PCR protocol

RBRC 00305 **Strain name** TNFκB

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	Exon 4	AGATAGCAAATCGGCTGACGG	21		Depositor's primer
4	neomycin	CCTTCTATCGCCTTCTTGACG	21		Depositor's primer
5	Exon 2	ATCAGTTCTATGGCCCAGACC	21		Depositor's primer

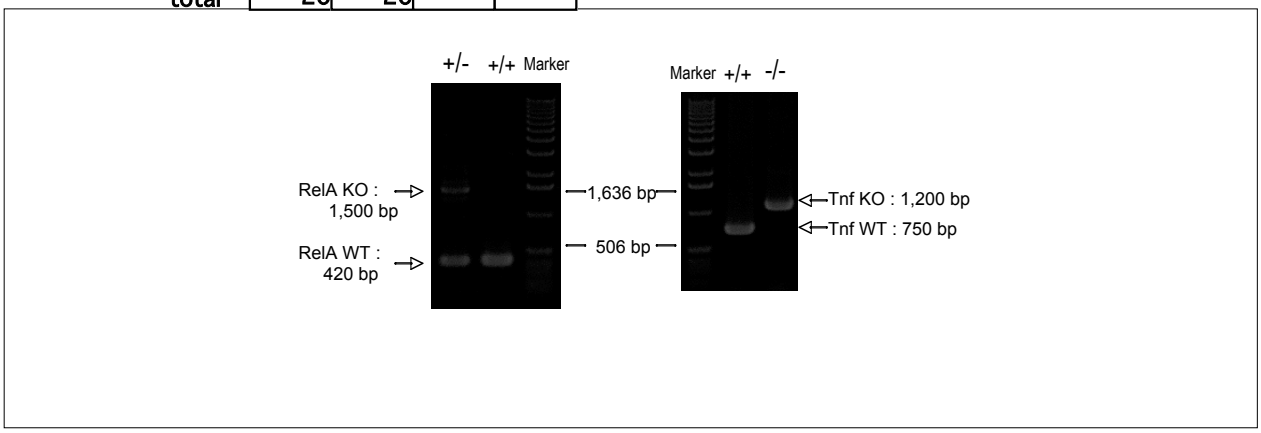
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		5.3	4.3		
PCR buffer	2 X GC I	10.0	10.0		
dNTP	2.5 mM	1.6	1.6		
primer 1	10 pmol/μl	1.0	0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	0	1.0		
primer 4	10 pmol/μl	0	1.0		
primer 5	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq	5U/μl	0.1	0.1		
	total	20	20		

Reaction Conditions

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



Marker

1 kb DNA ladder (Invitrogen, Cat. 15615-024)

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 2	420 bp	WT(RelA)
primer 1	primer 2	1,500 bp	KO(RelA)
primer 3	primer 5	750 bp	WT(Tnf)
primer 3	primer 4	1,200 bp	KO(Tnf)

Comment