

# Genotyping protocol

EM:13208

## B6N.129-Tfamtm1Lrsn/Kctt

### Primers

Tfam-A F1                    5' CTG CCT TCC TCT AGC CCG GG 3'  
Tfam-B R1                    5' GTA ACA GCA GAC AAC TTG TG 3'

### PCR profile

95 °C, 3 min  
  
95 °C, 30 s  
53 °C, 45 s                    30 cycles  
72 °C, 45 s  
  
72 °C, 7 min  
4 °C, ∞

### PCR mix

10 x PCR Buffer II (Life technologies)	2.0 µl
MgCl <sub>2</sub> (25 mM)	1.2 µl
dNTP mix (25 mM of each nucleotide)	0.16 µl
Tfam-A F1 (100 µM)	0.1 µl
Tfam-B R1 (100 µM)	0.1 µl
AmpliTaq Gold (5 U/µl)	0.13 µl
DNA template (~ 0.5 µg tail DNA)	1.0 µl
H <sub>2</sub> O	<u>15.31 µl</u>
	20 µl

### Post-PCR analysis

1.5% agarose gel.  
Expected pattern is wt:404bp, mut:437bp