

PROTOCOL FOR Rotatin XV-53 MOUSE GENOTYPING

Procedure

Genotyping of offspring from Rotatin XV-53 breeding colony is based on PCR.

PCR primers

5' forward primer (lacZ – F) 5' **cgt cac act acg tct gaa cgt cg** 3'
3' reverse primer (lacZ – R) 5' **cag acg att cat tgg cac cat gc** 3'

PCR profile

95 °C, 10 min

95 °C, 30 s
65 °C, 30 s
72 °C, 40 s

40 cycles

72 °C, 10 min

4 °C, ∞

PCR mix

10 x PCR Gold buffer (Perkin Elmer)	5.0 µl
MgCl ₂ (25 mM)	3.0 µl
dNTPs (10 mM)	1.0 µl
lacZ – F (20 µM)	0.5 µl
lacZ – R (20 µM)	0.5 µl
AmpliTaq Gold (5 U/µl)	0.25 µl
DNA template (~ 0.5 µg tail DNA)	1.0 µl
ddH ₂ O	<u>38.75 µl</u>
	50 µl

Post-PCR analysis

Load 10 µl of the PCR reaction on a 1.5 % - 2% agarose gel.

Expected results; One band - 400 bp (Gives heterozygous animals, since the homozygous animals dies at 11.5 dpc).