PCR protocol

Sheet ID PS_06089
Release date : 3/2/2021
Print date : 3/2/2021

RBRC 01491

Strain name B6;B6CB-Lrrn4<tm1>/Rbrc

Primer	primer_name	primer_seq	length		Reference
1	WT_Fw	CGTCTCCACCTGCTCACTGAC	21		RIKEN BRC
2	WTAS	AGGTTGTGACTGAGGTCGAGCTCAC	25		Mol. Cell. Biol., 25, 4166-4175 (2005).
3	3'lacZ-2F	ACTATCCCGACCGCCTTACT	20		Dev Biol., 336(2):222-31 (2009).

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	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			

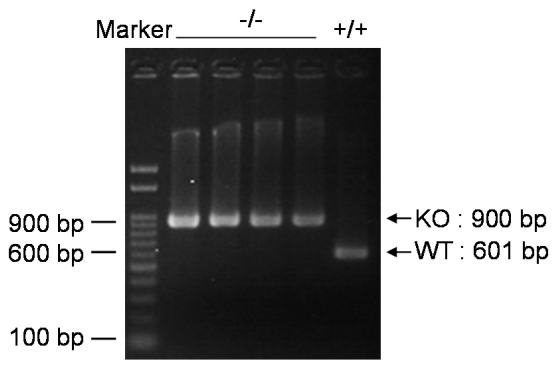
(μL)

Reaction Conditions

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

Marker Gene Ladder 100 (Wako, Cat. 316-06951)

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



primer_set		product_size	product (allele type)
primer 1	primer 2	601 bp	WT
primer 1	primer 3	900 bp	KO

+/+ (wild-type): 601 bp
-/- (homozygous): 900 bp
+/- (heterozygous): 601 bp and 900 bp

Comment

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

PCR protocol

Sheet ID PS_00620
Release date : 6/20/2006
Print date : 3/2/2021

RBRC 01491

Strain name NLRR4-KO(B6)

Primer	primer_name	primer_seq	length		Reference
1	WTS	TACGGACTCTCTCTGTGTAGGACTCCC	27		Depositor's primer
2	WTAS	AGGTTGTGACTGAGGTCGAGCTCAC	25		Depositor's primer
3	NLRR4KOS	GCTGTGCTCGACGTTGTCACTGAAG	25		Depositor's primer
4	NLRR4KOAS	ATACCGTAAAGCACGAGGAAGCGGTC	26		Depositor's primer

Reaction Components

Enzyme: Roche Taq (Cat. No. BM1418432)

Reagent	Conc	Reaction 1	2	3	4
H2O		15.3			
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	25			

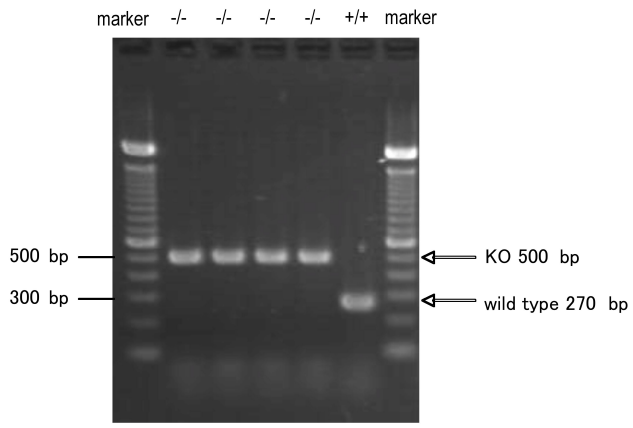
(μL)

Reaction Conditions

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

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

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



primer_set		product_size	product (allele type)
primer 1	primer 2	270 bp	WT
primer 3	primer 4	500 bp	KO

Comment Standard PCR