Genotyping of Lck-Cre mice

1. Method used in our animal resource bank

This section describes our method we are routinely using for genotyping Lck-Cre mice.

1.1) Primer sequences:

- Primer1: Lck-pro-F
  - Sequence: 5'- CCTTGGTGGAGGAGGGTGGAATGAA -3' (25-mer)
- Primer2: cre-cr-R
  - Sequence: 5'- AATGTTGCTGGATAGTTTTTACTGC -3' (25-mer)
- Primer3: Lck-cr-R
  - Sequence: 5'- TAGAGCCCTGTTCTGGAAGTTACAA -3' (25-mer)

1.2) Reaction mixture:

	Tube (μL)
Water	7.5
Primer1 (Lck-pro-F,10 μM)	0.5
Primer2 (cre-cr-R,10 μM)	0.5
Primer3 (Lck-cr-R,10 μM)	0.5
Taq polymerase (U/μL)	10
DNA extracted from tail (diluted 500 times)	1
total	20

Taq polymerase: HotStarTaq Master Mix Kit (Qiagen). The enzyme is a chemically modified Taq polymerase for hot start PCR and needs 15-min incubation at 95 °C for activation. Master Mix contains enzyme, dNTP, Mg, etc at 2 x concentration. Please see Qiagen's website for details (http://www1.qiagen.com/Products/Pcr/HotStarTaqSystem/HotStarTaqMasterMix.aspx).

1.3) Thermal cycles:

95 °C	15 min	Enzyme activation and first denature
93 °C	1 min	40 cycles
55 °C	1 min	
72 °C	2 min	
72 °C	5 min	once
4 °C	∞	

Thermal cycler: Veriti with 0.2mL tubes.

1.4) Product size:

Primers 1 and 2: 600 bp for Tg alleles

Primers 1 and 3: 350 bp for wild-type alleles

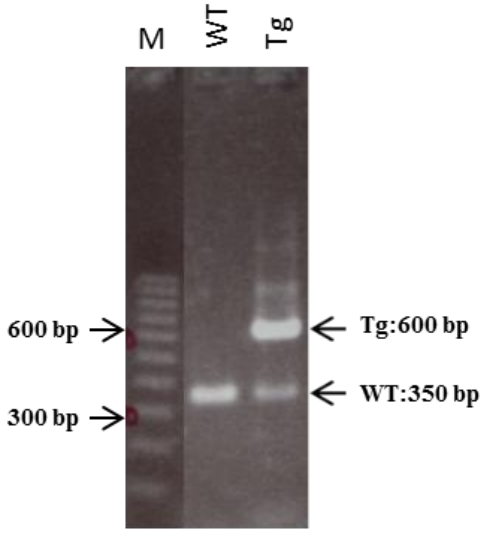

Fig.1. Electropherogram of PCR products from wild and heterozygous mice.

1.5) Reference

Takahama Y, Ohishi K, Tokoro Y, Sugawara T, Yoshimura Y, Okabe M, Kinoshita T, Takeda J. Functional competence of T cells in the absence of glycosylphosphatidylinositol-anchored proteins caused by T cell-specific disruption of the Pig-a gene. Eur J Immunol. 1998 Jul;28(7):2159-66.

Nibio_ID=025, May 31st, 2012