^{tm208}

Nature of the mutation

The *tm208* allele contains a single A-to-T point mutation at nucleotide position 877 of the *tbx1* coding region. This mutation introduces a premature stop codon near the end of the T-box, leading to the deletion of the entire C-terminus of the protein (Piotrowski et al., Development 130: 5043-5052, 2003).

Genotyping assay

Genotyping of the *tm208* 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 *tm208* mutation abolishes a site recognized by the AcuI restriction enzyme.

Primers:
TBX_03: 5' CAC AAG GCT CTG GAA TGA ACT TG 3'
TBX_04: 5' GCT ACA AAA CAA AGC ACA GTT ATG 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: 360 bp

Digestion of the PCR product with the AcuI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	cleaved	188 bp and 172 bp
PCR product containing the mutation	unaffected	360 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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