

RMRC Genotyping Protocol-PCR

No.	RMRC13359	Strain Name	C57BL/6- <i>Gnb4</i> ^{em1Fmj} /Narl
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A. Primer

	Primer name	Sequence
1	VF2	cagaactggtaaggcagc
2	VR2	ctcttgacagcagaagagttc

B. Reaction Conditions

Component	Concentration	Volume		Step	Temperature, °C	Time, sec	Number of Cycles	
H ₂ O	--	7.4.	μl	1	94	300		
5X buffer A	10x	2	μl	2	94	30	40	
dNTP	10	mM	0.04	μl	3	55		30
Primer 1	10	μM(ρmol/μl)	0.02	μl	4	72		30
Primer 2	10	μM(ρmol/μl)	0.02	μl	5	72	420	
DNA	--	0.5	μl	6				
KAPA	5	U/μl	0.02	μl	7			
Total	10	μl	10	8				

C. Enzyme Digestion

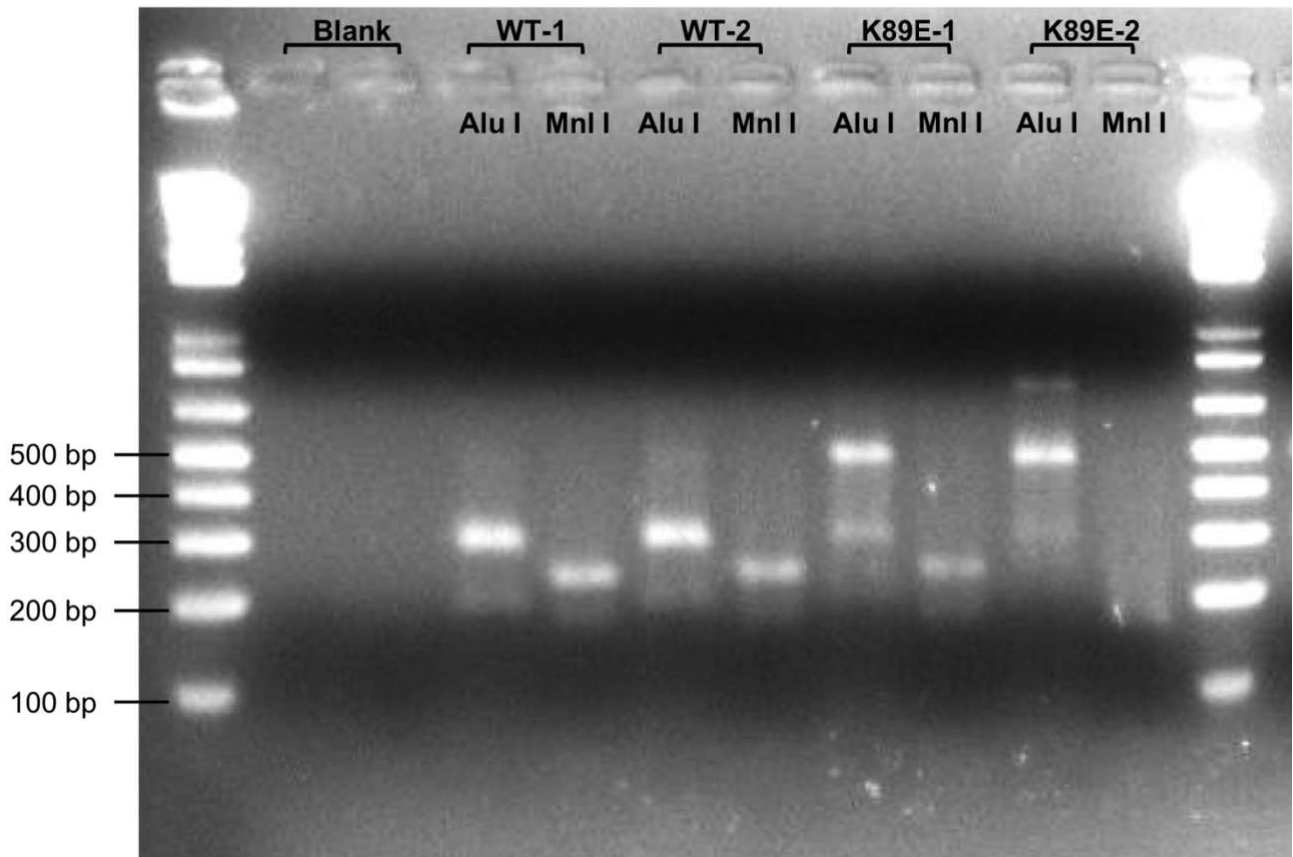
Component	Concentration	Volume		Component	Concentration	Volume			
H ₂ O	--	3.8.	μl	H ₂ O	--	3.8	μl		
10X Buffer Tango	10x	1	μl	10X Buffer G	10x	1	μl		
PCR product	--	5	μl	PCR product	--	5	μl		
AluI	10	U/μl	0.2	μl	MnII	10	U/μl	0.2	μl
Total	10	μl	10	μl	Total	--	10	μl	

37°C for 2 hr

D. PCR Product

Primer set	PCR product		
	-RE	Alu I	Mnl I
VF2+VR2	453 bp (WT)	177 bp+275 bp (WT)	29 bp+ 37 bp+165 bp+221 bp (WT)
	453 bp (K89E)	453 bp (K89E)	29 bp+37 bp+51bp+165bp + 170 bp (K89E)

E. Gel Photo



以AluI的pattern為判斷依據 (因MnlI切得不明顯) , 再拿以AluI切的pattern認為是K89E的小鼠送去定序確認。