edg5^{te273}

Nature of the mutation

The *te273* allele contains a single C-to-T point mutation that causes arginine to cysteine substitution at amino acid residue 167 of the Edg5 protein (Kupperman et al., Nature 406: 192-195, 2000).

Genotyping assay

Genotyping of the *te273* 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 *te273* mutation creates a site recognized by the ApeKI restriction enzyme.

Primers:
EDG_01: 5' CAT TTC ATT TGA CTC CTG TCC AGT G 3'
EDG_02: 5' TAG TAT CGG GTG TTG AGG GGC 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: 280 bp

Digestion of the PCR product with the ApeKI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	280 bp
PCR product containing the mutation	cleaved	148 bp and 132 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