PCR protocol

RBRC 02322 **Strain name** B6.129P2-Cmah<tm1Ykoz>

Primer

	primer_name	primer_seq	length	
1	ZSP-S1	GGGAGAAAGGGTAGTCACTATTGG	24	
2	A0162	GAGGTTTCTTGCATGCCTTTAG	22	
3	NEOA4	AGAGGCCACTTGTGTAGCGCCAAGT	25	

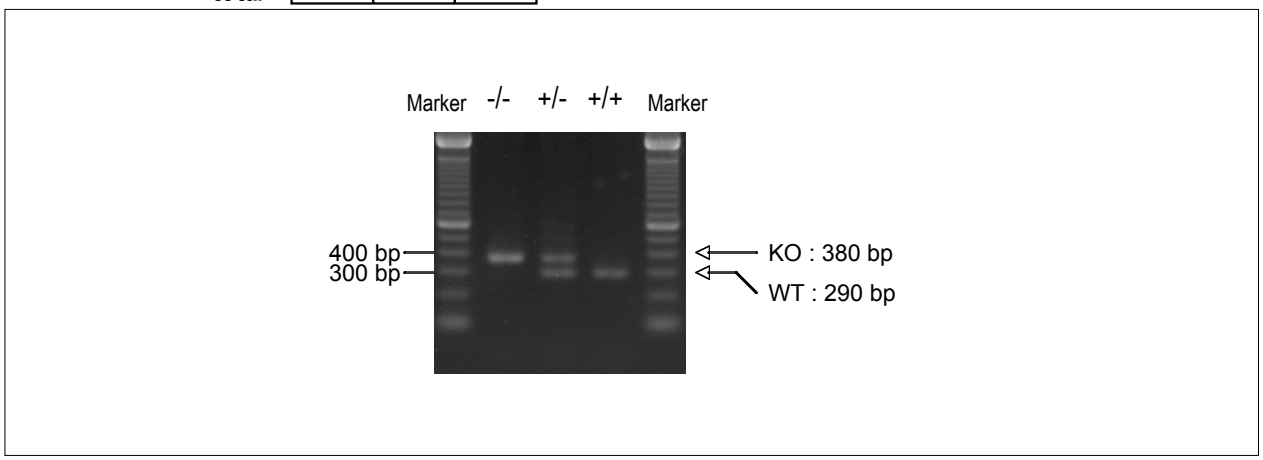
Reaction Components

Enzyme Roche Taq (Cat. No. BM1418432)

	Conc	Run 1	Run 2	Run 3
H2O		16.3		
PCR buffer	10 X	2.5		
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	25		

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30*	
3	60	30*	
4	72	60*	*repeat for 35 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	290 bp	wild
primer 1	primer 3	380 bp	KO

Comment