

PROTOCOL FOR Ret KO MOUSE GENOTYPING

Procedure

Genotyping of offspring from Ret KO breeding colony is based on PCR.

PCR primers

wt allele:

5' forward primer (Ret forw.wt) - 5' **gct cca ctc tta ctc tgc tga** 3'

3' reverse primer (Ret rev.wt/ Ret rev.KO)

- 5' **gca cat aca ccg gca cac aca** 3'

mutant allele:

5' forward primer (Ret forw.KO) - 5' **aca gtg tct ggg att cca ctt g** 3'

3' reverse primer (Ret rev.wt/ Ret rev.KO)

- 5' **gca cat aca ccg gca cac aca** 3'

PCR profile; programme RET KO

95 °C, 6 min

94 °C, 45 s

35 cycles

60 °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)	1.0 µl
dNTPs (10 mM)	0.5 µl
Ret.forw.wt (20 µM)	0.5 µl
Ret.rev.wt (20 µM)	0.5 µl
AmpliTaq Gold (5 U/µl)	0.2 µl
DNA template (~ 0.5 µg tail DNA)	2.0 µl
H ₂ O	<u>20.8 µl</u>
	30 µl

Post-PCR analysis

Load 12 µl of the PCR reaction on a 1% agarose gel.

Expected pattern; one band 489 bp.

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