PROTOCOL FOR Ukko;Hk2+/- MOUSE GENOTYPING

Procedure

Genotyping of offspring from Ukko;Hk2+/- breeding colony is based on PCR. Run all three primers in the same reaction.

PCR primers

5' forward primer (Hk2KO-F2) - 5´ **act ctc ctg ccg ccc tgc** 3´
3' reverse primer (Hk2KO-R1) - 5´ **ccc ctc atc gcc acc gc** 3´
3' reverse primer (Neo-R1) - 5´ **gtg ccc agt cat agc cga ata gc** 3´

PCR profile; programme UKKO

95 ˚C, 6 min

94 ˚C, 30 s 40 cycles
56 ˚C, 30 s
72 ˚C, 1 min

72 ˚C, 10 min
4 ˚C, ∞

PCR mix

10 x PCR Gold buffer (Perkin Elmer)	3.0 µl
$MgCl_2$ (25 mM)	1.5 µl
dNTPs (10 mM)	0.5 µl
Hk2KO-F2 (20 µM)	1.0 µl
Hk2KO-R1 (20 µM)	0.5 µl
Neo-R1 (20 µM)	0.5 µl
AmpliTaq Gold (5 U/µl)	0.2 µl
DNA template (~ 0.5 µg tail DNA)	2.0 µl
H_2O	20.8 µl
	30 µl

Post-PCR analysis

Load 12 µl of the PCR reaction on a 1% agarose gel.
Expected pattern; for wt 239 bp and for mutant 432 bp.

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