tbx1^{tu285}

Nature of the mutation

The *tu285* allele contains a single C-to-T point mutation at nucleotide position 364 of the *tbx1* coding region which introduces a premature stop codon and deletes 98% of the T-box as well as the whole C terminus of the protein (Piotrowski et al., Development 130: 5043-5052, 2003).

Genotyping assay

Genotyping of the *tu285* 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 *tu285* mutation creates a site recognized by the PacI restriction enzyme.

Primers:
TBX_09: 5' TCC AAC TCA GCA CAA GCC CC 3'
TBX_10: 5' CCA ATC AAG TGC ATT GAC GAT G 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: 438 bp

Digestion of the PCR product with the PacI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	438 bp
PCR product containing the mutation	cleaved	276 bp and 162 bp

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