

PROTOCOL FOR Zbed6 MOUSE GENOTYPING

PCR primers

5' forward primer (Zbed6_Lox_PCR_F) - 5' **ggg aaa gcc agt gtt gtt gt** 3'

3' reverse primer (Zbed6_Lox_PCR_R1) - 5' **ggg gag tca aat tcc ctt tc** 3'

3' reverse primer (Zbed6_Lox_PCR_R2) - 5' **aag tcc ttc aac gct tca ctg** 3'

F+R1= floxed allele 385bp

F+R1=wt allele 328bp

F+R2=KO allele 295bp

PCR profile

95 °C, 5 min

95 °C, 30 s 5 cycles

60 °C, 30 s

72 °C, 30 s

95 °C, 30 s 30 cycles

56 °C, 30 s

72 °C, 30 s

72 °C, 5 min

4 °C, ∞

PCR mix

10 x PCR buffer	2.5 µl
MgCl ₂ (25 mM)	1.3 µl
dNTPs (25 mM each)	0.2 µl
Lox F (100 µM)	0.075 µl
Lox R1 (100 µM)	0.15 ul
Lox R2 (100 µM)	0.075 µl
AmpliTaq Gold (5 U/µl)	1 µl
DNA template (~ 0.5 µg tail DNA)	2.0 µl
H ₂ O	<u>17.7 µl</u>
	25 µl

Post-PCR analysis

Load 12 µl of the PCR reaction on a 3% agarose gel , run for 1.5-2h.

Expected pattern; for wt 328 bp and for KO 295 bp.