Genotyping of VE-cadherin-Cre mice

1. Method used in our animal resource bank

This section describes our method we are routinely using for genotyping VE-cadherin-Cre mice.

1.1) Primer sequences:

Primer1: Cre F

- Sequence: 5'- GTTCGCAAGAACCTGATGGACA -3' (22-mer)

Primer2: Cre R

- Sequence: 5'- CTAGAGCCTGTTTTGCAGGTTC -3' (22-mer)

1.2) Reaction mixture:

	Tube (μL)
Water	8
Primer1 (Cre F,10 μM)	0.5
Primer2 (Cre R,10 μM)	0.5
Taq polymerase (U/μL)	10
DNA (diluted 100 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
58 °C	30 sec	
72 °C	1 min	
72 °C	5 min	once
4 °C	∞	

Thermal cycler:Veriti with 0.2mL tubes.

1.4) Product size:

Primers 1 and 2: 350 bp for Tg alleles

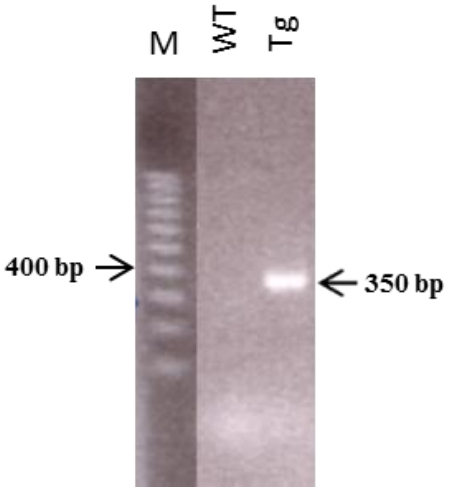

Fig.1. Electropherogram of PCR products from wild and Tg mice.

1.5) Reference

Kogata N, Arai Y, Pearson JT, Hashimoto K, Hidaka K, Koyama T, Somekawa S, Nakaoka Y, Ogawa M, Adams RH, Okada M, Mochizuki N. Cardiac ischemia activates vascular endothelial cadherin promoter in both preexisting vascular cells and bone marrow cells involved in neovascularization. Circ Res. 2006 Apr 14;98(7):897-904. PMID: 16543497

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