Genotyping of L1 EGFP mice

1. Method used in our animal resource bank

This section describes our method we are routinely using for genotyping L1 EGFP mice. This method is a little bit modified from the original.

1.1) Primer sequences:

- Primer1: L1-U1
  - Sequence: 5'- CATTTGGGCTGGAGTAGATT -3' (20-mer)
- Primer2: L1-L1
  - Sequence: 5'- AAGGAGGACGGCAACAT -3' (17-mer)
- Primer3: L1-L2
  - Sequence: 5'- AGTGCTTCAGCCGCTAC -3' (17-mer)

1.2) Reaction mixture:

	Tube 1 (µL)	Tube 2 (µL)
Water	8	8
Primer1 (L1-U1,10 µM)	0.5	0.5
Primer2 (L1-L1,10 µM)	0.5	
primer3 (L1-L2, 5 pmol)		0.5
Taq polymerase (U/µL)	10	10-
DNA extracted from tail (diluted 50 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	40 cycles
55 °C	30 sec	
72 °C	30 sec	
72 °C	10 min	once
4 °C	∞	

Thermal cycler: Veriti with 0.2mL tubes.

1.4) Product size:

Primers 1 and 2: 506 bp for Tg alleles

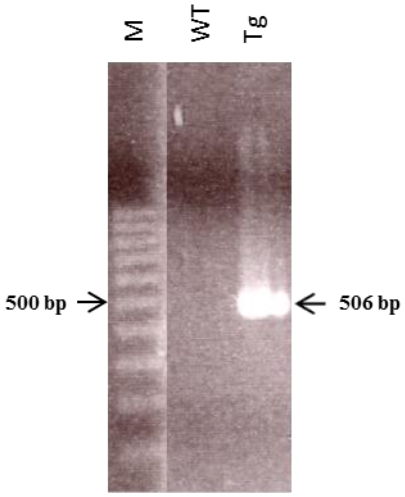

Fig.1. Electropherogram of PCR products from wild and Tg mice.

Primers 1 and 3: 692 bp for Tg alleles

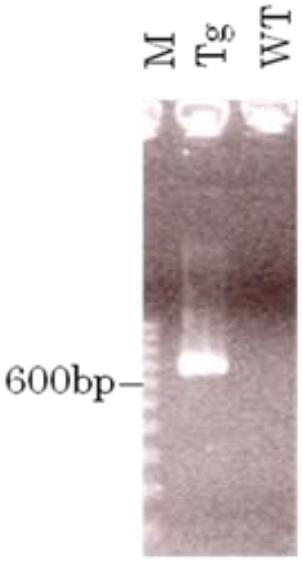

Fig.2. Electropherogram of PCR products from wild and Tg mice.

Nbio_ID=138-153; July 1st, 2014