PROTOCOL FOR Hoxb4 KO MOUSE GENOTYPING

PCR primers

For wt:
Primer (Hox-3) 5´ **gga agc aag aaa agg agg aag aaa gga** 3´
Primer (Hox-4) 5´ **caa agt ggg tac aga cag gga gga aag** 3´

For mut:
Primer (Hox-4) 5´ **caa agt ggg tac aga cag gga gga aag** 3´
Primer (Hox-5) 5´ **gtt gac ata aac act ccg ctc ata** 3´

Cycling parameters – named HOX

94 ˚C, 6 min

94 ˚C, 40 s 35 cycles
60 ˚C, 40 s
72 ˚C, 40 s

72 ˚C, 10 min
4 ˚C, ∞

PCR mix	**for wt**	**for mut**
10 x PCR Gold buffer (Perkin Elmer)	3.0 µl	3.0 µl
$MgCl_2$ (25 mM)	2.0 µl	2.0 µl
dNTPs (10 mM)	0.5 µl	0.5 µl
Hox-3 (20 µM)	0.5 µl	-
Hox-4 (20 µM)	0.5 µl	-
Hox-4 (20 µM)	-	0.5 µl
Hox-5 (20 µM)	-	0.5 µl
AmpliTaq Gold (5 U/µl)	0.2 µl	0.2 µl
DNA template (~ 0.5 µg tail DNA)	2.0 µl	2.0 µl
ddH_2O	21.3 µl	21.3 µl
	30 µl	30 µl

Post-PCR analysis

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