

tfap2a*^{m819}*Nature of the mutation**

The *m819* allele contains a single A-to-T point mutation that introduces a stop codon at residue 264 of the Tfap2a protein (Holzschuh et al., Development 130: 5741-5754, 2003).

Genotyping assay

Genotyping of the *m819* 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 *m819* mutation creates a site recognized by the EcoO109I restriction enzyme.

Primers:

TFA_01: 5' GAT GGA CAC TTG TAA CCA TAG TCT GG 3'

TFA_02: 5' CAG GAG AGT GAC ATT GGC AGC 3'

PCR program (53_30_30):

1. 94°C for 3 min
2. 94°C for 30 sec
3. **53°C for 30 sec**
4. 72°C for **30 sec**
5. Go to step 2 (above) for 39 cycles
6. 72°C for 5 min
7. 8.0°C hold
8. END

Product size: 370 bp**Digestion of the PCR product with the EcoO109I restriction enzyme:**

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	370 bp
PCR product containing the mutation	cleaved	267 bp and 103 bp

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