Genotyping of microRNA 182 KO mice

1. Method used in our animal resource bank

This section describes our method we are routinely using for genotyping microRNA 182 KO mice.

1.1) Primer sequences:

- Primer1: 108-03K-F
  - Sequence: 5'- GGA CCA TAC AGG CCG AAG GAC -3' (21-mer)
- Primer2: Neo-R1
  - Sequence: 5'- CCT TCT ATC GCC TTC TTG ACG AGT TC -3' (26-mer)
- Primer3: 182-R4
  - Sequence: 5'- CCC AAG TCC TTT TCA CCG AGA AGA G -3' (25-mer)

1.2) Reaction mixture:

	For KO allele	For WT allele
	Tube 1(µL)	Tube 2(µL)
Water	8	8
Primer1 (108-03K-F,10 µM)	0.5	0.5
Primer2 (Neo-R1,10 µM)	0.5	
Primer3 (182-R4,10 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).

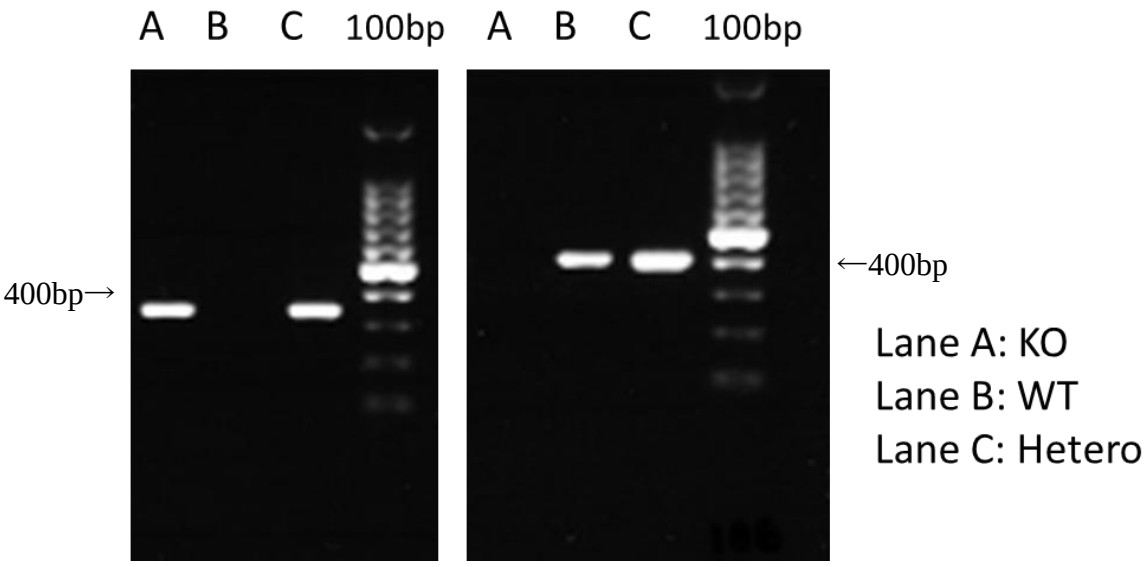
1.3) Thermal cycles:

95 °C	15 min	Enzyme activation and first denature
94 °C	30 sec	33 cycles
60 °C	30 sec	
72 °C	1 min	
72 °C	3 min	once
4 °C	∞	

Thermal cycler: Veriti with 0.2mL tubes.

1.4) Product size:

Primers 1 and 2: approx. 311 bp
Primers 1 and 3: approx. 442 bp



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