Genotyping protocol

EM:09449

B6;129-Dsel^{tm1Mmac}/Kctt

Primers

Dsel fw	5´ ACG TGG TCA AAT GGC TTC AT 3´
Dsel rev wt	5´ GCT GTG AAA TCC AGG TGA CAT 3´
Dsel rev ko	5´ ATT AAG GGC CAG CTC ATT CC 3´

PCR profile

95 ˚C, 5 min

94 ˚C, 30 s — 35 cycles
58 ˚C, 45s
72 ˚C, 1 min

72 ˚C, 10 min
4 ˚C, ∞

PCR mix

10 x PCR Buffer II (Life technologies)	3.0 µl
$MgCl_2$ (25 mM)	1.8 µl
dNTP mix (25 mM of each nucleotide)	0.24 µl
Dsel fw (100 µM)	0.15 µl
Dsel rev wt (100 µM)	0.15 µl
Dsel rev ko (100 µM)	0.15 µl
AmpliTaq Gold (5 U/µl)	0.15 µl
DNA template (~ 0.5 µg tail DNA)	2.0 µl
H_2O	22.36 µl
	30 µl

Post-PCR analysis

1.5% agarose gel.
Expected pattern is **mutant: 476bp**
Wt: 274bp