

Genotyping protocol

EM:09215

129-Gria3^{tm1Rsp}/Kctt

Primers

RC2 5' **CCA ATG TTG TGC TTT AGC CTT TGC** 3'
RC3 5' **GGT ATA TCT TCC CAG CCC CAA G** 3'

PCR profile

95 °C, 5 min

94 °C, 30 s 35 cycles

55 °C, 45s

72 °C, 45 s

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
RC2 (100 µM)	0.15 µl
RC3 (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 **wt: 238 bp**
Tg: 300 bp