PROTOCOL FOR Pdgfrb-849N MOUSE GENOTYPING

PCR primers

5' forward primer (HH31) 5´ **ttt ggg tta gac act tag gaa t** 3´
3' reverse primer (wt158) 5´ **cag cag agg gtt atg ggt tac t** 3´

Cycling parameters – named PDGFRB-D

95 ˚C, 6 min

94 ˚C, 30 s — 40 cycles
53 ˚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)	2.0 µl
dNTPs (10 mM)	0.5 µl
HH31 (20 µM)	0.5 µl
Wt158 (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	22.3 µl
	30 µl

Post-PCR analysis

Wt fragment: roughly 150 bp
Mut fragment (includes loxP-site): 184 bp