bmp2b^{tc300a}

Nature of the mutation

The *tc300a* allele contains a single T-to-G point mutation that changes amino acid 344 from cysteine to a tryptophan. This missense mutation is the third of seven highly conserved cysteine residues, known to form disulfide bonds in other TGF-β molecules (Kishimoto et al., Development 124: 4457-4466, 1997; Nguyen et al., Developmental Biology 199: 93-110, 1998).

Genotyping assay

Genotyping of the *tc300a* 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 *tc300a* mutation creates a site recognized by the HaeIII restriction enzyme.

Primers:
SWI_03: 5' AAA AGC CGA GGA GAA AGC AC 3'
SWI_04: 5' AGT CTT CAT TGG GGA GAT TGT TC 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: 364 bp

Digestion of the PCR product with the HaeIII restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	364 bp
PCR product containing the mutation	cleaved	228 bp and 136 bp

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