

Genotyping protocol

EM:09212

Gria2tm2Rsp

Primers

VM10 5' **GTT GTC TAA CAA GTT GTT GAC C** 3'
VM12 5' **GCG TAA GCC TGT GAA ATA CCT G** 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-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>22.51 μl</u>
	30 μl

Post-PCR analysis

1.5% agarose gel.
Expected pattern is **mutant: 348 bp**
Wt: 254 bp