Protocol for Genotyping Knockout Mice

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I. Genomic DNA Extraction from Mouse Tails

1. Label 1.5ml tubes with appropriate animal numbers.

2. Cut a small piece (approx. 1 mm^3) of mouse tail, using a scalpel. Samples can be stored at -70°C if needed.

3. Add 100ul Proteinase K buffer, vortex briefly, and incubate 8 hrs. / overnight at 50°C.

Prot K digestion buffer:
10mM Tris pH 8
100mM NaCl
10mM EDTA
0.1mg/ml Prot K
0.5% SDS

4. NEXT DAY: Add 200ul ddH_2O, Cfg for 2min at top speed to bring down moisture on sides of tubes.

5. Pour supernatant to a new tube,

6. Add 250ul PC-8 (phenol:chloroform)

7. Vortex 10s →Centrifuge 2min. at top speed.

6. Transfer the aqueous phase to a clean labeled 1.5ml tube. (Pull up ~250uL w/ pipet)

7. Add 700ul cold ethanol and invert to mix. (DNA ppt may not be visible.)

8. Cfg in microfuge for 5min at RT.

9. Aspirate supernatant carefully; add 500ul of 70% ethanol, vortex well, and spin down for 1 min. Repeat once.

10. Aspirate supernatant; Spin down for 10 sec and aspirate the residual liquid completely. Pellets may not be clear.

11. Add 200ul ddH_2O to each pellet and vortex briefly. (Optional: let sit several min. in ddH_2O before vortexing

12. Store samples at -20°C.

NOTE: When storing, divide into two aliquots of 100uL each. Store one 100uL aliquot, undiluted, in the "backup DNA" box. For the other aliquot, add 100uL more ddH_2O (for a total volume of 200uL) and label the tube as a 1:1 dilution. You will use this 1:1diluted DNA for PCR.

II. Genotyping of Mouse Genomic DNA using PCR

1) Set up the following reaction for each set of primers (LacZ, Neo, G, H)

	1x
Template DNA (1:1)*	3.0 µL *
H_2O	7.35 µL
10x PCR	1.5 µL
dNTP	1.5µL
DMSO	0.9 µL
Primer #1	0.3 µL
Primer #2	0.3 µL
Taq	0.15 µL
TOTAL	15.0 µL

*see note at the bottom of section I

2) Use the following Touchdown PCR program

95°C – 4 min	
92°C – 20s	Repeat 13x
68°C – 20s	
INC by -1°C	
72°C – 45s	
92°C – 20s	Repeat 32x
55°C – 20s	
72°C – 45s	
72°C – 5min.	
4°C – to infinity… and beyond	

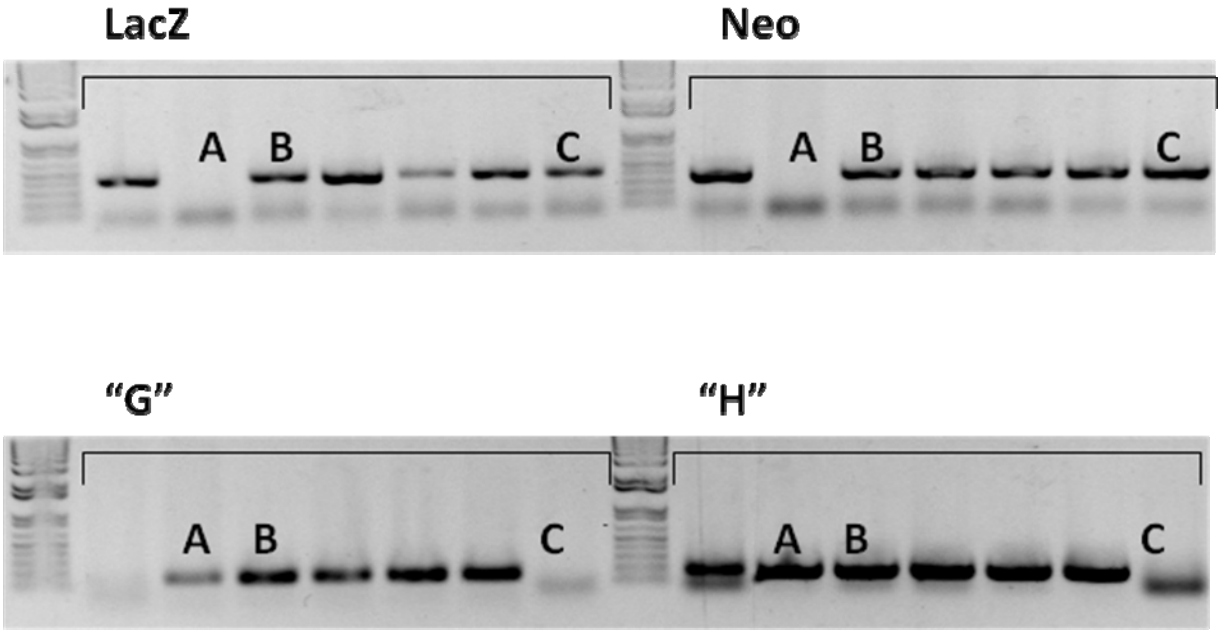
3) Run on a 1% agarose gel. Use the following scheme to determine genotype

	LacZ	**Neo**	**G**	**H**
Homozygote/KO	**+**	**+**	**-**	**-**
Heterozygote	**+**	**+**	**+**	**+**
Wild Type	**-**	**-**	**+**	**+**

Primers for PCR (using BMP-9/Gdf2 KO as an example)

	Primer #	Description	Primer sequence
"LacZ"	TCH2427	5'Universal LacZ-Rev	GCTGGCTTGGTCTGTCTGTCCTA
	TCH2311	BMP9-K/O: SU	CAGTAGTCAGCATCCTTTCC
"Neo"	TCH2428	3'Universal Neo- Fwd	GCAGCCTCTGTTCCACATACACTTCA
	TCH2312	BMP9-K/O: SD	AGTTTCTGCCTGGTTTCCTG
"G"	TCH2457	BMP9 internal KO1 Fwd	TGAGTCCCATCTCCATCCTC
	TCH2458	BMP9 internal KO1 Rev	ATGCAGGACCGTACCAGAAC
"H"	TCH2459	BMP9 internal KO2 Fwd	GGCATCTTGCTCTGAAGGAC
	TCH2460	BMP9 internal KO2 Rev	GGGCAGTCAGAAAACTCAGC

III. Representative Genotyping Gel



Using the Table shown in II-3, we can see that:

A= Wild Type mouse

B = Heterozygous mouse

C = Homozygous BMP9 KO mouse