

RBRC 00407

Strain name TCRd KO

Primer	primer_name	primer_seq	length	
1		CTTGCAGTTCAGAGGTCACAGCAG	24	RIKEN BRC
2		GTGGTGGTGAAGGGTCAGTCTG	22	RIKEN BRC
3	AGN	TCGTGCTTTACGGTATCGCCG	21	

Reaction Components

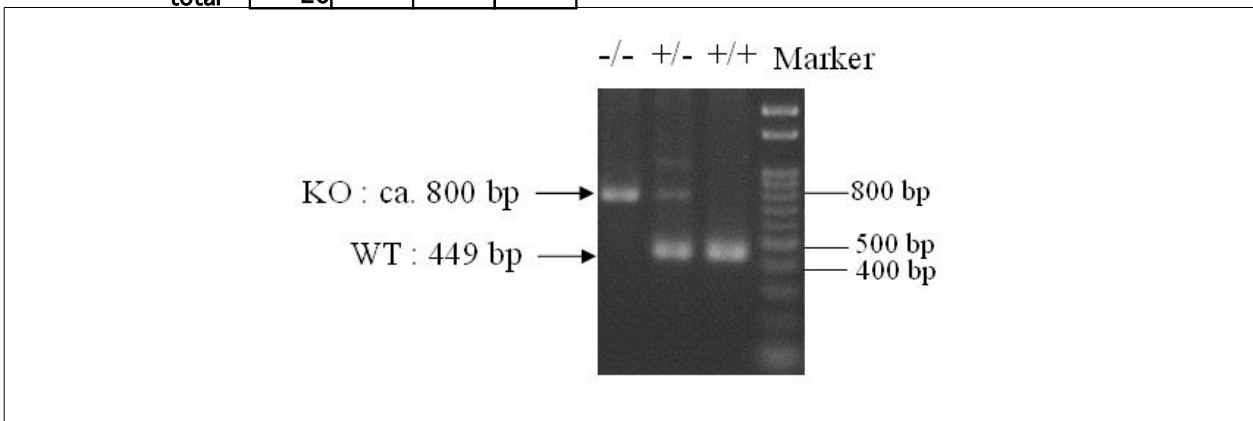
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

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

Standard PCR



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 1	primer 2		449 bp	WT
primer 3	primer 2		ca. 800 bp	KO

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

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Primer	primer_name	primer_seq	length	
1	oIMR0013	CTTGGGTGGAGAGGCTATTC	20	JAX Genotyping Protocol
2	oIMR0014	AGGTGAGATGACAGGAGATC	20	JAX Genotyping Protocol
3	WT TCRδallele1	AAAAGCCAGCCTCCGGCCAAA	21	Depositor's primer
4	WT TCRδallele2	AACTGAACATGTCACCTGAATT	21	Depositor's primer

Reaction Components

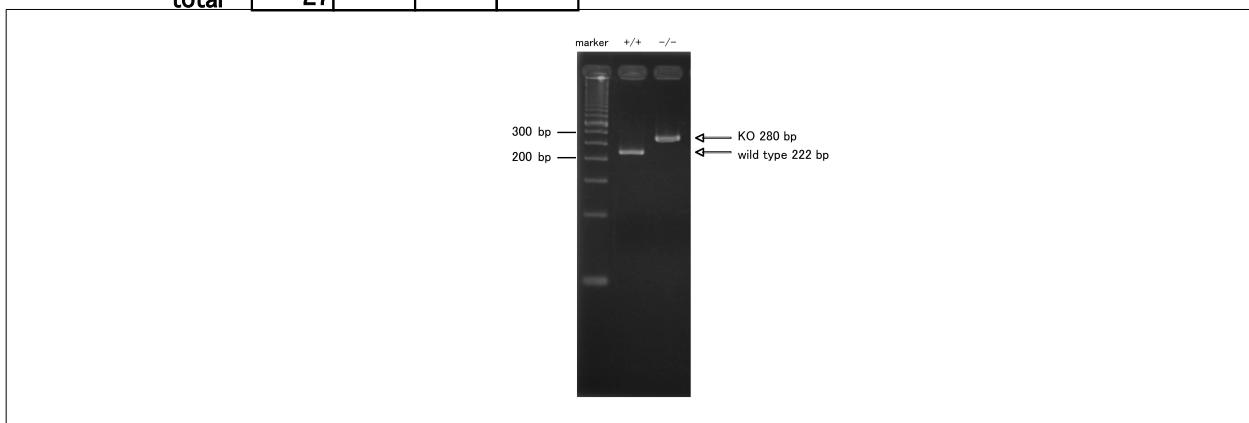
Enzyme Roche Taq (Cat. No. BM1418432)

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	300	
2	94	30*	
3	55	30*	
4	72	60*	*Repeat for 35 cycles
5	72	300	

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		17.3			
PCR buffer	10 X	2.5			
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			
primer 4	10 pmol/μl	1.0			
DNA		1.0			
Taq		0.2			
total		27			

Standard PCR



Marker

50 bp ladder (Invitrogen, Cat. 10416-014)

Gel

4% Nusieve in 0.5 X TBE (100 V, 45 min)

PCR products

primer_set	primer_1	primer_2	product_size	product
primer 1	primer 2		280 bp	KO
primer 3	primer 4		222 bp	WT

Comment