Genotyping of LECT2 KO mice

1. Method used in our animal resource bank

This section describes our method we are routinely using for genotyping LECT2 KO mice. This method is a little bit modified from the original (see the next section).

1.1) Primer sequences:

- Primer1: LECT2-common
  - Sequence: 5'- CCACCCCACCTAAGATGTATGCTGC -3' (25-mer)
- Primer2: LECT2-wild-type
  - Sequence: 5'- CCAGGATTCTAATGTCGTCCTTGTTGG -3' (25-mer)
- Primer3: LECT2-knockout
  - Sequence: 5'- CCTTCTTGACGAGTTCTTCTGAGGGG -3' (25-mer)

1.2) Reaction mixture:

	For wild allele	For KO allele
	Tube 1 (µL)	Tube 2 (µL)
Water	8	8
Primer1 (LECT2-common,10 µM)	0.5	0.5
Primer2 (LECT2-wild-type,10 µM)	0.5	-
Primer3 (knockout,10 µM)	-	0.5
Taq polymerase (U/µL)	10	10
DNA extracted from tail (diluted 200 times)	1	1
total	20	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).

See section 2 for primer info.

1.3) Thermal cycles:

95 °C	15 min	Enzyme activation and first denature
94 °C	20 sec	35 cycles
60 °C	30 sec	
72 °C	1 min	
72 °C	5 min	once
4 °C	∞	

Thermal cycler: Veriti with 0.2mL tubes.

1.4) Product size:

Primers 1 and 2: 517 bp for wild-type alleles
Primers 1 and 3: 385 bp for KO alleles

2. The original method provided by the original depositors

This chapter describes the genotyping method the original depositors provided.

2.1) Reaction mixture:

	Tube 1(μL)	Tube 2 (μL)
Water	8.75	8.75
10 x Buffer	1.25	1.25
2.5 mM dNTP	1	1
Primer1 (LECT2-common, 100 μM)	0.25	0.25
primer2 (LECT2-wild-type, 100 μM)	0.25	-
primer3 (knockout, 100 μM)	-	0.25
Taq polymerase (5 U/μL)	0.25	0.25
DNA (0.2 μg/μL)	0.5	0.5
total	12.5	12.5

Taq polymerase: TaKaRa Ex Taq

2.2) Thermal cycles:

94 °C	1 min	once
94 °C	20 sec	30 cycles
60 °C	30 sec	
72 °C	1 min	
72 °C	5 min	once

Thermal cycler:

2.3) Reference

Saito T, Okumura A, Watanabe H, Asano M, Ishida-Okawara A, Sakagami J, Sudo K, Hatano-Yokoe Y, Bezbradica JS, Joyce S, Abo T, Iwakura Y, Suzuki K, Yamagoe S. Increase in hepatic NKT cells in leukocyte cell-derived chemotaxin 2-deficient mice contributes to severe concanavalin A-induced hepatitis. J Immunol. 2004 Jul 1; 173(1):579-85. PMID: 15210819

Nbio_ID=176, 177, May 30th, 2012