Zebrafish International Resource Center

sox32^{ta56}

Nature of the mutation

The *ta56* allele contains a single T-to-G point mutation that introduces a premature stop codon at residue 170 of the Sox32 protein and leads to a truncation of the protein shortly after the HMG domain (Dickmeis et al., Genes and Development 15: 1487-1492, 2001; Kikuchi et al., Genes and Development 15: 1493-1505, 2001).

Genotyping assay

Genotyping of the *ta56* 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 *ta56* mutation creates a site recognized by the BfaI restriction enzyme.

Primers:
CAS_03: 5' CCA GCA TAC CAT TGA CTA TCC TAA C 3'
CAS_04: 5' CCA CTT GAT GAT GTT GCC TCG 3'

PCR program (55_40_40):

1. 94ºC for 3 min
2. 94ºC for 30 sec
3. **55**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: 252 bp

Digestion of the PCR product with the BfaI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	252 bp
PCR product containing the mutation	cleaved	129 bp and 123 bp

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