acvr1^{tm110b}

Nature of the mutation

The *tm110b* allele contains a single T-to-C point mutation that substitutes Cys by Arg at amino acid position 91 in the extracellular domain of the Alk8 receptor (Bauer et al., Development 128: 849-858, 2001; Mintzer et al., Development 128: 859-869, 2001).

Genotyping assay

Genotyping of the *tm110b* 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 *tm110b* mutation creates a site recognized by the FspI restriction enzyme.

Primers:
ALK_01: 5' ATG GCT ACA GAC GAG TTC CTC TTT C 3'
ALK_02: 5' AGC TCC AGA TGT CGA TTC TCC TG 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: 367 bp

Digestion of the PCR product with the FspI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	367 bp
PCR product containing the mutation	cleaved	272 bp and 95 bp

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