PCR protocol

RBRC 02683 Strain name B6J.Cg-Atp2b2<jog>/Oda

Primer	primer_name	primer_seq	length	
1		AGCAAACTGGCCTTCGCCAGCCGC	24	
2		GGGGACACTGAAGCCATCTGCCGG	24	
3		TGCCCTTGCCACCTGGAGGTAGGG	24	

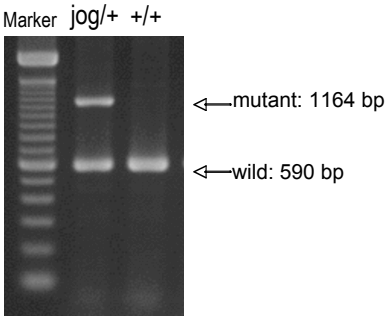
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		3.8			
PCR buffer	2 X	10.0			
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	20			

Reaction Conditions

step	temp (℃)	time (sec)		note
1	94	180		
2	94	30	*	
3	60	30	*	*Repeat for 30 cycles
4	72	60	*	
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	1164 bp	mutant
primer 2	primer 3	590 bp	wild

Comment