PROTOCOL FOR FoxB1 MOUSE GENOTYPING

Procedure

Genotyping of offspring from FoxB1 breeding colony is based on PCR.

PCR primers

5′ forward primer (SG1) - 5′ **cac tgg gat ggc ggg caa cgt ctg** 3′
3′ reverse primer (SG2) - 5′ **cat cgc tag gga gta caa gat gcc** 3′
3′ reverse primer (SG3) - 5′ **cga tgc ctg ctt gcc gaa tat cat gg** 3′

PCR profile

94 ˚C, 5 min

94 ˚C, 30 s	35 cycles
65 ˚C, 40 s	
72 ˚C, 40 s + 5 second autoext./cyle	

72 ˚C, 10 min
4 ˚C, ∞

PCR mix

10 x PCR Gold buffer (Amersham)	2.5 µl
dNTPs (10 mM each)	0.5 µl
primer SG1 (20 µM)	0.75 µl
primer SG2 (20 µM)	0.25 µl
primer SG3 (20 µM)	0.5 µl
polymerase (5 U/µl)	0.25 µl
DNA template (~ 0.5 µg tail DNA)	1.0 µl
ddH_2O	19.25 µl
	25 µl

Post-PCR analysis

Load 10 µl of the PCR reaction on a 1.5 % agarose gel.
Expected results; wt ~ 250 bp and tg ~ 600 bp