PROTOCOL FOR en1-PDGFB-IRES-lacZ MOUSE GENOTYPING

Procedure

Genotyping of offspring from en1-PDGFB-IRES-lacZ breeding colony is based on PCR.

PCR primers

5' forward primer (A4691) - 5´ **tgc tgc tac ctg cgt ctg** 3´
3' reverse primer (A4692) - 5´ **ttc ttc cgc aca atc tcg** 3´

PCR profile

94 ˚C, 10 min

94 ˚C, 30 s 40 cycles
55 ˚C, 30 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)	3.0 µl
dNTPs (10 mM)	0.50 µl
A4691 primer (20 µM)	0.25 µl
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AmpliTaq Gold (5 U/µl)	0.20 µl
DNA template (~ 0.5 µg tail DNA)	2.0 µl
H_2O	20.80 µl
	30 µl

Post-PCR analysis

Load 10 µl of the PCR reaction on a 1% agarose gel.
Expected pattern is one band; 452 bp