PCR protocol

RBRC 02399 **Strain name** C57BL/6-PRAT4A<tm1Kmiy>

Primer	primer_name	primer_seq	length	
1	PRAT4A Wild Rv primer(b)	GGGACTGCGCACGTGCCTCTGGGTAAC	27	
2	02399	AGCTTCCCAACAGGCAAACCTCGAG	25	
3	DT5'	GCCTTCTATCGCCTTCTTGACGAGTTCTTC	30	

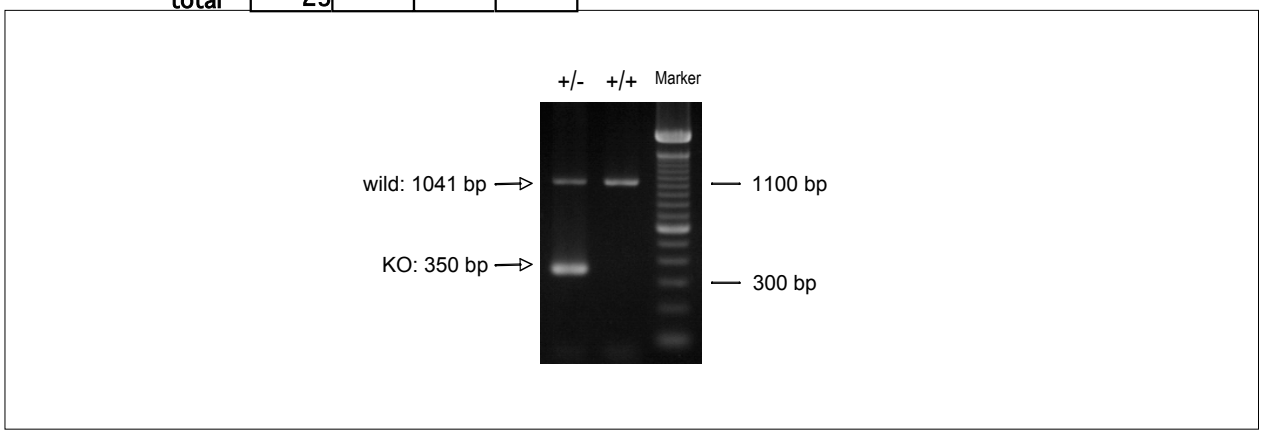
Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		16.3			
PCR buffer		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 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 2	1,041 bp	wild
primer 2	primer 3	350 bp	KO

Comment