

RMRC Genotyping Protocol-PCR

No.	RMRC13302	Strain Name	C57BL/6- <i>ApoE</i> ^{em1Narl} /Narl
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A. Primer

	Primer name	Sequence
1	mApoE_F	CCTCCAGACCCACTTCAAAGAGAC
2	mApoE_R	AAAGCCAATCGACCAAGCAAG

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		10.9	μl	1	94	180	1
PCR buffer	10x		1.5	μl	2	94	30	35
dNTP	10	mM	0.3	μl	3	60	30	35
Primer 1	10	μM(μmol/μl)	0.25	μl	4	72	30	35
Primer 2	10	μM(μmol/μl)	0.25	μl	5	72	180	1
gDNA	50~250 ng/ul		1	μl	6			
Taq	5	u/μl	0.3	μl	7			
Formamide	1	mM	0.5	μl	8			
Total			15	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	mApoE_F	mApoE-R	437 bp	PCR product 需進行定序, 須具備符合以下序列片段: MT : gga(2252)-g-(2253)agagctgca (WT : gga-agagctgca)
2			bp	

D. Gel Photo