PCR protocol

Sheet ID PS_04151
Release date : 7/24/2014
Print date : 7/24/2014

RBRC 04883 Strain name B6.129-Fcnb<tm1Tefu>

Primer	primer_name	primer_seq	length		
1	U1	TGTGTGGATTTGCACATGCATGTGTCCTGG	30		Depositor's primer
2	L1	AGATAGTCACTTCCTGGAGGCCTCTGGTTG	30		Depositor's primer
3	Pr-C	GCTTGCCGAATATCATGGTGGAAAATGGCC	30		Depositor's primer

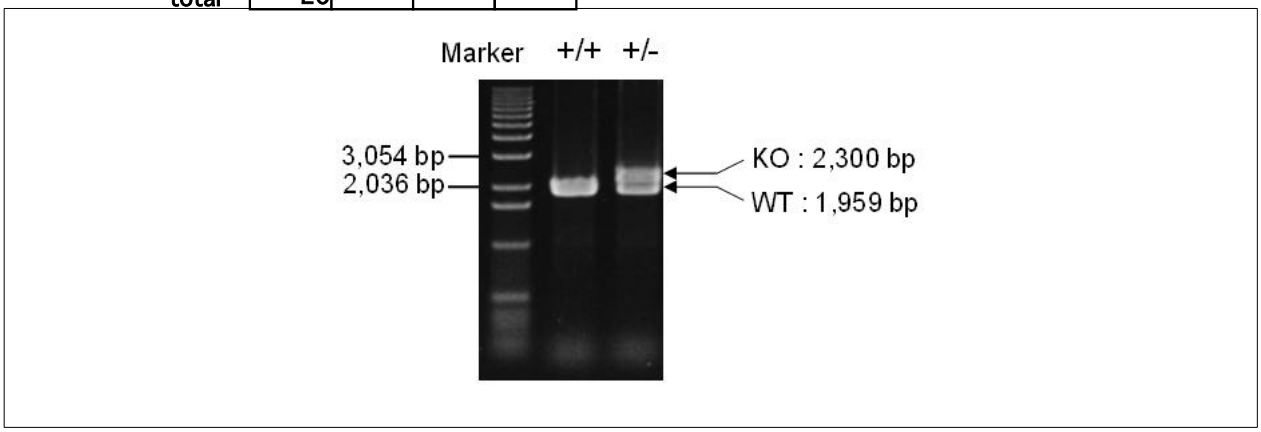
Reaction Components

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 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	68	180	* *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	1,959 bp	WT
primer 1	primer 3	2,300 bp	KO

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