PROTOCOL FOR Tie1-Cre MOUSE GENOTYPING

Procedure

Genotyping of offspring from Tie1-Cre breeding colony is based on PCR. Homozygous animals are breed.

PCR primers

5' forward primer (cre-forward) - 5´ **aac atg ctt cat cgt cgg** 3´
3' reverse primer (cre-reverse) - 5´ **ttc gga tca tca gct aca cc** 3´

PCR profile, program TIE1-CRE

95 ˚C, 10 min

94 ˚C, 30 s	10 cycles, one degree lower/cycle
63 ˚C, 30 s	
72 ˚C, 30 s	
95 ˚C, 30 s	34 cycles
53 ˚C, 30 s	
72 ˚C, 30 s	

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	
MyHCa (20 µM)	0.5 µl	
KCNE1 (20 µM)	0.5 µl	
AmpliTaq Gold (5 U/µl)	0.2 µl	
DNA template (~ 0.5 µg tail DNA)		1.0 µl
ddH_2O		22.3 µl
	30 µl	

Post-PCR analysis

Load 10 µl of the PCR reaction on a 1% agarose gel.
Expected results; one band is given ~ 450 bp