Genotyping of IKKi KO mice

1. Method used in our animal resource bank

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

1.1) Primer sequences:

- Primer1: IKKi wild
  - Sequence: 5'- TGC TAT TCT TCC TGA GGA CCC GAG TGC -3' (27-mer)
- Primer2: IKKi extra2
  - Sequence: 5'- AAG AAA CCG GAA ATG AGA GCT GCC AGC -3' (27-mer)
- Primer3: MC1-RC
  - Sequence: 5'- CTT CCT CTT GCA AAA CCA CAC TGC TCG -3' (27-mer)

1.2) Reaction mixture:

	For wild allele	For KO allele
	Tube 1 (µL)	Tube 2 (µL)
Water	7	7
Primer1 (IKKi wild,10 µM)	1	-
Primer2 (IKKi extra,10 µM)	1	1
Primer3 (MC1-RC(neo),10 µM)	-	1
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
67 °C	1 min	
72 °C	1 min	
72 °C	10 min	once
4 °C	∞	

Thermal cycler: Veriti with 0.2mL tubes.

1.4) Product size:

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

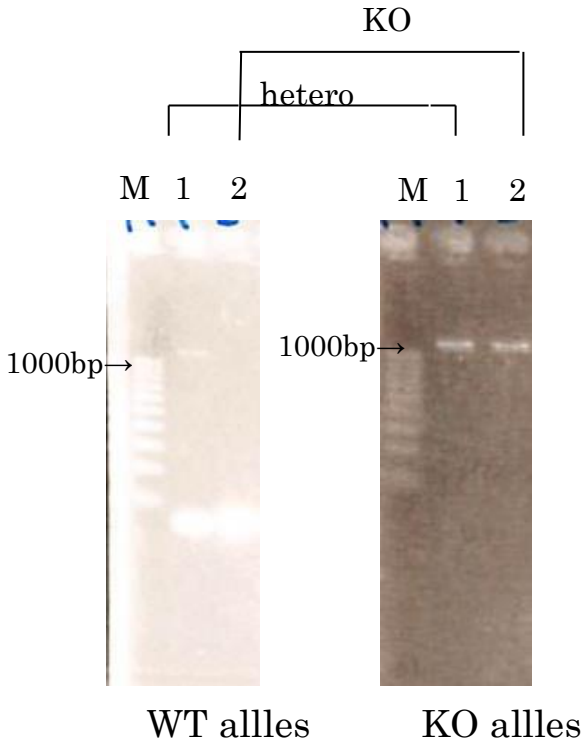

Fig.1. Electropherogram of PCR products from wild and heterozygous mice.

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