PCR protocol

RBRC 01765 **Strain name** tob-gene KO (B6)

Primer	primer_name	primer_seq	length		
1	Tob-F	ACCACTCTCTTTGGATGCCTGCTCG	25		
2	PGK1 PRIMER	GCTAAAGCGCATGCTCCAGACTGCCTTG	28	PGK promoter	
3	Tob-R	GTGGCTTCGCAGCTATGCAGCTTGA	25		

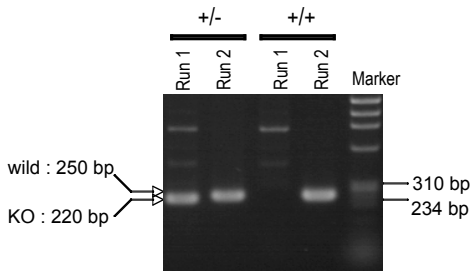
Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		17.3	17.3		
PCR buffer	10 X	2.5	2.5		
dNTP	2.5 mM	2.0	2.0		
primer 1	10 pmol/μl	1.0	1.0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq		0.2	0.2		
	total	25	25		

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30*	
3	58	30*	
4	72	60*	*Repeat for 30 cycles
5	72	300	



Marker

ϕX174 Hae III

Gel

2% agarose in 1 X TAE (100 V, 35 min)

PCR products

primer_set		product_size	product
primer 1	primer 2	220 bp	KO
primer 1	primer 3	250 bp	WT

Comment