stil ^{cz65}

Nature of the mutation

The *cz65* allele contains a single G-to-A point mutation at nucleotide position 820 of the coding region resulting in a premature stop codon and protein truncation (Pfaff et al., Mol. Cell. Biol. 27(16): 5887-5897, 2007).

Genotyping assay

Genotyping of the *cz65* 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 *cz65* mutation abolishes a site recognized by the BccI restriction enzyme.

Primers:
STL_03: 5' TAA AAT GTG CTG TTC TGT CTC AGG 3'
STL_04: 5' GAT GTA TGG GCT ACT CAC TTG TCC 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: 363 bp

Digestion of the PCR product with the BccI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	cleaved	252 bp and 111 bp
PCR product containing the mutation	unaffected	363 bp

IMPORTANT NOTE: It is highly recommended to use WT positive controls to monitor whether enzyme digestion has been carried out to completion. Without this control, partially digested WT samples can be mistakenly regarded as heterozygous samples.

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