cdh2^{m117}

Nature of the mutation

The *m117* allele contains a single T-to-G point mutation that substitutes tryptophan by glycine in the N-cadherin protein (Malicki et al., Developmental Biology 259: 95-108, 2003).

Genotyping assay

Genotyping of the *m117* 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 *m117* mutation creates a site recognized by the BsmBI restriction enzyme.

Primers:
NCA_03: 5' AGT GAT ACT GTT TCC TCG GCA C 3'
NCA_04: 5' TTT TGG TCT GCT CCT GGT CC 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: 333 bp

Digestion of the PCR product with the BsmBI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	333 bp
PCR product containing the mutation	cleaved	273 bp and 60 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