Genotyping of TNF KO mice

1. Method used in our animal resource bank

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

1.1) Primer sequences:

- Primer1: TNF Sense1
  - Sequence: 5'- AGA TGG AGA AGG GCA GTT AG -3' (20-mer)
- Primer2: TNF AntiS1
  - Sequence: 5'- ATA CCA GGG TTT GAG CTC AG -3' (20-mer)
- Primer3: TNF AntiS2
  - Sequence: 5'- TAC TTT GTT AAG AAG GGT GAG A -3' (22-mer)

1.2) Reaction mixture:

	For wild allele	For KO allele
	Tube 1 (μL)	Tube 2 (μL)
Water	8	8
Primer1 (TNF Sense1,10 μM)	0.5	0.5
Primer2 (TNF AntiS1,10 μM)	0.5	-
Primer3 (TNF AntiS2,10 μM)	-	0.5
Taq polymerase (U/μL)	10	10
DNA extracted from tail (purified)	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:

Tube 1 (WT : primer1 and 2)

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	∞	

Tube 2 (KO：primer1 and 3)

95 °C	15 min	Enzyme activation and first denature
94 °C	30 sec	35 cycles
56 °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: 800 bp for wild-type alleles
Primers 1 and 3: 500 bp for KO alleles

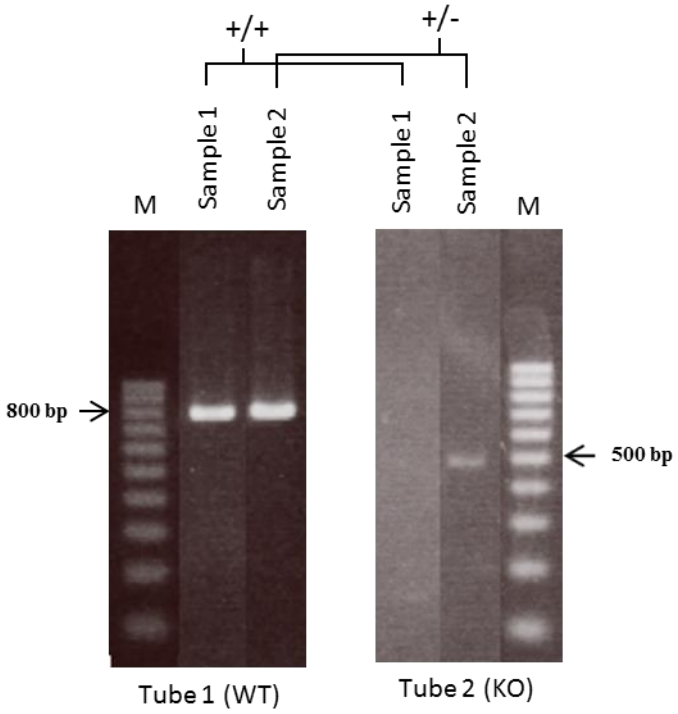

Fig.1. Electropherogram of PCR products from wild and heterozygous mice.

Nibio_ID=156;11/07/2012