

RMRC Genotyping Protocol-PCR

No.	RMRC13163	Strain Name	129-Cacna1h<tm2IBMS/CCC>
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A. Primer

	Primer name	Sequence
1	A1H_gene_F2	tgccctggaacgcagccgt
2	A1H_gene_R	TCCCGCAACACGCACACTGG
3	A1H_gene_neo	ATGATCGGAATTCCTGCAGCCCCG

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		5.1	μl	1	94	120	1
PCR buffer	10x		1	μl	2	94	15	40
dNTP	2.5 each	mM	0.5	μl	3	61	30	40
Primer 1	10	μM(ρmol/μl)	1	μl	4	72	30	40
Primer 2	10	μM(ρmol/μl)	1	μl	5	72	300	1
Primer 3	10	μM(ρmol/μl)	0.5	μl				
DMSO	100	%	0.4	μl				
DNA	--		2	μl				
Taq	2	U/μl	0.1	μl				
Mg ²⁺	50	mM	0	μl				
Total			10	μl				

C. PCR Product

	Primer set		Product size	Target region
1	1	2	443 bp	wildtype
2	2	3	247 bp	KnockOut

D. Gel Photo

knockout

