PCR protocol

Sheet ID PS_04133
Release date : 7/24/2014
Print date : 7/24/2014

RBRC 04884 **Strain name** B6;129S4-Cacna1b<tm1Ttan>

Primer	primer_name	primer_seq	length		
1	BIIIR2	ACCTGCATAGACTCGGATTC	20		Depositor's primer
2	lacZR1	GTGCGGGCCTCTTCGCTATTACGC	24		Depositor's primer
3	Cacna1b_Fw	GAGGTCCAGGCAGCTCGCTG	20		RIKEN BRC

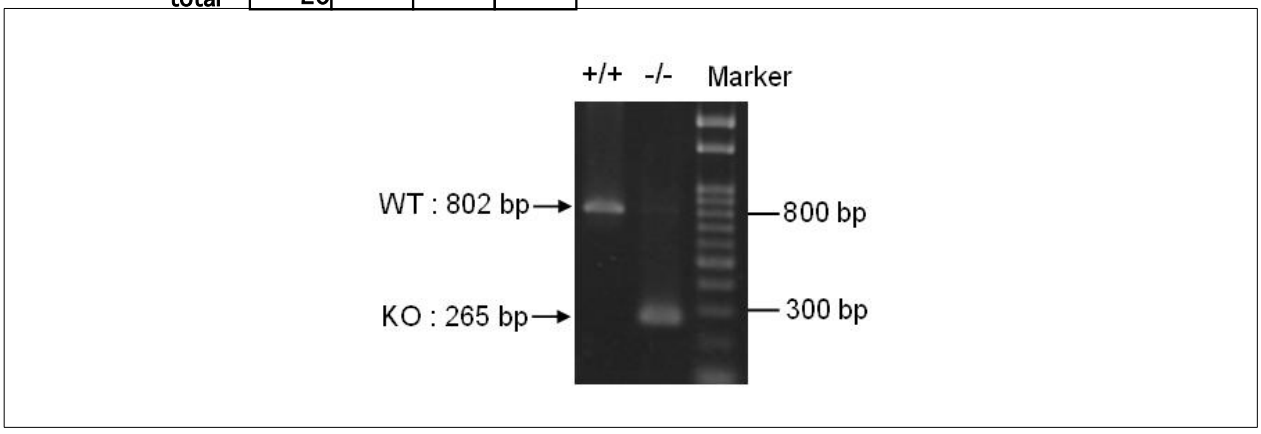
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.6			
PCR buffer	10 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	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 3	802 bp	WT
primer 2	primer 3	265 bp	KO

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