

RMRC Genotyping Protocol-PCR

No.	RMRC13283	Strain Name	B6.129-Sept12 ^{tm1(D197N)Kpln}
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A. Primer

	Primer name	Sequence
1	SEPT12-knock in-F	ATCGCCCGGGCTAACAGCC
2	SEPT12-knock in-R	TAGCGGGCCCGATTGTCGG

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--			μl	1	95	300	1
PCR buffer	10x		2.5	μl	2	95	30	35
dNTP	25	mM	0.2	μl	3	56	30	35
Primer 1	10	μM(μmol/μl)	0.5	μl	4	72	26	35
Primer 2	10	μM(μmol/μl)	0.5	μl	5	72	600	1
DNA	--			μl	6	4	-	-
Taq		U/μl	0.5	μl	7			
Mg ²⁺		mM		μl	8			
Total			25	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	SEPT12-knock in-F	SEPT12-knock in-R	~ 350 bp	Human septin12 CDS
2				

D. Gel Photo

