PCR protocol

RBRC 04545 **Strain name** 129-Rnf135<tm1>

Primer	primer_name	primer_seq	length		
1	Ex2 check F	GGTGACAGTACAGAAAAGTACC	22		Depositor's primer
2	Ex2 check R	GTCCCTCTTGAACTTACCGTGG	22		Depositor's primer
3	NeoS	TGCATACGCTTGATCCGGCTAC	22		Depositor's primer

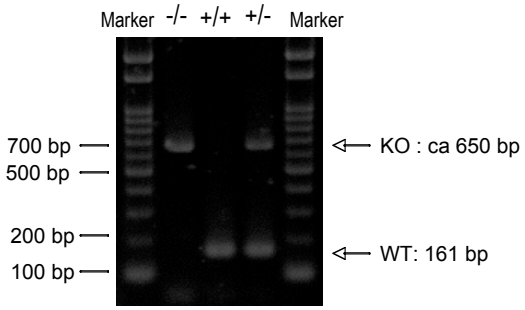
Reaction Components

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	161 bp	WT
primer 1	primer 3	ca 650 bp	KO

Comment