PCR protocol

RBRC 00300 **Strain name** RKO-1

Primer	primer_name	primer_seq	length		
1	LCB 1070 (R4)	CTCTGGCGAATGGCTTTACTTTAG	24		Depositor's primer
2	LCB 1071 (R6)	GGGAAACTGAGTCAGATACTTTCTTCC	27		Depositor's primer
3	3Neo	CGGCCACAGTCGATGAATCC	20		Proc. Natl. Acad. Sci. USA, 94, 13069-13074 (1997).

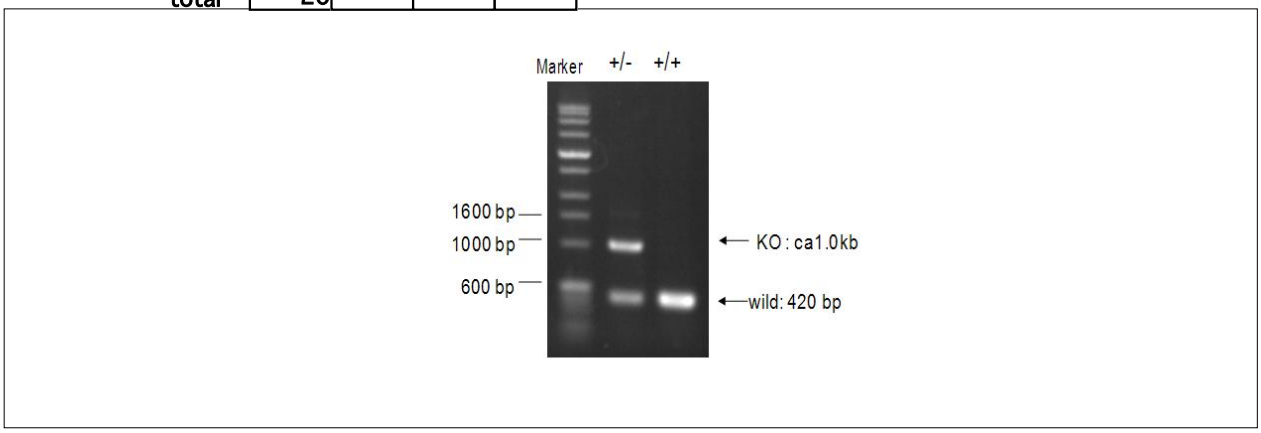
Reaction Components

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

	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/μl	1.0			
primer 2	10 pmol/μl	1.0			
primer 3	10 pmol/μl	1.0			
DNA		1.0			
Taq		0.2			
	total	20			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	300	
2	94	60	*
3	60	60	*
4	72	90	* *Repeat for 30 cycles
5	72	300	



Marker

1 kb DNA laddwe(Affymetrix, Cat. 76714 500 UL)

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 3	ca 1000 bp	KO
primer 1	primer 2	420 bp	WT

Comment