

# Genotyping protocol

EM:09257

TgTet0GFP-GluA1

## 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 <sub>2</sub> (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 <sub>2</sub> O	<u>22.36 μl</u>
	30 μl

## Post-PCR analysis

1.5% agarose gel.

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