

RBRC 00306

Strain name TNF.K.O

Primer	primer_name	primer_seq	length	
1	Exon 4	AGATAGCAAATCGGCTGACGG	21	Depositor's primer
2	Exon 2	ATCAGTTCTATGGCCAGACC	21	Depositor's primer
3	neomycin	CCTTCTATCGCCTTCTTGACG	21	Depositor's primer

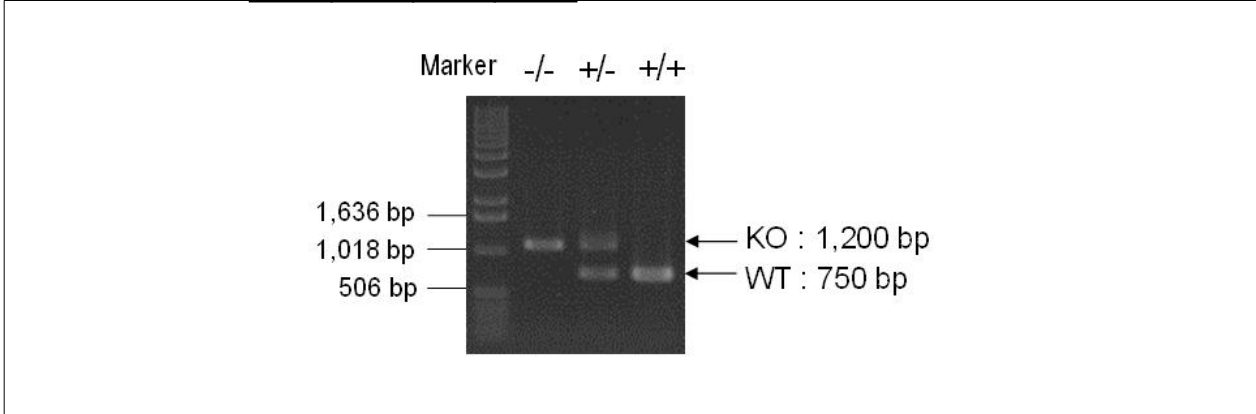
Reaction Components

Enzyme	TOYOBO KOD FX (Code.No : KFX-101)
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Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	90*	*Repeat for 30 cycles

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8			
PCR buffer	2 X	10.0			
dNTP	2.0 mM	4.0			
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	1 U/μl	0.2			
total		20			



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	750 bp	WT
primer 1	primer 3	1,200 bp	KO

Comment Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.

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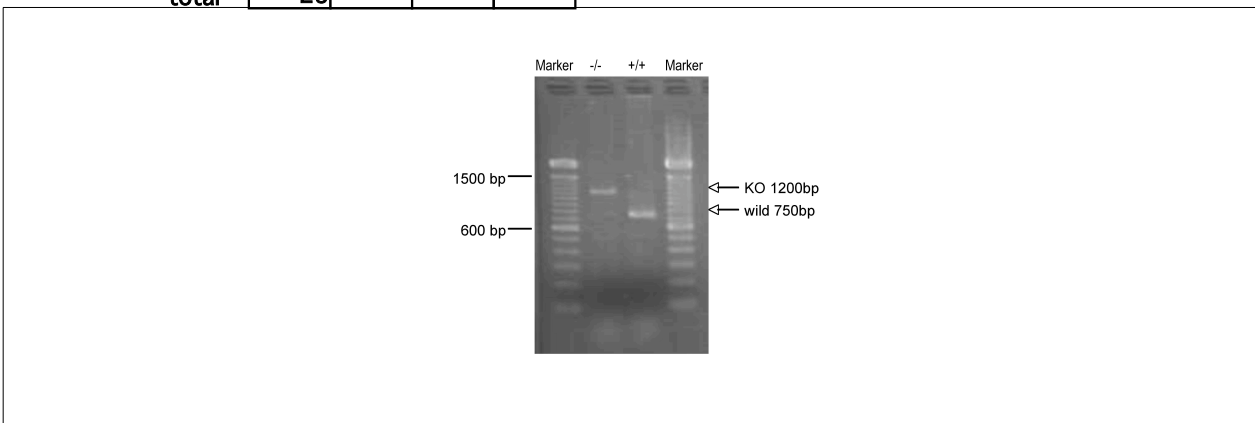
Reaction Components

Enzyme Roche Taq (Cat. No. BM1418432)

Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		15.3			
PCR buffer	10 X	2.5			
dNTP	2.5 mM	2.0			
primer 1	10 pmol/ μ l	2.0			
primer 2	10 pmol/ μ l	1.0			
primer 3	10 pmol/ μ l	1.0			
DNA		1.0			
Taq		0.2			
total		25			

step	temp (°C)	time (sec)	note
1	94	600	
2	94	60*	
3	58	60*	
4	72	120*	*Repeat for 30 cycles
5	72	300	



Marker
100 bp ladder (Invitrogen, Cat. 15628-050)

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

PCR products			
	primer_set	product_size	product
primer 1	primer 2	1200 bp	KO
primer 1	primer 3	750 bp	WT

Comment