PCR protocol

Sheet ID PS_06175
Release date : 4/20/2017
Print date : 4/20/2017

RBRC 02796 Strain name B6.Cg-Stab2<tm1>

Primer	primer_name	primer_seq	length		
1		AATCCTGTCACTCAGCCAGGAC	22		RIKEN BRC
2	stbZWT-rv	ACTGAAGCTTAGTGGGATTAGTTTGCT	27		Depositor's primer
3	lac Z	GCGGATTGACCGTAATGGGATAGG	24		Biochem. Biophys. Res. Commun., 236, 313

Reaction Components

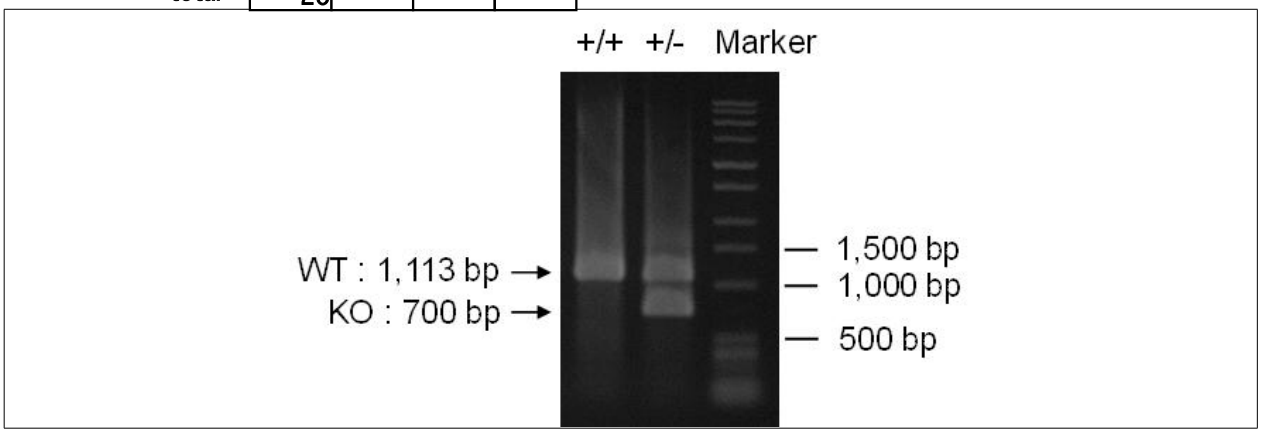
Enzyme: TOYOBO KOD FX (Code.No : KFX-101)

	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	90	* *Repeat for 30 cycles

Standard PCR



Marker
1 kb DNA ladder (Invitrogen, Cat. 15615-024)

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

PCR products	primer_set	product_size	product
primer 1	primer 2	1,113 bp	WT
primer 1	primer 3	ca 700 bp	KO

Comment

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

PCR protocol

Sheet ID PS_01940
Release date : 10/20/2008
Print date : 4/20/2017

RBRC 02796 Strain name B6.Cg-Stab2<tm1>

Primer	primer_name	primer_seq	length		
1	LacZ-rv	TTCAATCAACTGTTTACCTTGTGGAG	26		Depositor's primer
2	LacZ-rw	GGTGGATCAGTCGCTGATTAAATATG	26		Depositor's primer
3	stbZWT-rv	ACTGAAGCTTAGTGGGATTAGTTTGCT	27		Depositor's primer
4	stbZWT-rw	GGGCTACCTTATCACTCTGGGTATCTA	27		Depositor's primer

Reaction Components

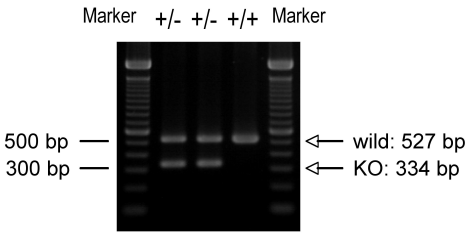
Enzyme: TaKaRa Ex Taq (Cat. No. RR001A)

	Conc	Run 1	Run 2	Run 3	Run 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			

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	

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	334 bp	KO
primer 3	primer 4	527 bp	WT

Comment