

RMRC Genotyping Protocol-PCR

No.	RMRC23006	Strain Name	SD-Apoe ^{em1Narl/} Narl
-----	-----------	-------------	----------------------------------

A. Primers

No.	Primer name	Sequence
1-1	rApoE_1706F	AACATGGTGGGAAGGAGAGAGCG
1-2	rApoE_2448B	GAGGAAGGAGAATTGCAGAGCC
2-1	rApoE_884F	ATC TCC AGG AGT ATG GGA CTG TCG
2-2	rApoE_3207B	ATG TCT TCC ACT AGC GGC TCG AAC

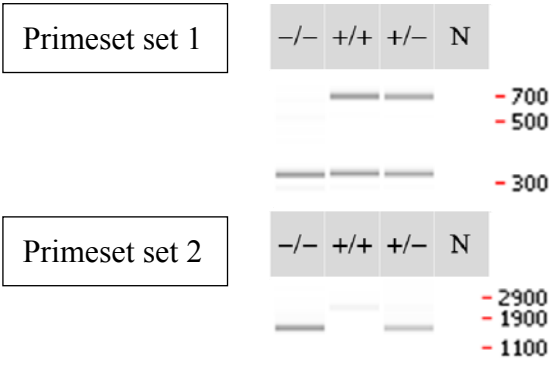
B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		10.9	μl	1	94	3'	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(ρmol/μl)	0.25	μl	4	72	30''	35
Primer 2	10	μM(ρmol/μl)	0.25	μl	5	72	3'	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

No.	Primer set		Product size	Target region
1	rApoE_1706F	rApoE_2448B	743bp	Wildtype: 743 bp
2	rApoE_884F	rApoE_3207B		Wildtype: 約 2.4K，由於尺寸大，易 miss，需以組別 1 結果輔助判讀。 2140-2888 deletion : 1.7K

D. Gel Photo



Wild type: +

2140-2888 deletion(Line 27.1 mutant): -