PCR protocol

Sheet ID PS_04207
Release date : 5/9/2022
Print date : 5/9/2022

RBRC 00305

Strain name B6.129S-Tnf<tm1Ljo> Rela<tm1Yuo>/Rbrc

Primer	primer_name	primer_seq	length		Reference
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)

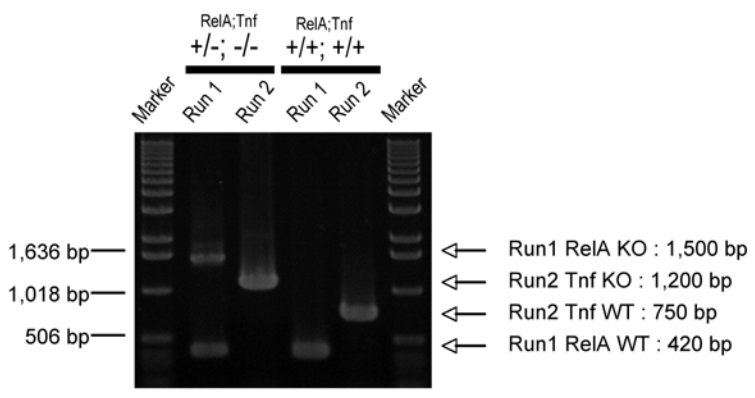
Reagent	Conc	Reaction 1	2	3	4	
H2O		4.9	3.9			(μL)
PCR buffer	2 X GC I	10.0	10.0			
dNTP	2.5 mM	2.0	2.0			
primer 1	10 pmol/μl	1.0	0			
primer 2	10 pmol/μl	1.0	0			
primer 3	10 pmol/μl	0	1.0			
primer 4	10 pmol/μl	0	1.0			
primer 5	10 pmol/μl	0	1.0			
DNA		1.0	1.0			
Taq	5 U/μl	0.1	0.1			
	total	20	20			

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)



primer_set		product_size	product (allele type)
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

PCR protocol

Sheet ID PS_03974
Release date : 03/23/2011
Print date : 5/9/2022

RBRC 00305

Strain name TNFκB

Primer	primer_name	primer_seq	length		Reference
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)

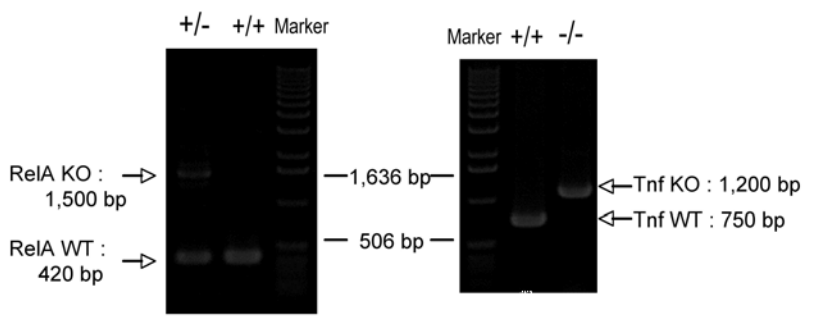
Reagent	Conc	Reaction 1	2	3	4	
H2O		5.3	4.3			(μL)
PCR buffer	2 X GC I	10.0	10.0			
dNTP	2.5 mM	1.6	1.6			
primer 1	10 pmol/μl	1.0	0			
primer 2	10 pmol/μl	1.0	0			
primer 3	10 pmol/μl	0	1.0			
primer 4	10 pmol/μl	0	1.0			
primer 5	10 pmol/μl	0	1.0			
DNA		1.0	1.0			
Taq	5 U/μl	0.1	0.1			
	total	20	20			

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)



primer_set		product_size	product (allele type)
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

PCR protocol

Sheet ID PS_00138
Release date : 11.19.2008
Print date : 5/9/2022

RBRC 00305

Strain name TNFκB

Primer	primer_name	primer_seq	length		Reference
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: Run1: TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)
Run2: Roche Taq (Cat. No. BM1418432)

Reagent	Conc	Reaction 1	2	3	4
H2O		4.8	15.3		
PCR buffer	2 X GC I	10.0	0		
PCR buffer2	10 X	0	2.5		
dNTP	2.5 mM	2.0	2.0		
primer 1	10 pmol/μl	1.0	0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	0	2.0		
primer 4	10 pmol/μl	0	1.0		
primer 5	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq		0.2	0.2		
	total	20	25		

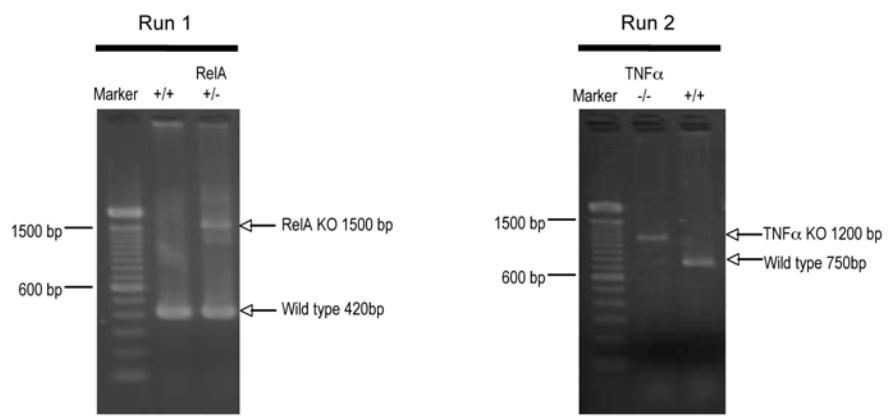
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	300	[Reaction 1]
2	94	60	*
3	60	60	*
4	72	120	* *Repeat for 38 cycles
5	72	300	
1	94	600	[Reaction 2]
2	94	60	*
3	58	60	*
4	72	120	* *Repeat for 30 cycles
5	72	300	

Marker: 100 bp DNA Ladder (TOYOBO, Cat. DNA-030)

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



primer_set		product_size	product (allele type)
primer 1	primer 2	420 bp	WT (Rela)
primer 1	primer 2	1.5 kb	KO (Rela)
primer 3	primer 4	1200 bp	KO (Tnfa)
primer 3	primer 5	750 bp	WT (Tnfa)

Comment Separated PCR