

RMRC Genotyping Protocol-PCR

No.	RMRC	Strain Name	Cacna1h ^{tm1IBMS/CCC}
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A. Primer

	Primer name	Sequence
1	#653_5loxp a1h F	aataccagcctatgtcctgt
2	#654_5loxp a1h R	gtataactggagggacatgg
3	#802_3loxp a1h R	cctgagacatggatgtttgg

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		5.4	μl	1	94	300	1
PCR buffer	10x		1	μl	2	94	15	30
dNTP	2.5each	mM	0.5	μl	3	58	30	30
Primer 1	10	μM(ρmol/μl)	0.5	μl	4	72	30	30
Primer 2	10	μM(ρmol/μl)	0.5	μl	5	72	120	1
DNA	--		2	μl	6			
Taq	2	U/μl	0.1	μl	7			
Mg ²⁺	50	mM	0	μl	8			
Total			10	μl	9			

C. PCR Product

	Primer set		Product size	Target region
	1	2		
1	1	2	330 bp	5'loxP inserted flanking region
2	1	3	244 bp	Deleted region generated from Cre

D. Gel Photo

