Genotyping of C57BL/6-Tg (Mx-Cre) Kyo mice

1. Method used in our animal resource bank

This section describes our method we are routinely using for genotyping C57BL/6-Tg (Mx-Cre) Kyo mice.

1.1) Primer sequences:

- Primer1: oIMR1084
  - Sequence: 5'- GCG GTC TGG CAG TAA AAA CTA TC -3' (23-mer)
- Primer2: oIMR1085
  - Sequence: 5'- GTG AAA CAG CAT TGC TGT CAC TT -3' (23-mer)
- Primer3: oIMR7338
  - Sequence: 5'- CTA GGC CAC AGA ATT GAA AGA TCT -3' (24-mer)
- Primer4: oIMR7339
  - Sequence: 5'- GTA GGT GGA AAT TCT AGC ATC ATC C -3' (25-mer)

1.2) Reaction mixture:

	For Tg allele	For WT allele
	Tube 1(μL)	Tube 2(μL)
Water	8	8
Primer1 (oIMR1084,10 μM)	0.5	
Primer2 (oIMR1085,10 μM)	0.5	
Primer3 (oIMR7338,10 μM)		0.5
Primer4 (oIMR7339,10 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:

95 °C	15 min	Enzyme activation and first denature
94 °C	30 sec	35 cycles
51.7 °C	1 min	
72 °C	1 min	
72 °C	2 min	once
4 °C	∞	

Thermal cycler: Veriti with 0.2mL tubes.

1.4) Product size:

Primers 1 and 2: approx. 100 bp
Primers 3 and 4: approx. 324 bp

Nbio_ID=227