PROTOCOL FOR Jnk1 KO MOUSE GENOTYPING

Procedure

Genotyping of offspring from Jnk1 KO breeding colony is based on PCR.

PCR primers

5' forward primer (J1-forw) 5´ **tcg acc cat gct aag cgc gcc** 3´
3' reverse primer (J1-rev) 5´ **cta ctt aat aac ggg ggt gga gga tca c** 3´
3' reverse primer (J1-lacZ) 5´ **cgg tgc ggg cct ctt cgc** 3'

PCR profile – JNKETT

95 ˚C, 10 min

95 ˚C, 45 s 35 cycles
60 ˚C, 45 s
72 ˚C, 1 min

72 ˚C, 10 min
4 ˚C, ∞

PCR mix - the PCR's for KO versus wt are run separate.

10 x PCR Gold buffer (Perkin Elmer)	3.0 µl
$MgCl_2$ (25 mM)	2.0 µl
dNTPs (10 mM)	0.5 µl
J2-forw (20 µM)	0.5 µl
J2-rev (20 µM)	0.5 µl
J2-lacZ	*0.5 µl*
AmpliTaq Gold (5 U/µl)	0.2 µl
DNA template (~ 0.5 µg tail DNA)	2.0 µl
ddH_2O	21.3 µl
	30 µl

Post-PCR analysis

Load 10 µl of the PCR reaction on a 1,5 % agarose gel.
Expected results; two bands – 150 bp (KO; J1-forw + J1-lacZ) and 200 bp (wt; J1-forw + J1-rev)