

PCR protocol

RBRC01916

Strain name pKF1K04.3.1

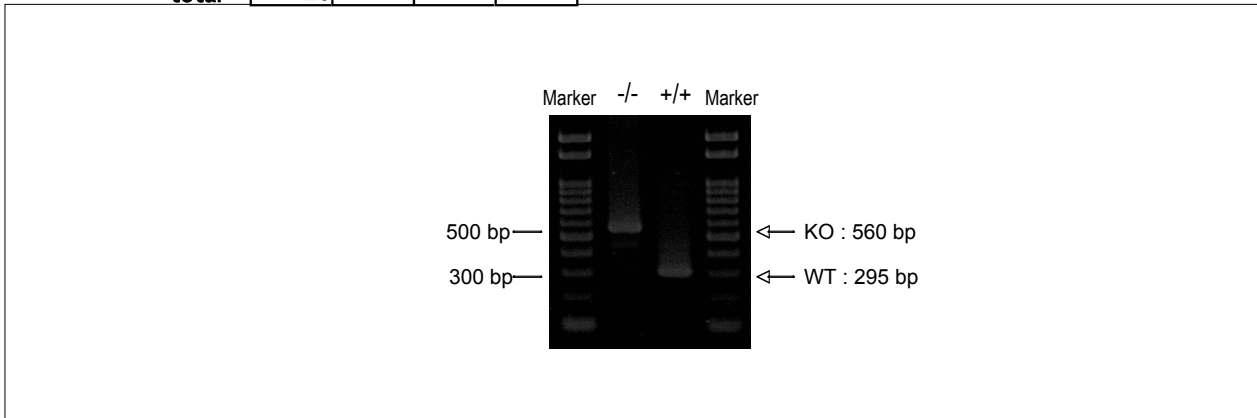
Primer	primer_name	primer_seq	length	
1	17436FW	AACTTGAAGTCGCTGTCTTTTGG	23	Depositor's primer
2	20292RV	CCCTATAAAATTCTTTCTATCC	24	Depositor's primer
3		caatctctgtccagcacctcttgg	24	RIKEN BRC

Reaction Components
Enzyme TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		3.8			
PCR buffer	2 X GC I	10.0			
dNTP	2.5 mM	2.0			
primer 1	10 pmol/ μ l	1.0			
primer 2	10 pmol/ μ l	1.0			
primer 3	10 pmol/ μ l	1.0			
DNA		1.0			
Taq		0.2			
total		20			

step	temp ($^{\circ}$ 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_set	product_size	product
primer 2 primer 3	295 bp	WT
primer 1 primer 2	560 bp	KO

Comment