lof^{j6g1}

Nature of the mutation

The *j6g1* allele contains a deletion of approximately 500 kb mapped to LG2 (Iovine and Johnson, Genomics 79(6): 756-759, 2002).

Genotyping assay

Genotyping of the *j6g1* allele is based on an assay in which individual samples obtained from single haploid embryos are tested for the presence or absence of genomic markers covered by the deletion. The absence of marker-specific PCR products demonstrates that the sample contains the deletion. An additional primer set is used as an internal positive control in this assay.

To genotype the *j6g1* allele, the z24212 marker is used to monitor the presence or the absence of the *j6g1* deletion. The primer set, FOA01 and FOA02, is used as an internal positive control.

z24212-specific primers:

z24212A: 5' CCC TGT GGA CAA CCT GTA CA 3'
z24212B: 5' TGG ACA CAC ACA TGG GAG AT 3'
(The concentrations of z24212A and z24212B in the PCR reaction are 0.5µM each.)

Control primers:

FOA01: 5' CCG TAG GAG AAG GAG CAC AAC G 3'
FOA02: 5' TGA TTC GTC GCT TTG GCT CTA TAA C 3'
(The concentrations of FOA01 and FOA02 in the PCR reaction are 0.25µM each.)

PCR program (55_30_30):

1. 94°C for 3 min
2. 94°C for 30 sec
3. **55**°C for **30** sec
4. 72°C for **30** sec
5. Go to step 2 (above) for 39 cycles
6. 72°C for 5 min
7. 8.0°C hold
8. END

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

PCR product size for z24212A and z24212B is 263 bp
PCR product size for FOA01 and FOA02 is 172 bp

Samples from haploid embryos containing the *j6g1* deletion only generate a 172-bp product. Both 172-bp and 263-bp products are detected for WT samples.

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