Genotyping protocol

EM:08916

B6.129-Dse^{tm1Mmac}/Kctt

Primers

5' forward primer (Dse_fwd) - 5´ **agc aca ttg cag ctc ggc tta c** 3´
3' reverse primer (Dse_rev_wt) - 5´ **gct gcc atc ctc tcc atg tag tc** 3´
3' reverse primer (Dse_rev_neo) - 5´ **tgg atg tgg aat gtg tgc gag g** 3´

PCR profile

95 ˚C, 5 min

95 ˚C, 40 s — 35 cycles
60 ˚C, 40s
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
forward primer (Dse_fwd) (100 µM)	0.15 µl
reverse primer (Dse_rev_wt) (**20 µM**)	0.15 µl
reverse primer (Dse_rev_neo) (100 µM)	0.15 µl
AmpliTaq Gold (5 U/µl)	0.2 µl
DNA template (~ 0.5 µg tail DNA)	2.0 µl
H_2O	22.46 µl
	30 µl

Post-PCR analysis

1.5% agarose gel.
Expected pattern is **mutant: 211 bp**
Wt: 454 bp