

RMRC Genotyping Protocol-PCR

No.	RMRC13352	Strain Name	C57BL/6- <i>Septin12^{tm1(S196E)Kpln}</i> /Narl
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A. Primer

	Primer name	Sequence
1	S196E-F	TTTCTTCCCTCACTCATCCAC
2	S196E-R	TCTACAGCATCTTACCCGAATC

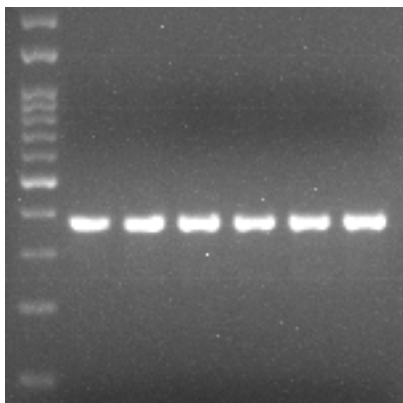
B. Reaction Conditions

Component	Concentration	Volume	Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--	19.5	μl	1	95	6:00
PCR buffer	10x	2.5	μl	2	95	30
dNTP	25	mM	0.2	μl	3	56
Primer 1	10	μM(ρmol/μl)	0.65	μl	4	72
Primer 2	10	μM(ρmol/μl)	0.65	μl	5	72
DNA	--	1	μl	6	4	1
Taq		U/μl	0.5	μl	7	
Mg ²⁺		mM	0	μl	8	
Total		25	μl	9		

C. PCR Product

	Primer set	Product size	Target region
1	S196E-F S196E-R	366 bp	SEPT12-exon6
2		bp	

D. Gel Photo



PCR 之後進行定序確認序列：S196E：GAG(Glutamate)，WT：AGC(Serine)。