PCR protocol

Sheet ID PS_05830
Release date : 5/22/2014
Print date : 5/22/2014

RBRC 06308 **Strain name** B6.129-(D1Mit501-Fcgr2b) Fcgr2b<tm1Ttk>/Hros

Primer	primer_name	primer_seq	length		
1	5'EC1(F)	AAACTCGACCCCCCGTGGATC	21		Depositor's primer
2	3'EC1(R)	TTGACTGTGGCCTTAAACGTGTAG	24		Depositor's primer
3	Neo	CTCGTGCTTTACGGTATCGCC	21		Depositor's primer

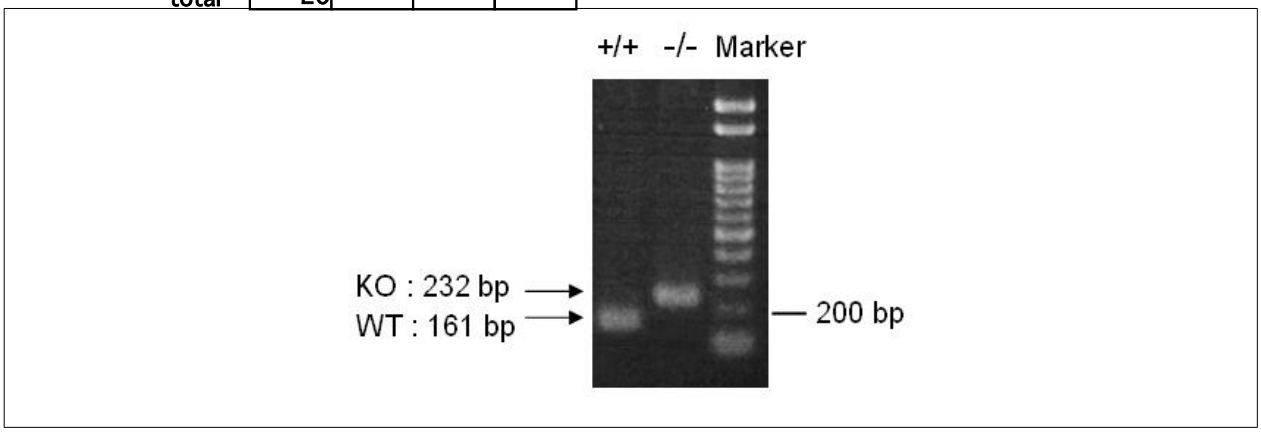
Reaction Components

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



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	161 bp	WT
primer 2	primer 3	232 bp	KO

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

PCR protocol

Sheet ID PS_05847
Release date : 5/22/2014
Print date : 5/22/2014

RBRC 06308 **Strain name** B6.129-(D1Mit501-Fcgr2b) Fcgr2b<tm1Ttk>/Hros

Primer	primer_name	primer_seq	length		
1	*D1Mit15-#1*	TCCACAGAACTGTCCCTCAA	20		Depositor's primer
2	*D1Mit15-#2*	ATACACTCACACCACCCCGT	20		Depositor's primer
3	D1Mit501	TCTGAGCACAGCAGAAGGAA	20		Depositor's primer
4	D1Mit501	GAATGATCTTTTACAACCAAAGTGG	25		Depositor's primer

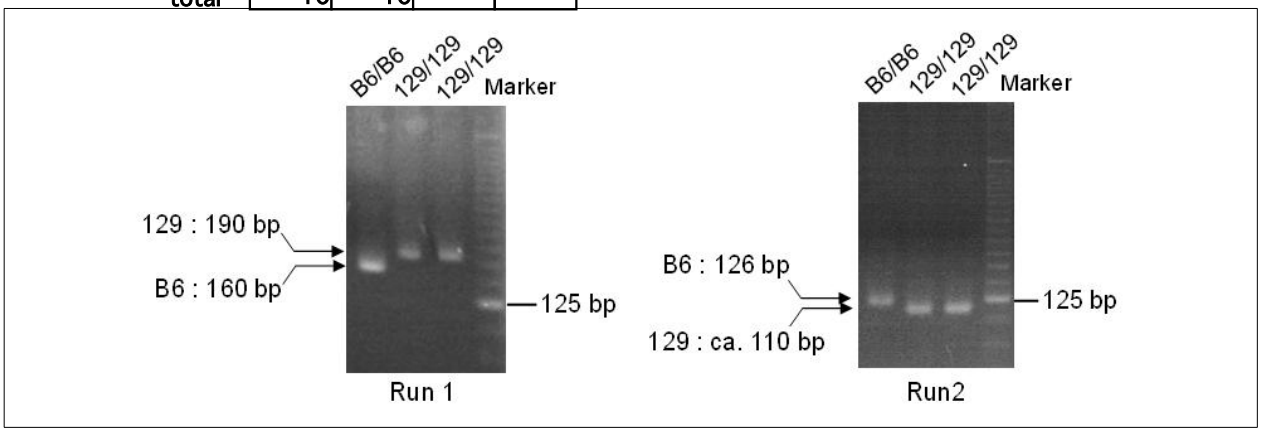
Reaction Components

Enzyme QIAGEN Multiplex PCR Kit (Cat. No. 206143)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		2.0	2.0		
Master Mix	2 X	5.0	5.0		
primer 1	10 pmol/μl	1.0	0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	0	1.0		
primer 4	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
	total	10	10		

Reaction Conditions

step	temp (℃)	time (sec)		note
1	95	900		
2	94	30	*	
3	60	90	*	
4	72	60	*	*Repeat for 30 cycles
5	60	1800		



Marker

25 bp DNA ladder (Invitrogen, Cat. 10597-011)

Gel

4% Nusieve in 0.5 X TBE (100 V, 80 min)

PCR products

primer_set		product_size	product
primer 1	primer 2	160 bp	B6
primer 1	primer 2	190 bp	129(KO2)
primer 3	primer 4	126 bp	B6
primer 3	primer 4	ca. 110 bp	129(KO2)

Comment