

## Genotyping protocol

EM:09286 and 09287

**B6.129-Grin1<tm1Phs>/Kctt****B6.129-Grin1<tm2Phs>/Kctt**

### Primers

Rspneo4      5' GGC TAT TCG GCT ATG ACT GGG C 3'  
 Rspneo5      5' GGG TAG CCA ACG CTA TGT CCT G 3'

### PCR profile

95 °C, 5 min  
 94 °C, 30 s  
 58 °C, 50 s                      35 cycles  
 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
rspneo4 (100 µM)	0.15 µl
rspneo5 (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

1.5% agarose gel.  
 Expected pattern is **Tg: 641 bp**