

PROTOCOL FOR WAP-SVt MOUSE GENOTYPING

Procedure

Genotyping of offspring from WAP-SVt breeding colony is based on PCR.

PCR primers

5' forward primer (WAPSVt-1); 5' **gct ttg caa aga tgg ata aag** 3'
3' reverse primer (WAPSVt-2); 5' **act aaa cac agc atg act c** 3'

PCR profile – WAP

95 °C, 10 min

95 °C, 45 s 40 cycles

55 °C, 45 s

72 °C, 1min

72 °C, 10 min

4 °C, ∞

PCR mix

10 x PCR Gold buffer (Perkin Elmer)	3.0 µl
MgCl ₂ (25 mM)	2.0 µl
dNTPs (10 mM)	0.5 µl
WAPSVt-1 (20 µM)	0.5 µl
WAPSVt-2 (20 µM)	0.5 µl
AmpliTaq Gold (5 U/µl)	0.2 µl
DNA template (~ 0.5 µg tail DNA)	2.0 µl
ddH ₂ O	<u>21.3 µl</u>
	30 µl

Post-PCR analysis

Load 10 µl of the PCR reaction on a 1 % agarose gel.
Expected results; one band – 813 bp fragment.