PROTOCOL FOR 3C7 MOUSE GENOTYPING

Procedure

Genotyping of offspring from 3C7 breeding colony is based on PCR.

PCR primers

For wt:
5' forward primer (primer 1) 5´ **cca atc ttg ctt ctt tgc tga gc** 3´
3' reverse primer (primer 3) 5´ **ggc tca tgc ttg aat gtt tca g** 3´

For tg:
5' forward primer (primer 1) 5´ **cca atc ttg ctt ctt tgc tga gc** 3´
3' reverse primer (primer 2) 5´ **atc atg caa gct ggt ggc tg** 3´

PCR profil; program 3C7-WT versus 3C7-TG

For wt:

94 ˚C, 10 min

94 ˚C, 1 min — 35 cycles
55 ˚C, 1 min
72 ˚C, 1 min

72 ˚C, 10 min
4 ˚C, ∞

For tg:

95 ˚C, 10 min

95 ˚C, 1 min — 35cycles
60 ˚C, 1 min
72 ˚C, 1 min + 1 s/cycle

72 ˚C, 10 min
4 ˚C, ∞

PCR mix

	For wt:	*For tg:*
10 x PCR Gold buffer (Perkin Elmer)	5.0 µl	5.0 µl
$MgCl_2$ (25 mM)	2.5 µl	3.0 µl
dNTPs (10 mM)	0.5 µl	0.5 µl
primer 1 (20 µM)	0.5 µl	0.5 µl
primer 2 (20 µM)	-	0.5 µl
primer 3 (20 µM)	0.5 µl	-
AmpliTaq Gold (5 U/µl)	0.25 µl	0.25 µl
DNA template (~ 0.5 µg tail DNA)	1.0 µl	1.0 µl
ddH_2O	18.75 µl	19.25 µl
	30 µl	30 µl

Post-PCR analysis

Load 10 µl of the PCR reaction on a 1.5 % agarose gel.
Expected results; wt ~ 280 bp and tg ~ 600 bp