dlat ^{a13}

Nature of the mutation

The *a13* allele contains a single C-to-T point mutation that results in a nonsense mutation at codon 554 (Taylor et al., Proc. Natl. Acad. Sci. USA 101(13): 4584-4589, 2004).

Genotyping assay

Genotyping of the *a13* 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 *a13* mutation creates a site recognized by the NlaIII restriction enzyme.

Primers:
NOA_08: 5' GAT GTT AGT GTG GCG GTC AG 3'
NOA_05: 5' ATG AGG TTG TAG TTT CCC ATC T 3'

PCR program (56_30_30):

1. 94ºC for 3 min
2. 94ºC for 30 sec
3. **56**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: 141 bp

Digestion of the PCR product with the NlaIII restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	141 bp
PCR product containing the mutation	cleaved	119 bp and 22 bp

Zebrafish International Resource Center (ZIRC)
5274 University of Oregon
Eugene, OR 97403-5274, USA
Phone: 541-346-6028
Email: genotyping@zebrafish.org
Web: http://zebrafish.org