

RMRC Genotyping Protocol-PCR

No.	RMRC13164	Strain Name	129-cacna1h<tm2IBMS/CCC>Tg(Myh7-YFP)IBMS/CCC
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A. Primer

	Primer name	Sequence
1	YFPBMHC-Frd	TACTCCTCTgCTgAggCTTCC
2	YFPBMHC-Rev	gTTTgCACATgAACACTgTgg
1'	A1H_gene_F2	tgccctggaacgcagccgt
2'	A1H_gene_R	TCCCGCAACACGCACACTGG
3'	A1H_gene_neo	ATGATCGGAATTCCTGCAGCCCG

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		5.4	μl	1	94	120	1
PCR buffer	10x		1	μl	2	94	15	30
dNTP	2.5 each	mM	0.5	μl	3	58	30	30
Primer 1	10	μM(μmol/μl)	0.5	μl	4	72	30	30
Primer 2	10	μM(μmol/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			
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(μmol/μl)	1	μl	4	72	30	40
Primer 2	10	μM(μmol/μl)	1	μl	5	72	300	1
Primer 3	10	μM(μmol/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	μ l				
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C. PCR Product

	Primer set		Product size	Target region
1	1	2	440 bp	Beta-myosin heavy chain-YFP
2	1'	2'	443 bp	wildtype
	2'	3'	247 bp	Cacna1H knockout

D. Gel Photo

