ache^{tm205}

Nature of the mutation

The *tm205* allele contains a single C-to-A point mutation that substitutes Tyr139 by a premature stop codon (Downes and Granato., Developmental Biology 270: 232-245, 2004).

Genotyping assay

Genotyping of the *tm205* 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 *tm205* mutation creates a site recognized by the HincII restriction enzyme.

Primers:
ACA_11: 5' TTG AAA TGT GGA ACC CCA ACA G 3'
ACA_12: 5' GTT ACC GCC AAA GAA GTG GAT G 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: 332 bp

Digestion of the PCR product with the HincII restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	332 bp
PCR product containing the mutation	cleaved	163 bp and 169 bp

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