Zebrafish International Resource Center

szl ^{tm305}

Nature of the mutation

The *tm305* allele contains a single G-to-A point mutation that changes aspartate to asparagine at residue 92 of the Szl protein (Yabe et al., Development 130: 2705-2716, 2003; Martyn and Schulte-Merker, Developmental Biology 260: 58-67, 2003).

Genotyping assay

Genotyping of the *tm305* 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 *tm305* mutation abolishes a site recognized by the Taq$^{\alpha}$I restriction enzyme.

Primers:
SZL_05: 5' CCT CGA TCT GAC GAC TTG AGG A 3'
SZL_03: 5' GCC AGT TCT AAA TCA TGA GCT ACA C 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: 300 bp

Digestion of the PCR product with the Taq$^{\alpha}$I restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	cleaved	210 bp and 90 bp
PCR product containing the mutation	unaffected	300 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