ZIR ZEBRAFISH INTERNATIONAL RESOURCE CENTER

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Tg(TOP:GFP)w25

Transgene description

The Tg(TOP:GFP)w25 transgene contains the EGFP cDNA under control of the 358-bp promoter/enhancer region containing 4 consensus LEF binding sites and a minimal 94bp promoter derived from the mouse cFos gene (Dorsky et al., Developmental Biology 241:229-237, 2002).

Genotyping assay

To genotype the Tg(TOP:GFP)w25 line, the transgene-specific primers (EGFP6 and EGFP8) are used.

Primers:

EGFP6: 5' TTC TTC AAG TCC GCC ATG CCC G 3' EGFP8: 5' GCA CGC TGC CGT CCT CGA TGT T 3'

PCR program (62 40 40):

1 94°C for 3 min 2 94°C for 30 s 3 **62°**C for **40** s 4 72°C for **40** s 5 Go to step 2 (above) for 34 cycles 6 72°C for 5 min 7 8.0°C hold 8 END

Product size: 283 bp

The 283-bp product is specific for the genomic DNA containing the Tg(TOP:GFP)w25 transgene. No PCR product is generated for wild-type genomic DNA.

IMPORTANT NOTE: It is possible that multiple copies of the transgene might have integrated into the genome during transgenesis and that some of these integrations are non-functional. Samples that contain only a non-functional transgene or its fragment will be identified falsely as positive in the genotyping assay. For this reason, it is recommended to use functional assays to verify individuals identified as positive in the genotyping assay.