PCR protocol

RBRC 01474 **Strain name** B6 Fas-KO

Primer	primer_name	primer_seq	length
1	neo	GCTTGAGTAAATACATCCCG	20
2	Fas antisense	TGTCCATCAATGGAAGGTCT	20
3	NeoR primer	GATTCGCAGCGCATCGCCTTCTATCG	26

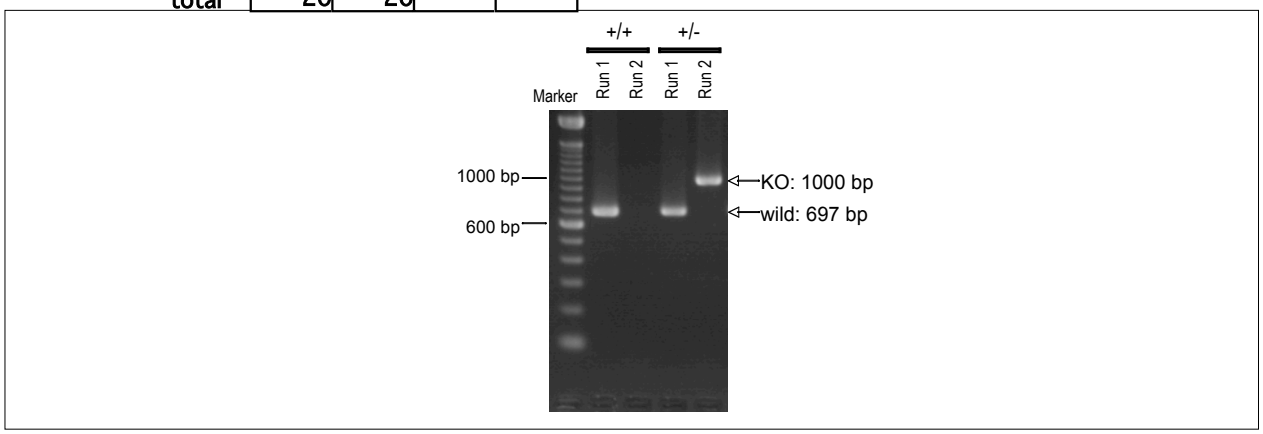
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		4.8	4.8		
PCR buffer	2 X GC I	10.0	10.0		
dNTP	2.5 mM	2.0	2.0		
primer 1	10 pmol/μl	1.0	0		
primer 2	10 pmol/μl	1.0	1.0		
primer 3	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq		0.2	0.2		
	total	20	20		

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30*	
3	57	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, 35 min)

PCR products

primer_set		product_size	product
primer 1	primer 2	697 bp	WT
primer 2	primer 3	1000 bp	KO

Comment