

PCR protocol

RBRC 03709

Strain name Disc1(-) (Acc.No. CDB0583K)

Primer	primer_name	primer_seq	length	
1	Wt	GGTCAGAGCTCAGAGAACTCCTTGTGG	27	Depositor's primer
2	Neo	CATCGCCTTCTATCGCCTTCTTGACG	26	Depositor's primer
3	Rev	GCTTCCTACAGAGCAGACTCCACACC	26	Depositor's primer

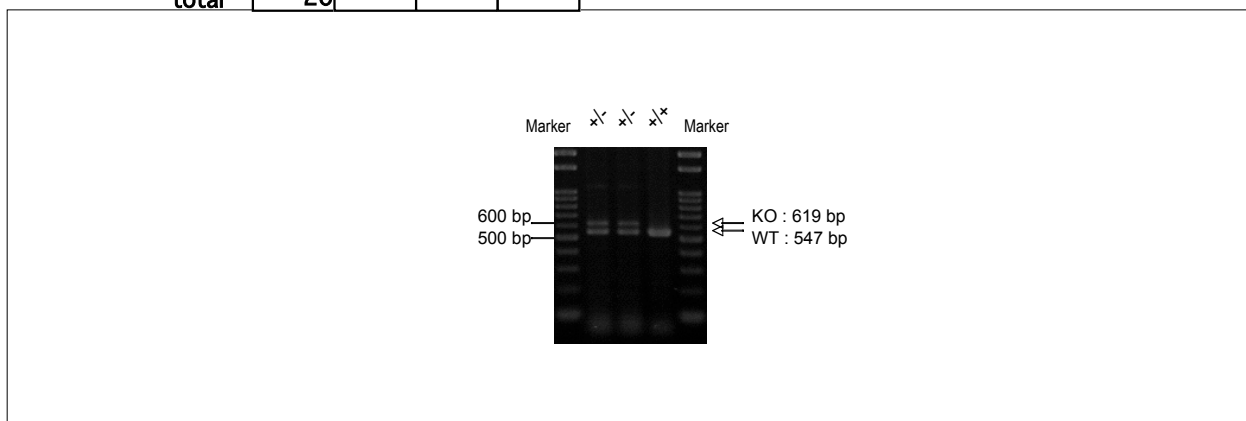
Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

Reaction Conditions

	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			

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	63	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 3	547 bp	WT
primer 2	primer 3	619 bp	KO

Comment