

RMRC Genotyping Protocol-PCR

No.	RMRC 13204	Strain Name	C57BL/6-Tg(ADRA2C-Cre)1Acch
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A. Primer

	Primer name	Sequence
1	rtTA-forward	5'-TTACGGGTCTACCATCGAGG-3'
2	rtTA-reverse	5'-CGTCTAAGTGGAGCTCGTCC-3'
3		

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--			μl	1	94	180	Denaturation
PCR buffer	10x (containing 15mM MgCl ₂)	2.5	μl	2		94	35	1
						62	45	
						72	45	
dNTP	2.5mM	2.0	μl	3		94	35	2
						61.5	45	
						72	45	
Primer 1	20	μM(μmol/μl)	1.5	μl	4	94	35	3
						61	45	
						72	45	
Primer 2	20	μM(μmol/μl)	0.75	μl	5	94	35	4
						60.5	30	
						72	45	
DNA	100ng/μl	2.0	μl	6		94	35	5-40
						60	30	
						72	45	
Taq		5U/μl	0.25	μl	7	72	120	final extension
Mg ²⁺		mM	---	μl	8	4	--	storage
Total			25	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	rtTA-forward	rtTA-reverse	167bp	
2				

D. Gel Photo