atp1a1^{m883}

Nature of the mutation

The *m883* allele contains a single C-to-T point mutation at nucleotide position 1881. The mutation creates a stop codon that truncates the protein after amino acid 568 (Ellertsdottir et al., Developmental Dynamics 235: 1794-1808, 2006).

Genotyping assay

Genotyping of the *m883* 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 *m883* mutation abolishes a site recognized by the HpyCH4III restriction enzyme.

Primers:
ATP_01: 5' AAG AGC AGC CTT TGG ATG ATG 3'
ATP_02: 5' TCT GAG ATG ATA CCC ACC CCC 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: 473 bp

Digestion of the PCR product with the HpyCH4III restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	cleaved	288 bp and 185 bp
PCR product containing the mutation	unaffected	473 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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