Genotyping protocol

EM:09285

B6;D-Tg(tetO-GFP,-lacZ)G3Rsp/Kctt

Primers

glob1 5´ **AGT GGT ATT TGT GAC CCA GGG CA** 3´
b-glob-as2 5´ **GAA TAT TTC TGC ATA TAA ATT CTG GCT GGC G** 3´
b-glob-as1 5´ **ATA ATT TGT CAG CAG TTT AAG GTT GCA AAC** 3´

PCR profile

95 ˚C, 5 min

94 ˚C, 30 s 35 cycles
55 ˚C, 45s
72 ˚C, 1 min

72 ˚C, 10 min
4 ˚C, ∞

PCR mix

10 x PCR Buffer II (Life technologies)	3.0 µl
$MgCl_2$ (25 mM)	1.8 µl
dNTP mix (25 mM of each nucleotide)	0.24 µl
glob1 (100 µM)	0.15 µl
b-glob-as2 (**50 µM**)	0.15 µl
b-glob-as1 (100 µM)	0.15 µl
AmpliTaq Gold (5 U/µl)	0.15 µl
DNA template (~ 0.5 µg tail DNA)	2.0 µl
H_2O	22.36 µl
	30 µl

Post-PCR analysis

1.5% agarose gel.
Expected pattern is **mutant: 321 bp**
Wt: 237 bp