

Genotyping protocol

EM:10417

B6.129-Shisa3^{tm1Ktry}/Kctt

Primers

KOR	5' GAA CTT CAG GGT CAG CTT GC 3'
WTR	5' TTG TGG CCC GAG CTA GTA AG 3'
WTL	5' CTG CTT CAG TCG CAG CCT AC 3'

PCR profile

95 °C, 5 min

95 °C, 30 s

55 °C, 45 s

35 cycles

72 °C, 45 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
KOR (100 µM)	0.1 µl
WTR (100 µM)	0.1 µl
WTL (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:606bp, mut:403bp