Zebrafish International Resource Center

rx3^{w29}

Nature of the mutation

The *w29* allele contains a single T-to-A point mutation that changes Tyr to a stop codon at residue 133 of the Rx3 protein (Kennedy et al., Developmental Biology 270: 336-349, 2004).

Genotyping assay

Genotyping of the *w29* 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 *w29* mutation abolishes a site recognized by the BspEI restriction enzyme.

Primers:
CHK_01: 5' CTC TCT CTC TTT ATG CAG GAG TTT G 3'
CHK_02: 5' AGT GTC TCT CAC CTG TAC TCG GAC 3'

PCR program (58_40_40):

1. 94ºC for 3 min
2. 94ºC for 30 sec
3. **58**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: 257 bp

Digestion of the PCR product with the BspEI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	cleaved	182 bp and 75 bp
PCR product containing the mutation	unaffected	257 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