PROTOCOL FOR Irf8 MOUSE GENOTYPING

PCR primers

5' Forward primer (Irf-1) 5´ **cat ggc act ggt cca gat gtc ttc c** 3´
3' Reverse primer (Irf-2) 5´ **ctt cca ggg gat acg gaa cat ggt c** 3´
3' Reverse primer (A545 mutant) 5´ **ttg gcg cct acc ggt gg** 3´

Cycling parameters – named IRF

94 ˚C, 6 min

94 ˚C, 30 s — 35 cycles
64 ˚C, 30 s
72 ˚C, 40 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
Irf-1 (20 μM)	1.0 μl
Irf-2 (20 μM)	0.5 μl
A545 mut (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	21.3 μl
	30 μl

Post-PCR analysis

Wt fragment: ~390 bp
Mut fragment: ~ 490 bp