mef2ca^{b631}

Nature of the mutation

The *b631* allele contains a single A-to-C point mutation that changes the start methionine codon (ATG) into a leucine codon (CTG) (Miller et al., Developmental Biology 308: 144-157, 2007).

Genotyping assay

Genotyping of the *b631* 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 *b631* mutation creates a site recognized by the BstNI restriction enzyme.

Primers:
HOO_03: 5' GGA AGG AGA AGG AGA CGA GTG T 3'
HOO_02: 5' AAT TAC AAG TGC TTG ATG TTT GGA 3'

PCR program (53_30_30):

1. 94ºC for 3 min
2. 94ºC for 30 sec
3. **53**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: 361 bp

Digestion of the PCR product with the BstNI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	361 bp
PCR product containing the mutation	cleaved	290 bp and 71 bp

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