PCR protocol

RBRC 00889 **Strain name** MINT-KO (ICR background)

Primer	primer_name	primer_seq	length	
1	RAM7F	GAGGGGAAACTGGCTGATAAAAAGGACGAG	30	
2	RAM7R	GTCACGACGGTGACGGTGACGGCAGGAAG	29	
3	PGKF	GCGCCTACCGGTGGATGTGGAATGTGTGCG	30	

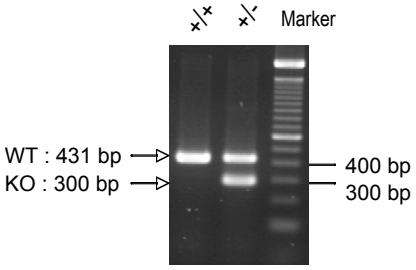
Reaction Components

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

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 3	300 bp	KO
primer 1	primer 2	431 bp	wild

Comment