PROTOCOL FOR Pdgfrbdelta P13K 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´
3' reverse primer (neo antisense) 5' **tgg cta ccc gtg ata ttg ct** 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)	1.0 μl
Wt158 (20 μM)	0.5 μl
Neo antis. (20μM)	0.5 μl
AmpliTaq Gold (5 U/μl)	0.2 μl
DNA template (~ 0.5 μg tail DNA)	1.0 μl
ddH_2O	21.3 μl
	30 μl

Post-PCR analysis

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