PCR protocol

Sheet ID PS_01838
Release date : 1/30/2017
Print date : 1/30/2017

RBRC 02674 Strain name B6;129S2-Phf21a<tm1Tba>

Primer	primer_name	primer_seq	length		
1	Bhc80-2	GCCTAGTCTAACTGCATCACAG	22		Depositor's primer
2	Bhc80-3	GGCTCTCTGAGATAGCATCTC	21		Depositor's primer
3	NP4	TGCTCGACGTTGTCACTGAAGC	22		Depositor's primer

Reaction Components

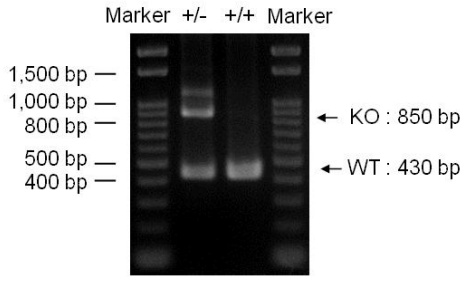
Enzyme: TOYOBO KOD FX (Code.No : KFX-101)

	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			

Reaction Conditions

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

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	430 bp	WT
primer 2	primer 3	850 bp	KO

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

PCR protocol

Sheet ID PS_01956
Release date : 11.19.2008
Print date : 1/30/2017

RBRC 02674 Strain name B6;129S2-Phf21a<tm1Tba>

Primer	primer_name	primer_seq	length		
1	Bhc80-2	GCCTAGTCTAACTGCATCACAG	22		Depositor's primer
2	Bhc80-3	GGCTCTCTGAGATAGCATCTC	21		Depositor's primer
3	IL3NEO	TGATCTGGACGAAGAGCATCA	21		

Reaction Components

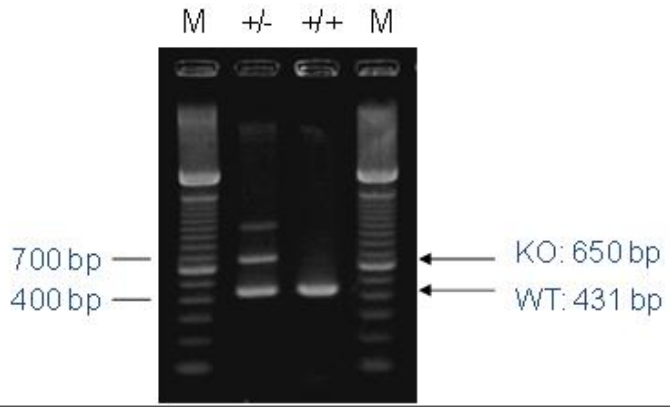
Enzyme: TaKaRa Ex Taq (Cat. No. RR001A)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		16.3			
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			
DNA		1.0			
Taq		0.2			
	total	25			

Reaction Conditions

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

Standard PCR



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 1	primer 2	431 bp	WT
primer 2	primer 3	650 bp	KO

Comment