

## PROTOCOL FOR Ret flox MOUSE GENOTYPING

### *Procedure*

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

### *PCR primers*

5' forward primer (Ret forw.wt) 5' **gct cca ctc tta ctc tgc tga** 3'  
3' reverse primer (Ret rev.wt) 5' **gca cat aca ccg gca cac aca** 3'

### *PCR profile; program RET WT*

95 °C, 6 min

94 °C, 45 s                          40 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 <sub>2</sub> (25 mM)	2.0 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
ddH <sub>2</sub> O	<u>21.3 µl</u>
	30 µl

### *Post-PCR analysis*

Load 13 µl of the PCR reaction on a 1 % agarose gel.  
Expected results; wt – 274 bp and for mutant – 489 bp