

## ***Tg(-6.5msxb:EGFP)zp4***

### **Transgene description**

The *Tg(-6.5msxb:EGFP)zp4* transgene contains the GFP cDNA under control of the regulatory regions of the *msxb* gene (Pyati et al., Development 133:2275-2284, 2006).

### **Genotyping assay**

To genotype the *Tg(-6.5msxb:EGFP)zp4* 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(-6.5msxb:EGFP)zp4* 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.