Genotyping of BKO mice

1. Method used in our animal resource bank

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

1.1) Primer sequences:

- Primer1: T-Neo 782F
  - Sequence: 5'- CTC GCG CCA GCC GAA CTG TT -3' (20-mer)
- Primer2: T-Neo1351R
  - Sequence: 5'- GTT CGA GGC CAC ACG CGT CA -3' (20-mer)
- Primer3: Wild 3
  - Sequence: 5'- CAT TCC TGC CAA GAC AGT AG -3' (20-mer)
- Primer4: BKO1415-1R
  - Sequence: 5'- ATG GCC TCA GTG TTC AGT GGG -3' (21-mer)

1.2) Reaction mixture:

	For KO allele	For Wild allele
	Tube 1(μL)	Tube 2(μL)
Water	8.6	8.6
Primer1 (T-Neo 782F,10 μM)	0.2	
Primer2 (T-Neo1351R,10 μM)	0.2	
Primer3 (Wild 3,10 μM)		0.2
Primer4 (BKO1415-1R,10 μM)		0.2
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	10 sec	35 cycles
60 °C	10 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: 570 bp for KO alleles
Primers 3 and 4: 242 bp for wild-type alleles

1.5) References:

1. Matsuda J et al. (1997) β-Galactosidase-deficient Mouse as an Animal Model for GM1-gangliosidosis. Glycoconjugate J, 14:729-736.

2. Itoh M et al. (2001) Development of lysosomal storage in mice with targeted disruption of the β-galactosidase gene: a model of human GM1-gangliosidosis. Brain Dev, 23: 379-384.

3. Matsuda J *et al.* (2003) Chemical chaperone therapy for brain pathology in GM1-gangliosidosis. Proc Natl Acad Sci USA, 100(26):15912-7.

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