Genotyping of Zbp1 KO mice

1. Method used in our animal resource bank

This section describes our method we are routinely using for genotyping Zbp1 KO mice.

1.1) Primer sequences:

Primer1: Zbp1 extra

- Sequence: 5'- ACTAAATTCAGGCTGACCCCTGTGT -3' (25-mer)

Primer2: Zbp1 wild

- Sequence: 5'- TGTATGTGCACCACATGCATACAGT -3' (25-mer)

Primer3: pgkrc2

- Sequence: 5'- CTAAAGCGCATGCTCCAGACTGCCTTG -3' (27-mer)

1.2) Reaction mixture:

	For wild allele	For KO allele
	Tube 1 (µL)	Tube 2 (µL)
Water	8	8
Primer1 (Zbp1 extra, 10µM)	0.5	0.5
Primer2 (Zbp1 wild, 10µM)	0.5	-
Primer3 (pgkrc2, 10µM)	-	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).

1.3) Thermal cycles:

95 °C	15 min	Enzyme activation and first denature
94 °C	30 sec	35 cycles
60 °C	1 min	
72 °C	1 min	
72 °C	10 min	once
4 °C	∞	

1.4) Product size:

Primers 1 and 2: approx. 1200 bp for wild-type alleles
Primers 1 and 3: approx. 1200 bp for KO alleles

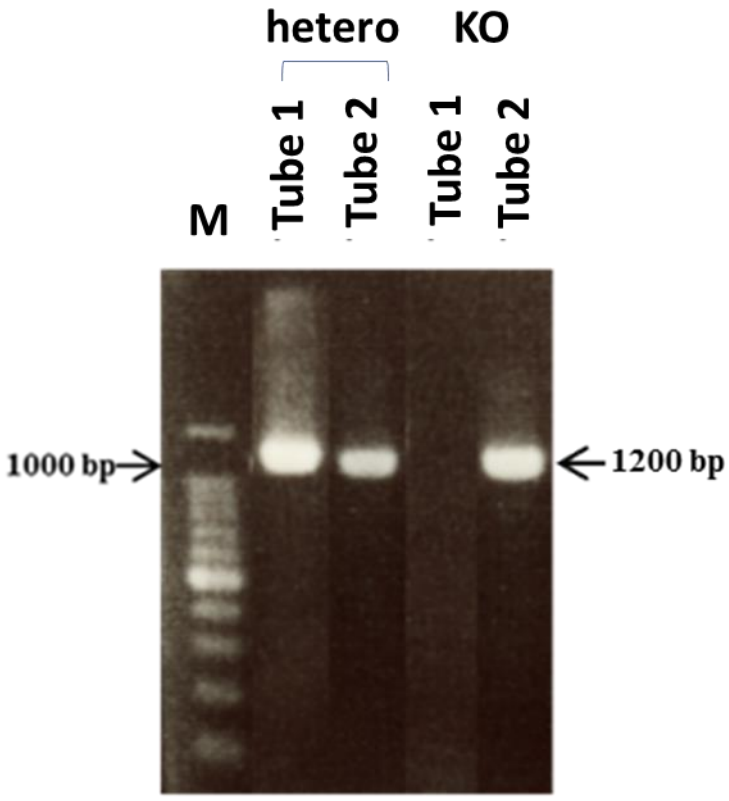

Fig.1. Electropherogram of PCR products from heterozygous and KO mice.

1.5) Reference

Ishii KJ, Kawagoe T, Koyama S, Matsui K, Kumar H, Kawai T, Uematsu S, Takeuchi O, Takeshita F, Coban C, Akira S. TANK-binding kinase-1 delineates innate and adaptive immune responses to DNA vaccines. Nature. 2008 Feb 7;451(7179):725-9. PMID:18256672

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