PCR protocol

Sheet ID PS_06539
Release date : 3/17/2022
Print date : 3/17/2022

RBRC 01306

Strain name B6.Cg-Hbegf<tm1.1Mek>/MekRbrc

Primer	primer_name	primer_seq	length		Reference
1	promoter	CAGCGGACAGTGCCTTAGTGG	21		RIKEN BRC
2	exon 1	GAGGAGGCCGCAATAAGACCC	21		RIKEN BRC
3	lacZ-R	TCTGCTTCAATCAGCGTGCC	20		Genes Cells, 1:59-72 (1996).

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	4
H2O		1.6			
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	0.4			
	total	20			

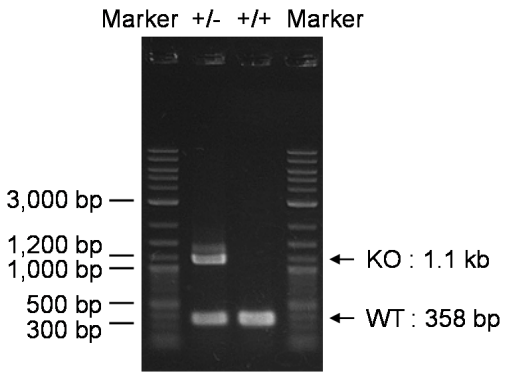
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	68	180	* *Repeat for 30 cycles

Marker: 2-Log DNA Ladder (NEB, Cat. N3200)

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



primer_set		product_size	product (allele type)
primer 1	primer 2	358 bp	WT
primer 1	primer 3	1.1 kb	KO

+/+ (wild-type): 358 bp
+/- (heterozygous): 358 bp and 1.1 kb

Comment

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

PCR protocol

Sheet ID PS_00093
Release date : 7/12/2005
Print date : 3/17/2022

RBRC 01306

Strain name HB-EGF Knockout mice(BL6)(HBdel#80/B6)

Primer	primer_name	primer_seq	length		Reference
1	Neo-geno-F1	ATGGGATCGGCCATTGAACA	20		Depositor's primer
2	Neo-geno-R1	GAAGAACTCGTCAAGAAGGC	20		Depositor's primer
3	HBint1	AGGGCAAGATCATGTGTCCTGCCTCAAGCC	30		Depositor's primer
4	5-5S2	CATGATGCTCCAGTGAGTAGGCTCTGATTAC	31		Depositor's primer

Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

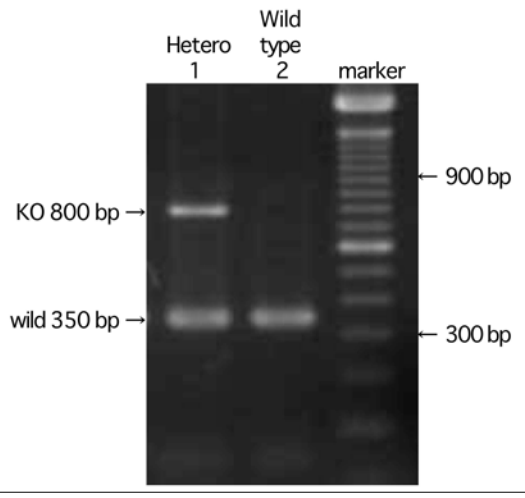
Reagent	Conc	Reaction 1	2	3	4	
H2O		15.3				(μL)
PCR buffer	10 X	2.5				
dNTP	2.5 mM	2.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
primer 4	10 pmol/μl	1.0				
DNA		1.0				
Taq		0.2				
	total	25				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30*	
3	57	30*	
4	72	60*	*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)



primer_set		product_size	product (allele type)
primer 1	primer 2	800 bp	KO
primer 3	primer 4	350 bp	WT

Comment Standard PCR