

Genotyping protocol

EM:09283

TgTet0GFP-GluA2/1

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 ₂ O	<u>22.36 µl</u>
	30 µl

Post-PCR analysis

1.5% agarose gel.

Expected pattern is **mutant: 321 bp****Wt: 237 bp**