PCR protocol

RBRC 01089 **Strain name** B6.129S-Nras<tm1Mok>

Primer	primer_name	primer_seq	length		
1	01089	CAAATGAAGTGTGCCAGCAGTGTG	24		RIKEN BRC
2		CTCAAACTCCGCAGACCCCGCC	22		RIKEN BRC
3	Neo10	ATCAGAGCAGCCGATTGTCTGTTG	24		Depositor's primer

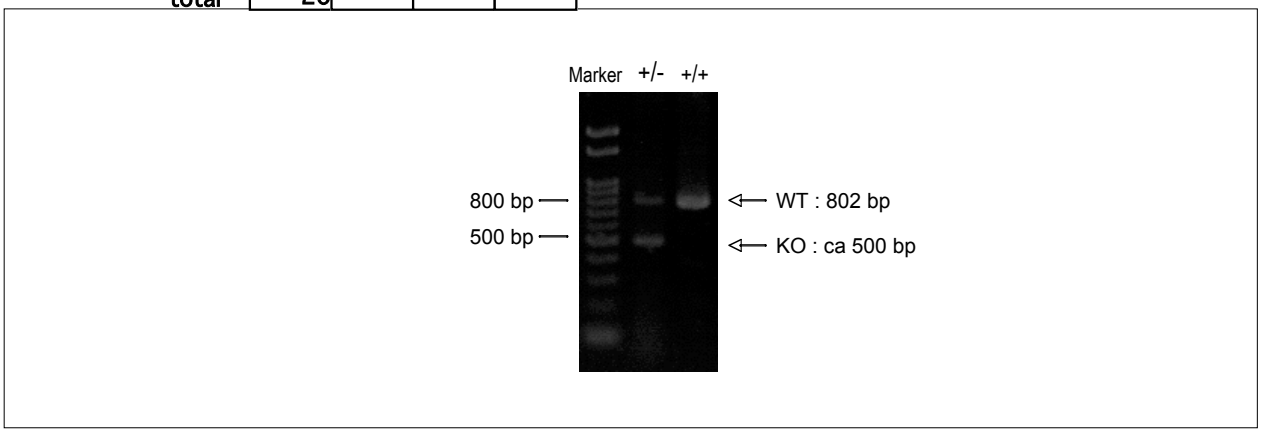
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		4.3			
PCR buffer	2 X GC I	10.0			
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	65	30	*
4	72	60	* *Repeat for 30 cycles
5	72	600	



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	802 bp	WT
primer 1	primer 3	ca 500 bp	KO

Comment