PCR protocol

Sheet ID PS_05471
Release date : 8/23/2017
Print date : 8/23/2017

RBRC 02369 Strain name B6;129-Rsph1<tm1Htan>

Primer	primer_name	primer_seq	length		
1	02369_R	GTAGAGGTAGGTGCCTTGCCCGTGC	25		RIKEN BRC
2		GGAGTAGCAAGTGATGTCAGGTCC	24		Depositor's primer
3	Neo10	ATCAGAGCAGCCGATTGTCTGTTG	24		Depositor's primer

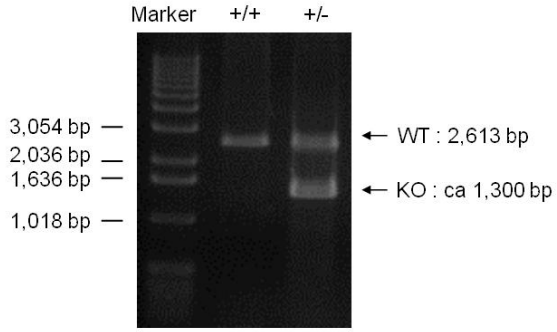
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.6			
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			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	30	*
4	68	240	* *Repeat for 30 cycles



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	2,613 bp	WT
primer 2	primer 3	ca 1,300 bp	KO

Comment

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