PROTOCOL FOR Testastin 3BE6 MOUSE GENOTYPING

PCR primers

5' forward primer (Testastin-P1) 5´ **cag agt ctc agg aca tag tc** 3´
3' reverse primer (Testastin-P2) 5´ **gtt cca tcc tgt agg cat** 3´
3' reverse primer (Testastin-Pneo) 5´ **cgc att gtc tga agt agg t** 3´

Cycling parameters – named TESTASTN

95 ˚C, 6 min

94 ˚C, 30 s 40 cycles
56 ˚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
Testastin-P1 (20 μM)	1.0 μl
Testastin-P2 (20 μM)	0.5 μl
Testastin-Pneo (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	20.3 μl
	30 μl

- It is sometimes preferble to run separate PCR's for wt and mut.

Post-PCR analysis

Wt fragment (P1/P2): 612 bp
Mut fragment (P1/Pneo): 349 bp