PCR protocol

Sheet ID PS_05515
Release date : 5/29/2014
Print date : 5/29/2014

RBRC 05501 Strain name 129-Obif<tm1Tfur>

Primer	primer_name	primer_seq	length		
1	Obif-51	GATTCTGGGAGCCTGGCCGGATAAC	25		Depositor's primer
2	Obif-31	CTACACAGGATGCCACCCGTCCTG	24		Depositor's primer
3	Obif-32	GAGCAAAGCTGCTATTGGCCGCTG	24		Depositor's primer

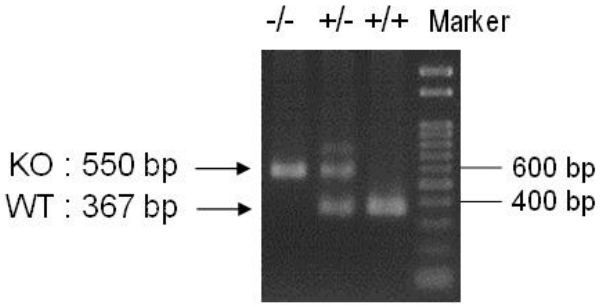
Reaction Components

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	367 bp	WT
primer 1	primer 3	550 bp	KO

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

PCR protocol

Sheet ID PS_04762
Release date : 02/18/2013
Print date : 5/29/2014

RBRC 05501 Strain name 129-Obif<tm1Tfur>

Primer	primer_name	primer_seq	length		
1	Obif-51	GATTCTGGGAGCCTGGCCGGATAAC	25		Depositor's primer
2	Obif-31	CTACACAGGATGCCACCCGTCCTG	24		Depositor's primer
3	Obif-32	GAGCAAAGCTGCTATTGGCCGCTG	24		Depositor's primer

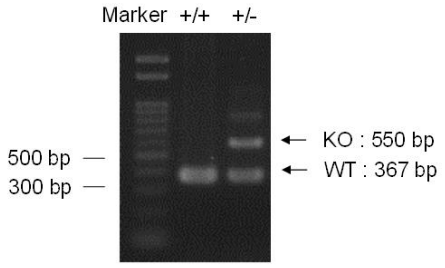
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		12.3			
PCR buffer	10 X	2.0			
dNTP	2.5 mM	1.6			
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	5 U/μl	0.1			
	total	20			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30	*
3	63	30	*
4	72	60	* *Repeat for 30 cycles
5	72	300	



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	367 bp	WT
primer 1	primer 3	550 bp	KO

Comment