PCR protocol

RBRC 04739 **Strain name** B6.B6CB-Ltb4r1<tm1Tksh>

Primer	primer_name	primer_seq	length		
1	5f(p1)	ATGGGTGTGTCCACTTTCACTCTCTAGGTC	30		Depositor's primer
2	3j(p2)	GCACCACAGATGAAACGCCGAGTT	24		Depositor's primer
3	3q(p3)	CCGGTATGGCCAGCAGAAAAGACACC	26		Depositor's primer

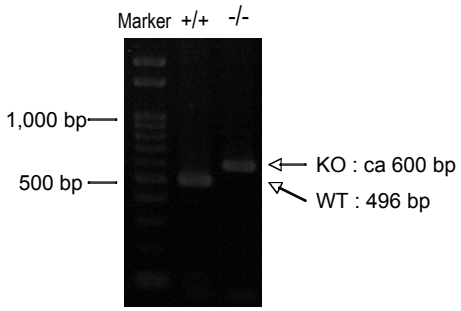
Reaction Components

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	5U/μl	0.1			
	total	20			

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	



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	ca 600 bp	KO
primer 1	primer 3	496 bp	WT

Comment