

# Genotyping protocol

EM:12392

B6;129-Acan<sup>tm1c(EUCOMM)Hmgu/Kctt</sup>

## Primers

GT3_F	5' GAG GGC ATA CAG TCC ACC AT 3'
GT3WT_R	5' CCA TCA TCA GGC ATT CTC CTT 3'
GT3_R	5' GTG GTA TCG TTA TGC GCC TT 3'

## PCR profile

95 °C, 5 min	
95 °C, 30 s	35 cycles
58 °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
GT3_F (100 µM)	0.15 µl
GT3WT_R or GT3_R (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>22.51 µl</u>
	30 µl

## Post-PCR analysis

1% agarose gel.  
 Expected pattern is **mutant: 650 bp**  
**Wt: 638 bp**