

PCR protocol

RBRC 02886

Strain name EOD/ThpNkc-Cno<cno-II>/Nkc

Primer	primer_name	primer_seq	length	
1	Cno_Wild	caggtcgccaaggccgaagcTg	22	RIKEN BRC
2	Cno_Mut	caggtcgccaaggccgaagcTa	22	RIKEN BRC
3	Cno_common	ctatctatcccagaaggctacag	23	RIKEN BRC
4		AGGTTACAAAAGGTCACCC	19	Clin. Exp. Immunol., 116 (1): 153-163
5		GATACGAAGATCCTTTCTGTG	22	Clin. Exp. Immunol., 116 (1): 153-163
6		CAAACGCAGTCAAATCTGCTC	21	Clin. Exp. Immunol., 116 (1): 153-163

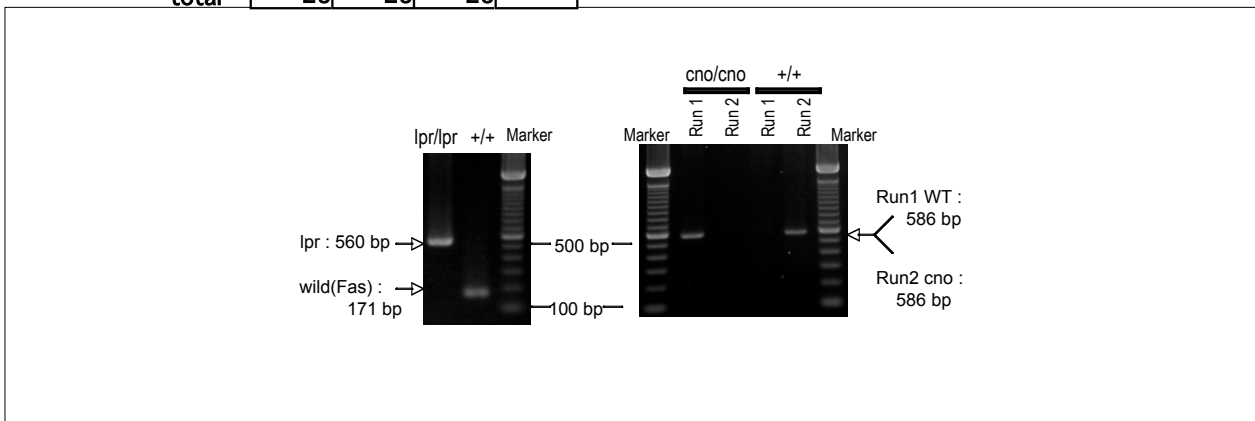
Reaction Components

Enzyme Run1, 2: Roche Taq (Cat. No. BM1418432) Run3:
TaKaRa Ex Taq (Cat. No. RR001A)

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	60	30*	
4	72	60*	*Repeat for 35 cycles
5	72	300	

Conc	Run 1	Run 2	Run 3	Run 4
H2O	17.3	17.3	16.3	
PCR buffer	2.5	2.5	2.5	
dNTP	2.0	2.0	2.0	
primer 1	10 pmol/μl	0	1.0	0
primer 2	10 pmol/μl	1.0	0	0
primer 3	10 pmol/μl	1.0	1.0	0
primer 4	10 pmol/μl	0	0	1.0
primer 5	10 pmol/μl	0	0	1.0
primer 6	10 pmol/μl	0	0	1.0
DNA	1.0	1.0	1.0	
Taq	0.2	0.2	0.2	
total	25	25	25	



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 2	586 bp	cno
primer 1	586 bp	WT
primer 4	171 bp	Fas
primer 4	560 bp	lpr

Comment Arthritis Rheum., 54, 2934-2943(2006).