PROTOCOL FOR bim MOUSE GENOTYPING

Procedure

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

PCR primers

5' forward primer (PB20) 5´ **cat tct cgt aag tcc gag tct** 3´
3' reverse primer (PB335(a)) 5´ **gtg cta act gaa acc aga tta g** 3´
3' reverse primer (PB65) 5´ **ctc agt cca ttc atc aac ag** 3´

PCR profile – BIM

95 ˚C, 10 min

94 ˚C, 30 s   35 cycles
58 ˚C, 30 s
72 ˚C, 45 s

72 ˚C, 10 min
4 ˚C, ∞

PCR mix

10 x PCR Gold buffer (Perkin Elmer)	3.0 µl	
$MgCl_2$ (25 mM)	2.5 µl	
dNTPs (10 mM)	0.5 µl	
PB20 (20 µM)	1.0 µl	
PB335(a) (20 µM)	0.5 µl	
PB65 (20 µM)	0.5 µl	
AmpliTaq Gold (5 U/µl)	0.2 µl	
DNA template (~ 0.5 µg tail DNA)		1.0 µl
ddH_2O		20.8 µl
	30 µl	

Post-PCR analysis

Load 10 µl of the PCR reaction on a 2.5 % agarose gel.
Expected results; two bands band – wt gives a 400 bp fragment and mutant a 540 bp fragment.