Genotyping protocol

EM:06222

STOCK Pmel<tm1.1Sson>/Kctt

Primers

primer A- 5´ **cca agt gta ggc acc act at** 3´
primer B - 5´ **aga tcc tgg gat cct tag tc** 3´
primer D- 5´**ttc cca ctc tag agg atc ag** 3´

PCR profile

95 ˚C, 6 min

94 ˚C, 30 s — 35 cycles
58 ˚C, 30 s
72 ˚C, 30 s

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
primer A (100 µM)	0.15 µl
primer B (100 µM)	0.15 µl
primer D (100 µM)	0.2 µl
AmpliTaq Gold (5 U/µl)	0.2 µl
DNA template (~ 0.5 µg tail DNA)	2.0 µl
H_2O	22.26 µl
	30 µl

Post-PCR analysis

2% agarose gel.
Expected pattern is **KO: 319 bp**
Wt: 236 bp