PCR protocol

Sheet ID PS_04769
Release date : 9/28/2015
Print date : 9/28/2015

RBRC 05512 **Strain name** C57BL/10ScCr

Primer	primer_name	primer_seq	length		
1	Tlr4-5	GCAACACCTGTAGAGATGAATACC	24		RIKEN BRC
2	Tlr4-3	CAAAGATACACCAACGGCTCTG	22		RIKEN BRC
3	ScCrTlr4-5	GCAAGTTTCTATATGCATTCTC	22		Depositor's primer
4	ScCrTlr4-3	CCTCCATTTCCAATAGGTAG	20		Depositor's primer
5	common	ATGCCTTATCCTACACAAACTGAG	24		RIKEN BRC
6	wild	GGTCTCTGCTTTCTAGCACCTAG	23		RIKEN BRC
7	mut	GGTCTCTGCTTTCTAGCACCTAC	23		RIKEN BRC

Reaction Components

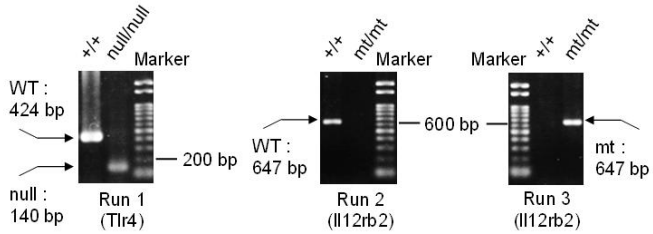
Enzyme Run1: TaKaRa Ex Taq (Cat. No. RR001A) Run2: RocheTaq (Cat. No.BM1418432)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		9.3	13.3	13.3	
PCR buffer	10 X	2.0	2.0	2.0	
dNTP	2.5 mM	1.6	1.6	1.6	
primer 1	10 pmol/μl	2.0	0	0	
primer 2	10 pmol/μl	2.0	0	0	
primer 3	10 pmol/μl	1.0	0	0	
primer 4	10 pmol/μl	1.0	0	0	
primer 5	10 pmol/μl	0	1.0	1.0	
primer 6	10 pmol/μl	0	1.0	0	
primer 7	10 pmol/μl	0	0	1.0	
DNA		1.0	1.0	1.0	
Taq	5 U/μl	0.1	0.1	0.1	
	total	20	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		

Allele specific PCR (ASP)



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	424 bp	WT (Tlr4)
primer 3	primer 4	140 bp	null (Tlr4)
primer 5	primer 6	647 bp	WT (Il12rb2)
primer 5	primer 7	647 bp	mutant (Il12rb2)

Comment