PCR protocol

RBRC 02233 **Strain name** STOCK Calr<tm1Osb>

Primer

	primer_name	primer_seq	length	
1	2621	GAGTGGAAACCAGGTGAAATTGACAACC	28	
2	2622	CTTCTCTGATAAGTTTTCCTCTGACCTC	28	
3	1927	AATGACCAGAGTTGATTCCAAGG	23	
4	2509	CCAGAATGCTGATCTTCATACCATGG	26	

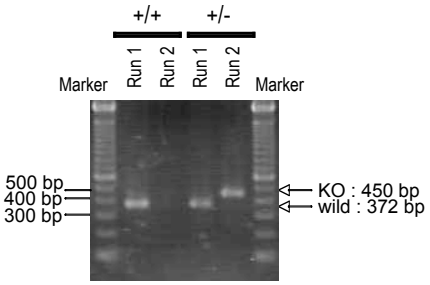
Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

	Conc	Run 1	Run 2	Run 3
H2O		17.3	17.3	
PCR buffer	10 X	2.5	2.5	
dNTP	2.5 mM	2.0	2.0	
primer 1	10 pmol/μl	1.0	0	
primer 2	10 pmol/μl	1.0	0	
primer 3	10 pmol/μl	0	1.0	
primer 4	10 pmol/μl	0	1.0	
DNA		1.0	1.0	
Taq		0.2	0.2	
	total	25	25	

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

100 bp ladder (Invitrogen, Cat. 15628-050)

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 2	372 bp	wild
primer 3	primer 4	450 bp	KO

Comment