

PCR protocol

RBRC05261

Strain name DW/J-Tpst2<grt>/Hkv

Primer	primer_name	primer_seq	length	
1	common	CCCTTTACTTACTGAGCCATACTGC	25	RIKEN BRC
2	WT	GGAGTGACACAGTCCTGCACCTC	23	RIKEN BRC
3	MT	GGAGTGACACAGTCCTGCACCTG	23	RIKEN BRC

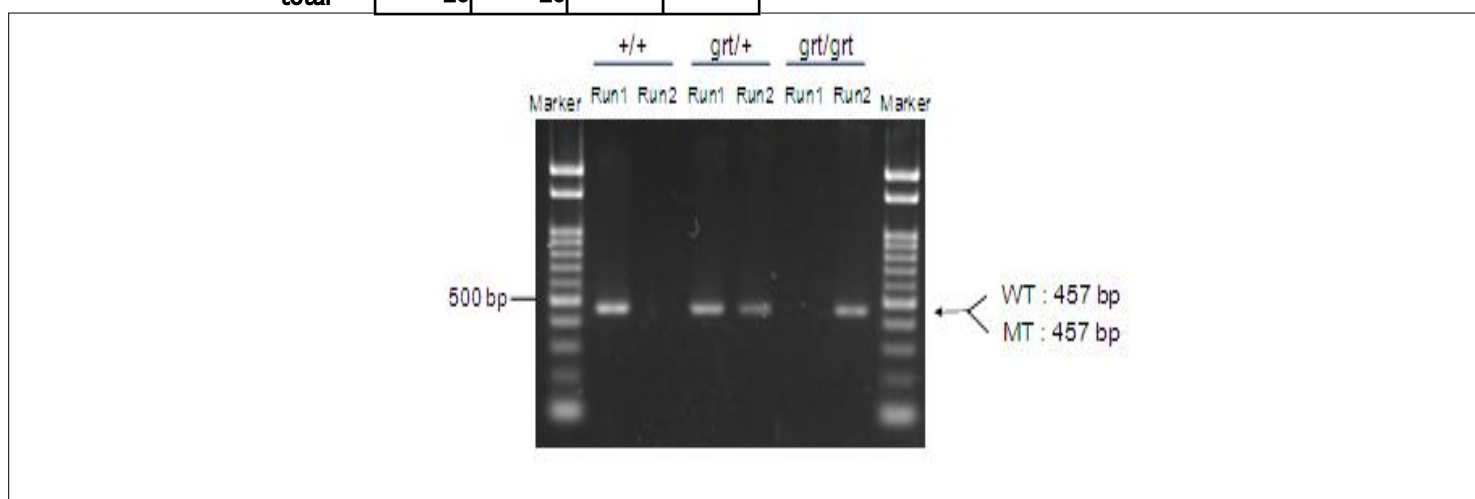
Reaction Components

 Enzyme

	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/μl	1.0	1.0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq	5 U/μl	0.1	0.1		
total		20	20		

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	63	30*	
4	72	60*	*Repeat for 35 cycles
5	72	300	



Marker	PCR products	primer_set	product_size	product
Gene Ladder 100 (Wako, Cat. 316-06951)	primer 1	primer 2	457 bp	WT
Gel	primer 1	primer 3	457 bp	mutant

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