

RBRC01360 Strain name C57BL/6-grm5<tm1(T840D)Kyo>

Primer	primer_name	primer_seq	length
1	G5-Ch	CCTCATTGCAAAGCCCAAACAGAT	24
2	G5-2a	GCTCAAAGCCTGAAGCACCTGTAT	24

**Reaction Components**

Enzyme Roche Taq (Cat. No. BM1418432)

	Conc	Run 1	Run 2	Run 3
H2O		17.3		
PCR buffer	10 X	2.5		
dNTP	2.5 mM	2.0		
primer 1	10 pmol/ $\mu$ l	1.0		
primer 2	10 pmol/ $\mu$ l	1.0		
DNA		1.0		
Taq		0.2		
total		25		

**Reaction Conditions**

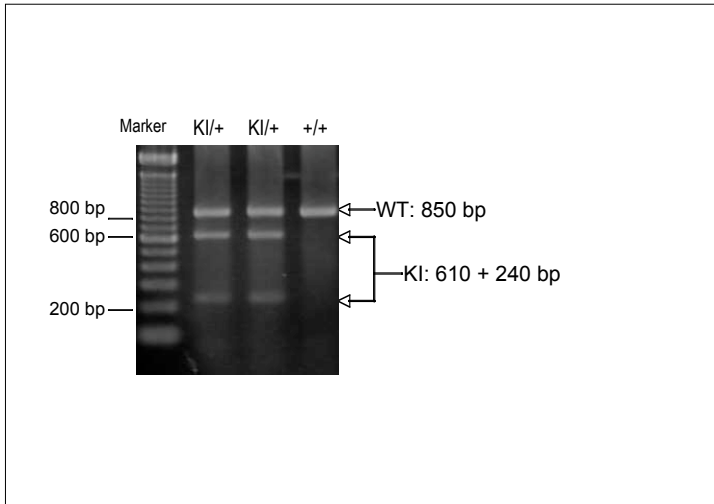
step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	60	30*	
4	72	60*	*Repeat for 35 cycles
5	72	300	

**Restriction\_Reactions**

PCR product 15.0  $\mu$ l  
 Aat II 1.0  $\mu$ l  
 ↓  
 37°C 120 min.

After Aat II digestion, run 15  $\mu$ l of digested products on a 2% agarose

+/+ 850 bp  
 KI/+ 850 bp + 610 bp + 240 bp  
 KI/ KI 610 bp + 240 bp



**Marker**

100 bp ladder (Invitrogen, Cat. 15628-050)

**Gel**  
 2% agarose in 1 X TAE (100 V, 30 min)

primer_set	Band_size	product
primer 1 primer 2	850 bp	WT
primer 1 primer 2	610, 240 bp	KI