

RMRC Genotyping Protocol-PCR

No.	RMRC13202	Strain Name	B6.129P2-Adra2c^{tm1(lacZ)Acch}
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A. Primer

	Primer name	Sequence
1	α2C-PCR1	5'-CGC GTA GCT CTC GGG AGG-3' (for both WT and KO alleles)
2	MC4N2	5'-TCC AGA CTA ATG GCA CAC AGG-3' (for WT);
3	lacZ-Asn	5'-GATCGATCTCGCCATACAGC-3' (for KO)

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 MgCl₂)	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	α2C-PCR1	MC4N2	456 bp	
2	α2C-PCR1	lacZ-Asn	200 bp	

D. Gel Photo