PCR protocol

RBRC 00994 **Strain name** C57BL/6･129 SH2-B -/-

Primer	primer_name	primer_seq	length		
1	SH2B-Fw	CTCCAGTGATGTTGTCCTTG	20		Depositor's primer
2	SH2B-Rv	CATCGGGGTAGCATCGGT	18		Depositor's primer
3	Neo	CAGGATCTCCTGTCATCTCACCTT	24		Depositor's primer

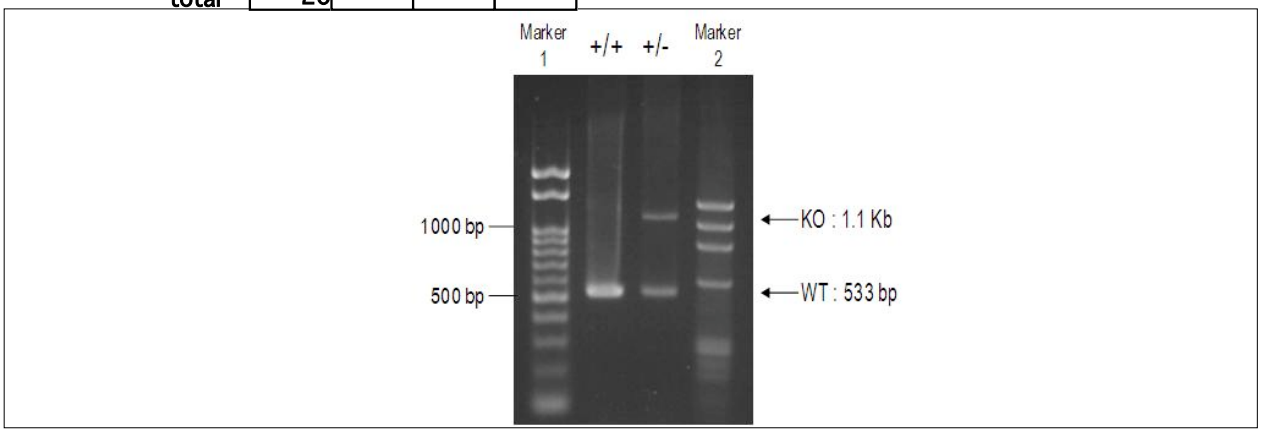
Reaction Components

Enzyme TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		3.8			
PCR buffer	2 X GC I	10.0			
dNTP	2.5 mM	2.0			
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		0.2			
	total	20			

Reaction Conditions

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



Marker

marker 1:Gene Ladder 100 (Wako, Cat. 316-06951), marker2:

Gel

2% agarose in 1 X TAE (100 V, 30 min)

PCR products

primer_set		product_size	product
primer 2	primer 3	1.1 kb	KO
primer 1	primer 2	533 bp	WT

Comment