Genotyping of FOXO1 Tg mice

1. Method used in our animal resource bank

This section describes our method we are routinely using for genotyping FOXO1 Tg mice.

1.1) Primer sequences:

- Primer1: HSA-5
  - Sequence: 5'- ATG GTT GGG GAG GCC TTT GG -3' (20-mer)
- Primer2: HSA-3
  - Sequence: 5'- GGA AGC GAG GCT TCA CTT GG -3' (20-mer)

1.2) Reaction mixture:

	Tube (μL)
Water	8
Primer1 (HSA-5,10 μM)	0.5
Primer2 (HSA-3,10 μM)	0.5
Taq polymerase (U/μL)	10
DNA (diluted 200 times)	1
total	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
55 °C	30 sec	
72 °C	30 sec	
72 °C	7 min	once
4 °C	∞	

Thermal cycler: Veriti with 0.2mL tubes.

1.4) Product size:

Primers 1 and 2: 412 bp for Tg alleles

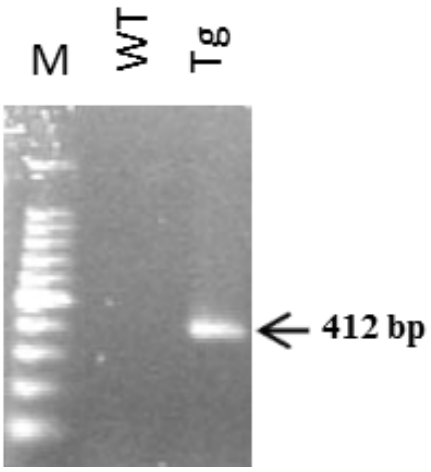

Fig.1. Electropherogram of PCR products from wild and Tg mice.

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