PCR protocol

RBRC 01120 **Strain name** AGT-KO (BL/6)

Primer	primer_name	primer_seq	length		
1	LCB355(NEO 602)	GTCTTGTCGATCAGGATGATCTGGA	25		Depositor's primer
2	mAGT5	GGGCTCTTCACTGCTCCAGGC	21		RIKEN BRC
3	mAGT3	CCACCGTAGTCTCAGGAGTATG	22		RIKEN BRC

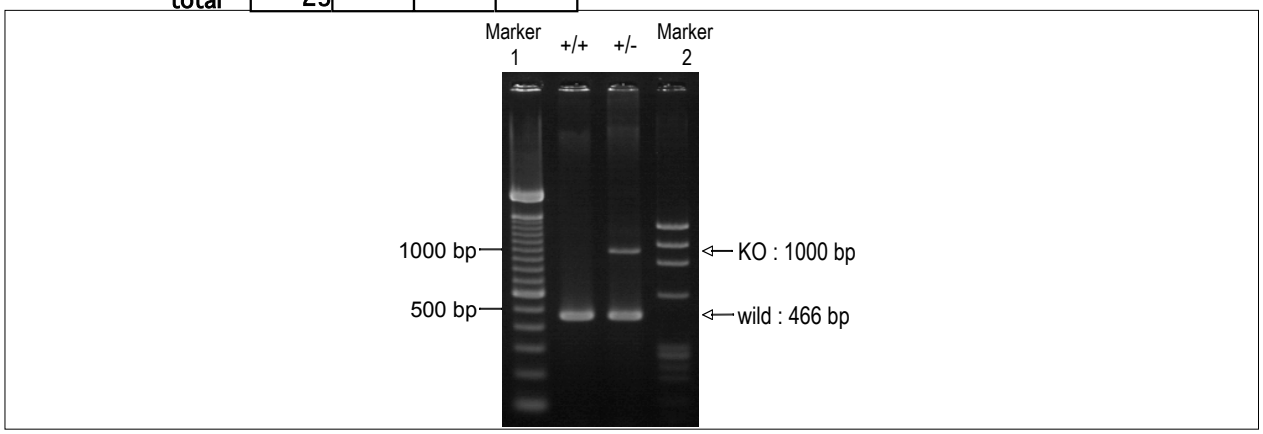
Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		16.8			
PCR buffer	10 X	2.5			
dNTP	2.5 mM	2.0			
primer 1	10 pmol/μl	1.0			
primer 2	10 pmol/μl	0.5			
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	58	30*	
4	72	60*	*Repeat for 30 cycles
5	72	300	



Marker

marker 1:100 bp ladder (Invitrogen, Cat. 15628-050), marker2: ϕX174 Hae III

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 3	1000 bp	KO
primer 2	primer 3	466 bp	WT

Comment