PROTOCOL FOR Jnk2 KO MOUSE GENOTYPING

Procedure

Genotyping of offspring from Jnk2 KO breeding colony is based on PCR.

PCR primers

5' forward primer (J2-forw) 5´ **cga agc agc agc cct cag gat cc** 3´
3' reverse primer (J2-rev) 5´ **ggt tct gac gtc ctg ggc tgg ac** 3´
3' reverse primer (J2-lacZ) 5´ **gcc tcc agt aca gcg cgg ctg** 3'

PCR profile – JNKTVA

95 ˚C, 5 min

94 ˚C, 45 s 40 cycles
58 ˚C, 45 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)		2.0 µl
dNTPs (10 mM)		0.5 µl
J2-forw (20 µM)		1.0 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		20.3 µl
		30 µl

Post-PCR analysis

Load 10 µl of the PCR reaction on a 1 % agarose gel.
Expected results; two bands – 750 bp (KO; J2-forw + J2-lacZ) and 600 bp (wt; J2-forw + J2-rev)