Genotyping of 11Enh-Cre mice

1. Method used in our animal resource bank

This section describes our method we are routinely using for genotyping 11Enh-Cre (LI-Cre) mice.

1.1) Primer sequences:

Primer1: Cre F

- Sequence: 5'- CAATTTACTGACCGTACACCA -3' (21-mer)

Primer2: Cre R

- Sequence: 5'- TCTTCAGGTTCTGCGGG -3' (17-mer)

1.2) Reaction mixture:

	Tube (μL)
Water	8.0
Primer1 (Cre F,10 μM)	0.5
Primer2 (Cre R,10 μM)	0.5
Taq polymerase (U/μL)	10
DNA extracted from tail (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
58 °C	30 sec	
72 °C	30 sec	
72 °C	7 min	once
4 °C	∞	

1.4) Product size:

Primers 1 and 2: 187 bp for TG alleles

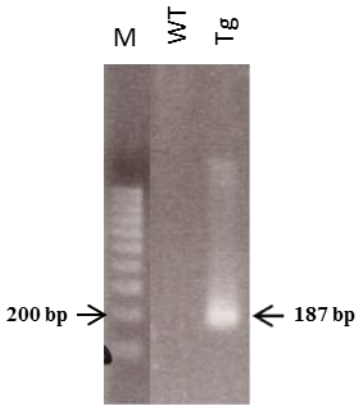
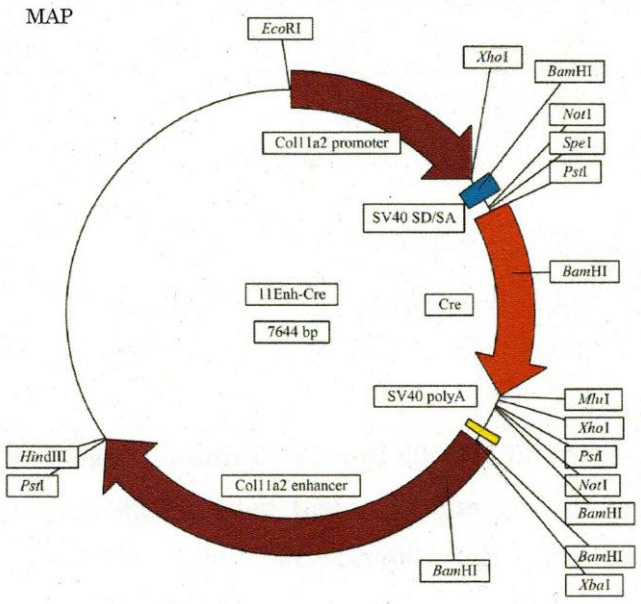


Fig.1. Electropherogram of PCR products from wild and Tg mice.

1.5) Vector map



1.6) Reference

Iwai T, Murai J, Yoshikawa H, Tsumaki N 2008 Smad7 Inhibits chondrocyte differentiation at multiple steps during endochondral bone formation and down-regulates p38 MAPK pathways. J Biol Chem 283:27154-27164.

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