

PCR protocol

RBRC GD000099 Strain name Srr<Rgsc01872>

Primer	primer_name	primer_seq	length	
1	Primer R	CTGCAACCAACCAAACTCC	20	Depositor's primer
2	wild	GCTCCTTTCTGTGCCAACAGTGT	23	RIKEN BRC
3	mut	GCTCCTTTCTGTGCCAACAGTGA	23	RIKEN BRC

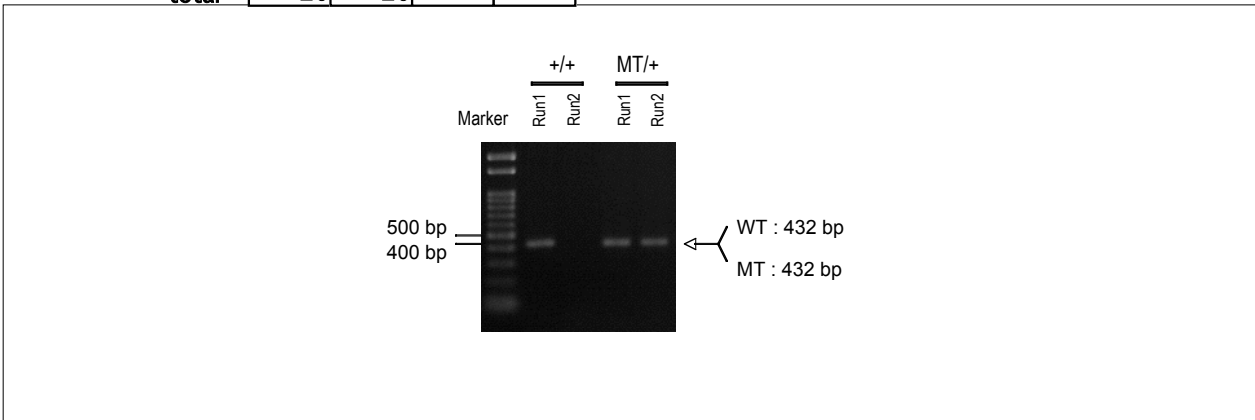
Reaction Components

 Enzyme Roche Taq (Cat. No. BM1418432)

Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		13.3	13.3		
PCR buffer	10 X	2.0	2.0		
dNTP	2.5 mM	1.6	1.6		
primer 1	10 pmol/ μ l	1.0	1.0		
primer 2	10 pmol/ μ l	1.0	0		
primer 3	10 pmol/ μ l	0	1.0		
DNA		1.0	1.0		
Taq	5U/ μ l	0.1	0.1		
total		20	20		

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	57	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 1	primer 2	product_size	product
primer 1	primer 2	432 bp	WT
primer 1	primer 3	432 bp	mutant

Comment