

Genotyping protocol

EM:05269

B6.129P2-lapp^{tm1Sgm}/Kctt

Primers

miAPP1	5' CGA ATA CAT ATG GCA AGA G 3'
miAPP2	5' ACA CAC AGT CAT CAA GCA C 3'
miAPP5	5' TTG GGT GGA GAG GCT ATT CG 3'
miAPP6	5' GGC AGG AGC AAG GTG AGA TG 3'

PCR profile

94 °C, 5 min	
94 °C, 20 s	
56 °C, 30 s	39 cycles
72 °C, 30 s	
72 °C, 10 min	
4 °C, ∞	

PCR mix

10 x PCR Buffer II (Life technologies)	2.0 µl
MgCl ₂ (25 mM)	1.2 µl
dNTP mix (25 mM of each nucleotide)	0.16 µl
miAPP1/miAPP5 (100 µM)	0.1 µl
miAPP2/miAPP6 (100 µM)	0.1 µl
AmpliTaq Gold (5 U/µl)	0.13 µl
DNA template (~ 0.5 µg tail DNA)	1.0 µl
H ₂ O	<u>15.31 µl</u>
	20 µl

Post-PCR analysis

1.5% agarose gel.
 Wt allele: Primers 1+2, product length 203bp
 Mut allele: Primers 5+6, product length 288bp