Genotyping of TBK1 KO mice

This document contains two methods, one is used in our animal resource bank and the other is supplied by the original developer.

1. Method used in our animal resource bank

This section describes our method we are routinely using for genotyping TBK1 KO mice.

1.1) Primer sequences:

- Primer1: TBK1-S23605
  - Sequence: 5'- AGTTCCAGGCTGGCCAGAACACTAT -3' (27-mer)
- Primer2: TBK1-R23985
  - Sequence: 5'- CTGAGCCAACTCTCCAGCCTTCTGT -3' (27-mer)
- Primer3: TBK1-KO-R23892
  - Sequence: 5'-CGTTGGCTACCCGTGATATTGCTGA -3' (27-mer)

1.2) Reaction mixture:

	(μL)
Water	21
Primer1 (TBK1-S23605,10 μM)	1
Primer2 (TBK1-R23985,10 μM)	1
Primer3 (TBK1-KO-R23892,10 μM)	1
Taq polymerase (U/μL)	25
DNA extracted from tail (purified)	1
Total	50

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:

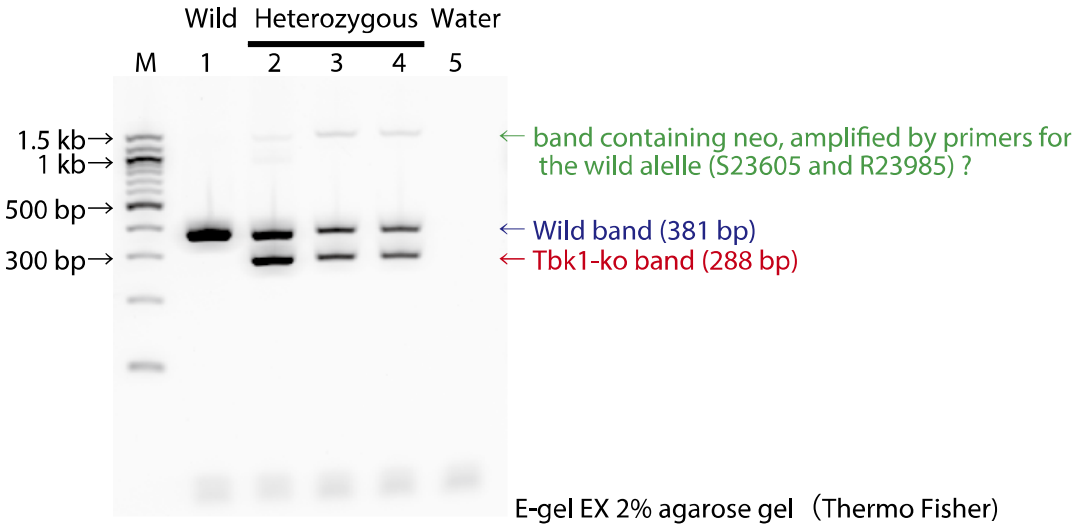
95 °C	15 min	Enzyme activation and the first denature
94 °C	30 sec	30 cycles
60 °C	30 sec	
72 °C	30 sec	
72 °C	5 min	Once
4 °C	∞	

Thermal cycler: Veriti with 0.2mL tubes.

1.4) Product size:

Primers 1 and 2: 381 bp for wild-type alleles

Primers 1 and 3: 288 bp for KO alleles



M: 100-bp ladder (NEB)
1: wild-type (purified)
2: heterozygous (purified)
3: heterozygous (crude)
4: heterozygous (crude)
5: Negative control (water only, no template)

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

2. Method supplied by the original developer with minor modifications

2.1) Primer sequences:

- Primer1: TBK1 extra
  - Sequence: 5'- TGC GTT CCT GTC CTG ACC GTG ATT GTG -3' (27-mer)
- Primer2: TBK1 wild
  - Sequence: 5'- CTA ATG GTT GTA GTC AGG GTC TCC TGC -3' (27-mer)
- Primer3: neo1500
  - Sequence: 5'- ATC GCC TTC TAT CGC CTT CTT GAC GAG -3' (27-mer)

2.2) Reaction mixture:

	For wild allele	For KO allele
	Tube 1 (μL)	Tube 2 (μL)
Water	7	7
Primer1 (TBK1 wild ,10 μM)	1	
Primer2 (TBK1 extra,10 μM)	1	1
Primer3 (neo1500,10 μM)	-	1
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).

2.3) Thermal cycles:

95 °C	15 min	Enzyme activation and first denature
94 °C	30 sec	35 cycles
67 °C	1 min	
74 °C	1 min	
72 °C	10 min	once
4 °C	∞	

Thermal cycler: Veriti with 0.2mL tubes.

2.4) Product size:

Primers 1 and 2: 1200 bp for wild-type alleles
Primers 1 and 3: 900 bp for KO alleles

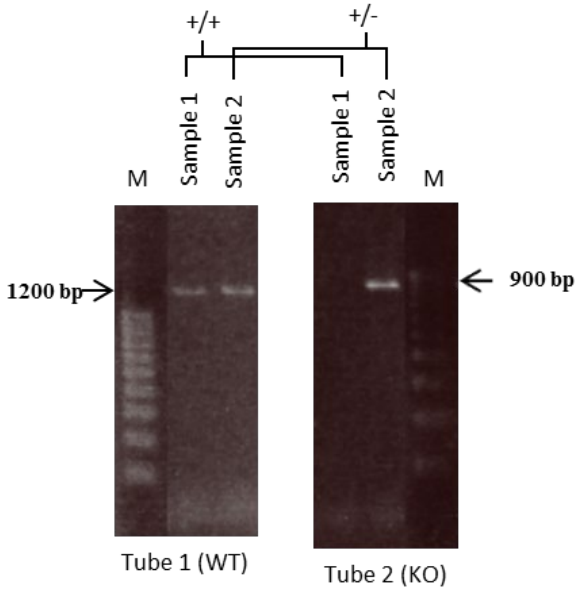


Figure 2. Electropherogram of PCR products from wild and heterozygous mice.

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