Zebrafish International Resource Center

mef2ca^{b1086}

Nature of the mutation

The *b1086* allele contains a single A-to-T point mutation that introduces an early stop codon upstream of the MEF2 domain (Miller et al., Developmental Biology 308: 144-157, 2007).

Genotyping assay

Genotyping of the *b1086* allele is based on the dCAPS assay (**d**erived **C**leaved **A**mplified **P**olymorphic **S**equence; Neff *et al.*, The Plant Journal 14(3): 387-392, 1998). In this assay, a restriction enzyme recognition site that includes the single nucleotide polymorphism (SNP) is introduced into the PCR product by a primer containing one or more mismatches to template DNA. The PCR product modified in this manner is then subjected to restriction enzyme digestion and the presence or absence of the SNP is determined by the resulting restriction pattern.

To genotype the *b1086* allele, a mismatch (marked in red) has been introduced into the reverse primer. During PCR, this mismatch and the *b1086* mutation create an SfcI restriction enzyme site in the amplified product. The SfcI site is not present in the PCR product derived from the WT DNA template.

Primers:
HOO_10: 5' ATT TCA TGT CAT GGA ACT AAA TCT GTT 3'
HOO_13d: 5' CGG CTC GTT GTA CTC GGT GTA CT 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: 243 bp

Digestion of the PCR product with the SfcI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	243 bp
PCR product containing the mutation	cleaved	217 bp and 26 bp