PROTOCOL FOR B2+(Th-IRES-BMPR2trunc-neomycin+) MOUSE GENOTYPING

Procedure

Genotyping of offspring from B2+(Th-IRES-BMPR2trunc-neomycin+) breeding colony is based on PCR.

PCR primers

For wt:
5' forward primer (5TH) 5´ **act gaa ttc acc ctg acc caa gca ct** 3´
3' reverse primer (HB22b) 5´ **tat gaa ttc cag cca tgg ctt cct cgc tgc atcg** 3´

For B2+:
5' forward primer (3TH) 5´ **act gaa ttc ttt cct tcc ttt att gag at** 3´
3' reverse primer (HB24) 5´ **ctg tct aga tca gag gtc ctc ttc gga aat aag ttt ctg ttc acg ctg ttt c** 3´

PCR profile – TEDX3

94 ˚C, 5 min

94 ˚C, 45 s 35 cycles
56 ˚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)	2.5 µl
dNTPs (10 mM)	0.5 µl
5TH (20 µM)	0.5 µl
HB22b (20 µM)	0.5 µl
3TH (20 µM)	0.5 µl
HB24 (20 µM)	0.5 µl
AmpliTaq Gold (5 U/µl)	0.2 µl
DNA template (~ 0.5 µg tail DNA)	2.0 µl
ddH_2O	19.8 µl
	30 µl

Post-PCR analysis

Load 10 µl of the PCR reaction on a 1 % agarose gel. Expected results; two bands – 280 bp (for wt-allele) and 550 bp (for B2+ allele).