RMRC Genotyping Protocol-PCR

No.	RMRC13129	Strain Name	*B6.129-Gas7<tm1Lin>*

A. Primer

	Primer name	Sequence
1	Gas7F1	**5'-GTTCAGCCTTCCTCCACTTGC-3'**
2	Gas7F19	**5'-CGACCACCAAGCGAAACATC-3'**
3	Gas7B15	**5'-AGTGAATGGGAAACTGACAGAGG-3'**

B. Reaction Conditions

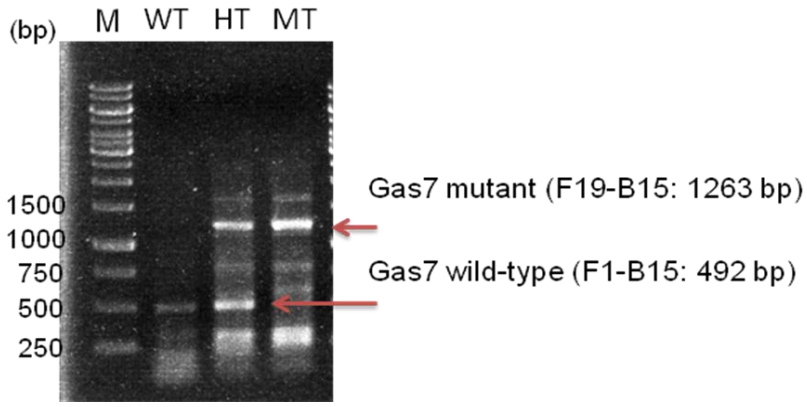
Component	Concentration		Volume		Step	Tempera-ture, ℃	Time, sec	Number of Cycles
H_2O	--		11.5	µl	1	95	300	1
PCR buffer	10x		2	µl	2	95	60	35
dNTP	10	mM	1	µl	3	56	60	35
Primer 1	10	µM(ρmol/µl)	1	µl	4	68	90	35
Primer 2	10	µM(ρmol/µl)	1	µl	5	68	420	1
Primer 3	10	µM(ρmol/µl)	1	µl	6	4	∞	1
DNA	--		1	µl	7			
Taq	0.5	U/µl	0.5	µl	8			
Mg^{2+}	2.5	mM	1	µl	9			
Total			20	µl				

C. PCR Product

	Primer set		Product size	Target region
1	Gas7F1	Gas7B15	492 bp	Gas7 exon6
2	Gas7F19	Gas7B15	1263bp	Neo gene and partial of Gas7 exon6

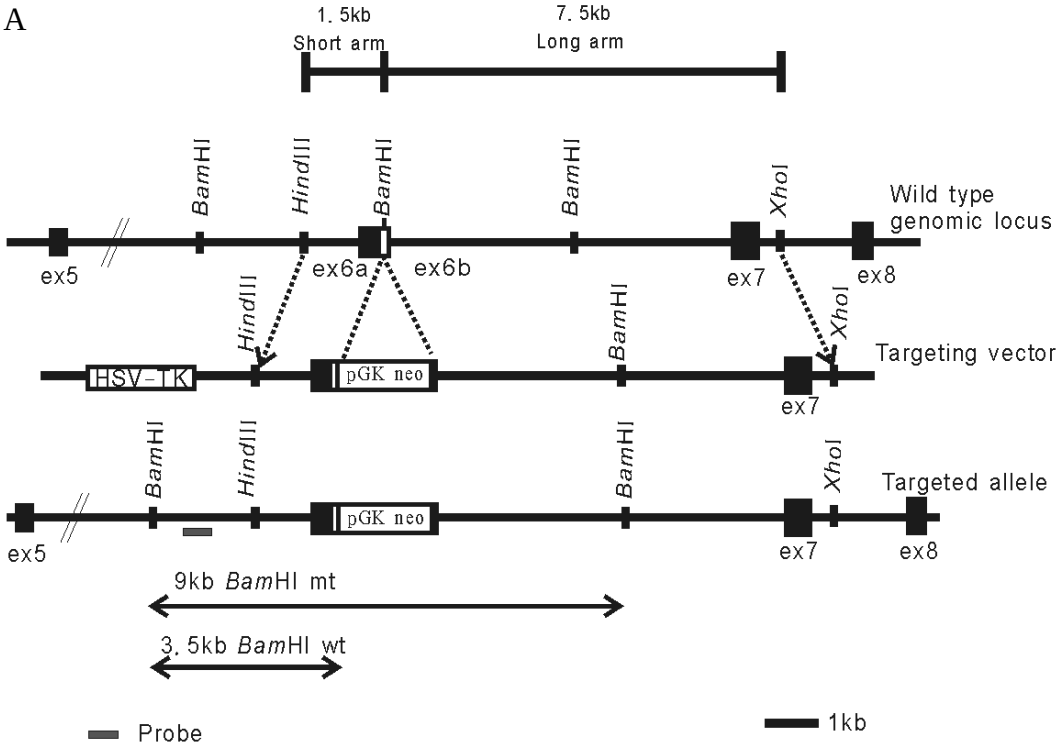
D. Gel Photo

Gas7 allele

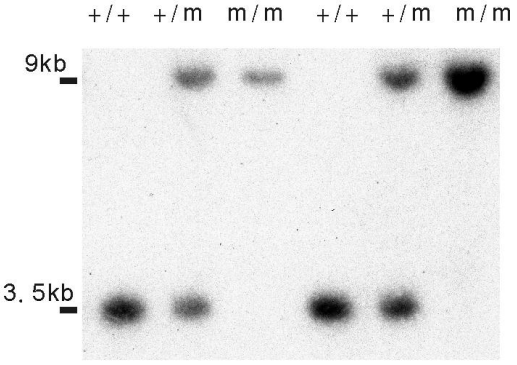


Southern Blot

A



B



DNA preparation and Southern analysis

Genomic DNA samples were prepared from mouse tail clippings. The tail clippings were digested overnight on a rotating tube rack at 55℃ with 0.7 ml of tail lysis buffer, containing 100 mM Tris-Cl (pH 8.5), 5 mM EDTA, 0.2 % sodium dodecyl sulfate (SDS), 200 mM NaCl, and 100 μg of proteinase K/ml. Supernatants from the lysates were centrifuged at 13,000 rpm in an Eppendorf centrifuge for 10 min and then transferred to prelabeled tubes containing 0.5 ml of isopropanol. The DNA was recovered from each of the samples by lifting the precipitates with disposable pipette tips and dissolving the genomic DNA overnight at 55℃ in Tris-EDTA buffer (100 μl).

Southern blot analyses were carried out to determine the site of integration of the gene trap sequence in the gas7 gene locus of the mice. A 600-bp short arm probe generated from Gas7 genomic DNA as figure A shown for mice screening. Genomic DNA samples (10 μg) were digested with *BamHI* and electrophoresed in 0.7% agarose gels then transferred the genomic DNA onto Zeta-probe membrane (Bio-Red). The DNA attached to the membrane was hybridized to the radiolabeled DNA probe generated by random primer (Strategy) using ^{32}P-dCTP, after hybridization at 65 ℃ O/N and washing. Expose it to X-ray film (Kodak). The signal shown that the wild-types is 3.5 Kb DNA band and mutant is only one 9 Kb DNA band, and heterozygous includes both 3.5 Kb and 9 Kb bands.