PCR protocol

RBRC 03983 **Strain name** B6;129(FVB)-Atp6v0c<tm1.1Ywd>

Primer

	primer_name	primer_seq	length		
1	GH133	GAGTCTCGATCGAGGTCGACAT	22		Depositor's primer
2	TA-Rv1	CAGAAGGAGGTGACACCGTGGGA	23		Depositor's primer
3	03983WT	GACCTATTCATGCGTCTGTGGAGC	24		RIKEN BRC

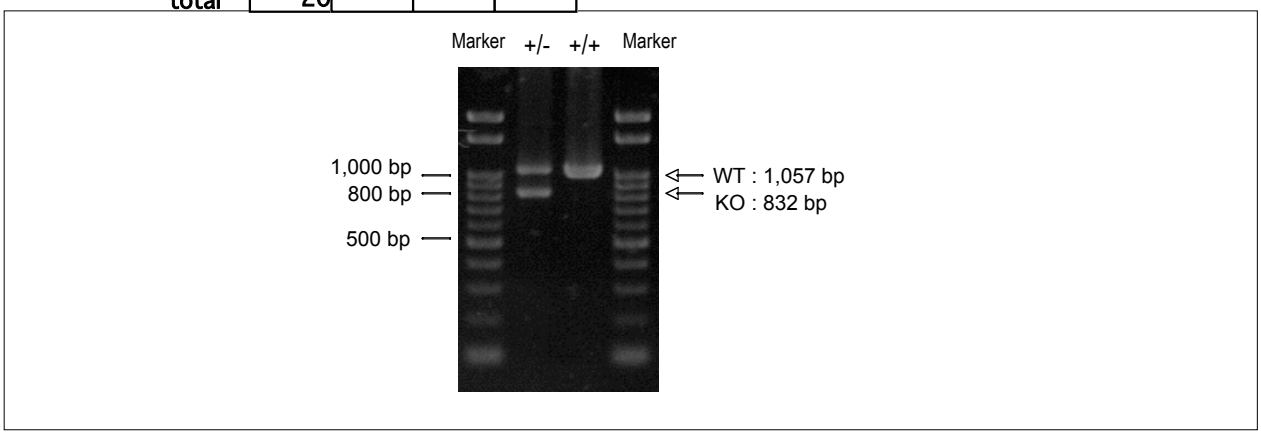
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		4.3			
PCR buffer	2 X GC I	10.0			
dNTP	2.5 mM	1.6			
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		0.1			
	total	20			

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

Gene Ladder 100 (Wako, Cat. 316-06951)

Gel

2% agarose in 1 X TAE (100 V, 30 min)

PCR products

primer_set		product_size	product
primer 1	primer 2	832 bp	KO
primer 2	primer 3	1,057 bp	WT

Comment