

PROTOCOL FOR Hoxb3/b4 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-5) 5' **gtt gac ata aac act ccg ctc ata** 3'

Primer (Hox-6) 5' **gag tgt cac caa tgc cct cct gct** 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-5 (20 µM)	-	0.5 µl
Hox-6 (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 ₂ O	<u>21.3 µl</u>	<u>21.3 µl</u>
	30 µl	30 µl

Post-PCR analysis

Wt fragment: ~ 390 bp

Mut fragment: ~ 390 bp