PCR protocol

RBRC 01222 **Strain name** MSM.HR1-Hr<hr>/Rbrc

Primer

	primer_name	primer_seq	length		
1	00187_F	GTGCCTGCTGCCACATATGTGG	22		RIKEN BRC
2	00187_R	GCATCCGAGGGCATGCGTGCAC	22		RIKEN BRC
3	00187_FM	CCACACTCGGCGCGCCAGTC	20		RIKEN BRC

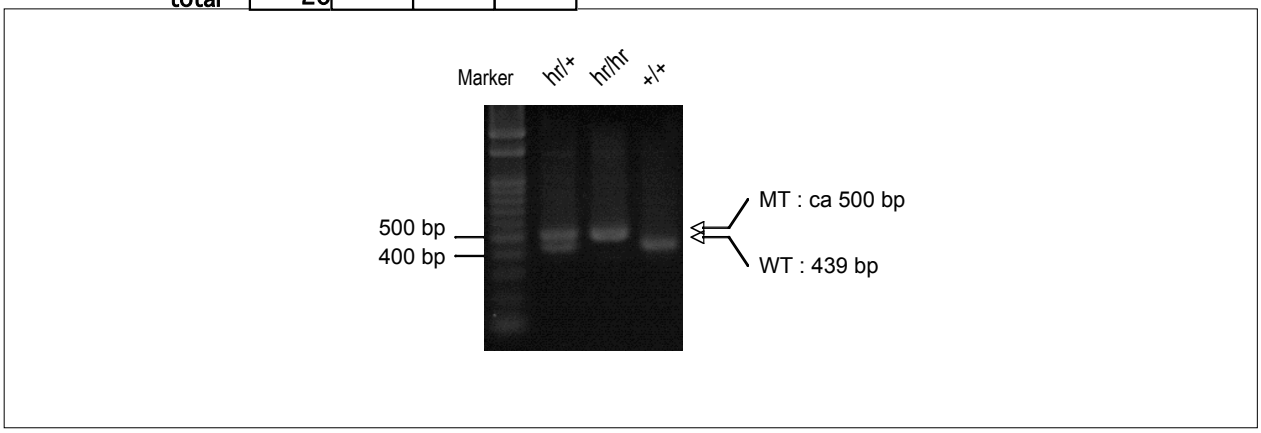
Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		12.3			
PCR buffer	10 X	2.0			
dNTP	2.5 mM	1.6			
primer 1	10 pmol/μl	1.0			
primer 2	10 pmol/μl	1.0			
primer 3	10 pmol/μl	1.0			
DNA		1.0			
Taq	5U/μl	0.1			
	total	20			

Reaction Conditions

step	temp (℃)	time (sec)		note
1	94	180		
2	94	30	*	
3	65	30	*	
4	72	60	*	*Repeat for 30 cycles
5	72	300		



Marker

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

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 2	439 bp	WT
primer 3	primer 2	ca 500 bp	mutant

Comment