PCR protocol

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RBRC 05843 **Strain name** B6.Cg-Actb<tm1(tetO-ChR2*E123T/T159C)Ahky>

Primer	primer_name	primer_seq	length		
1	WT_Fw	ATTTCTGAATGGCCCAGGTCTGAG	24		RIKEN BRC
2	WT_Rv	CTGCTCTGGTGTCTGTGTTACCTG	24		RIKEN BRC
3	ChR2PCRR	ACCGACCCTTTGGCACAGTATG	22		Cell Reports 2, 397-406 (2012).

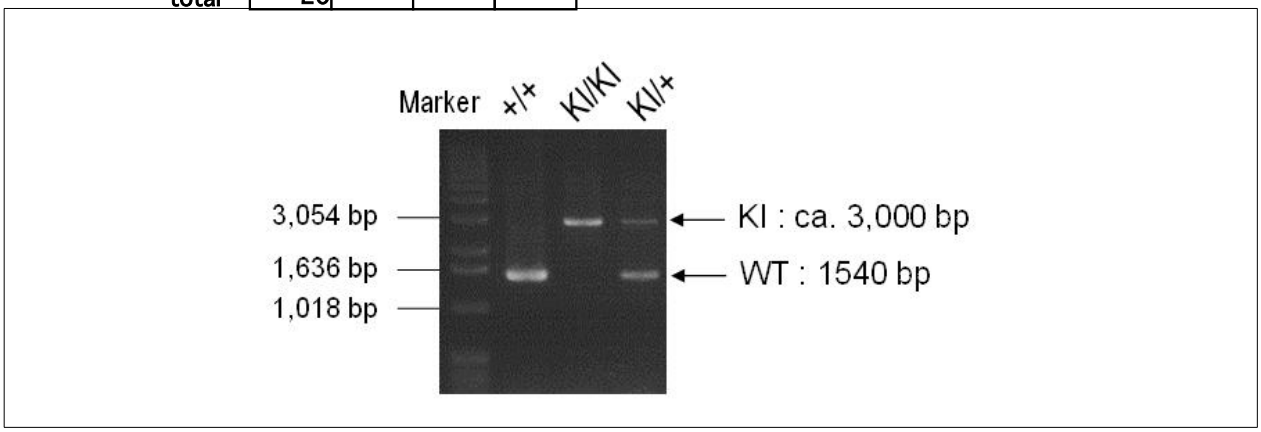
Reaction Components

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 GC I	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	5 U/μl	0.2			
	total	20			

Reaction Conditions

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



Marker

1 kb DNA ladder (Invitrogen, Cat. 15615-024)

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 2	1,540 bp	WT
primer 1	primer 3	ca. 3,000 bp	KI

Comment