PCR protocol

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RBRC 11850 **Strain name** C57BL/6-Madcam1<em1Nsas>

Primer	primer_name	primer_seq	length		Reference
1	Madcam1-F	GCTTCCCCTGGCGGTCACAAGACA	24		Depositor's primer
2	wt	TACCTCTGCTGAGCTGGTAGGGTAC	25		RIKEN BRC
3	mut	CCTCCTGCTGGCCCTGGCCCTAAC	24		RIKEN BRC
4	common	GATGGGACAACTGGGTCCCAGGTTC	25		RIKEN BRC

Reaction Components

Enzyme QIAGEN Multiplex PCR Kit (Cat. No. 206143)

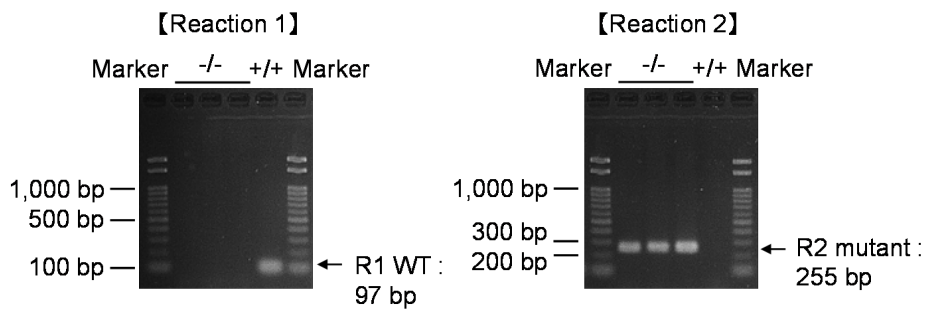
Reagent	Conc	Reaction 1	2	3	4	
H2O		2.0	2.0			(μL)
primer 1	10 pmol/μl	1.0	0			
primer 2	10 pmol/μl	1.0	0			
primer 3	10 pmol/μl	0	1.0			
primer 4	10 pmol/μl	0	1.0			
DNA		1.0	1.0			
Master Mix	2 X	5.0	5.0			
	total	10	10			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	95	900	
2	94	30	*
3	65	90	*
4	72	60	* *Repeat for 30 cycles

Marker Gene Ladder 100 (Wako, Cat. 316-06951)

Gel 2% agarose in 1 X TAE (100 V, 30 min)



primer_set		product_size	product (allele type)	
primer 1	primer 2	97 bp	WT	
primer 3	primer 4	255 bp	mutant	

Comment Allele specific PCR (ASP)