

Genotyping protocol

EM:13210

B6.129-Gt(ROSA)26Sortm1Lrsn/Kctt

Primers

Rosa26-1F	5' AAA GTC GCT CTG AGT TGT TAT 3'
Rosa26-2R1	5' GCG AAG AGT TTG TCC TCA ACC 3'
Rosa26-2R2	5' GGA GCG GGA GAA ATG GAT ATG 3'

PCR profile

95 °C, 3 min	
95 °C, 30 s	
57 °C, 30 s	35 cycles
72 °C, 45 s	
72 °C, 7 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
Rosa26-1F (100 µM)	0.1 µl
Rosa26-2R1 (100 µM)	0.1 µl
Rosa26-2R2 (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>15.21 µl</u>
	20 µl

Post-PCR analysis

1.5% agarose gel.
Expected pattern is wt:583bp, mut:300bp