PCR protocol

RBRC 02386 **Strain name** B6.129P2-Ly86<tm1Kmiy>

Primer	primer_name	primer_seq	length		
1	02386_Fw	GCCTGGCAGAATATTCAGCTAC	22		RIKEN BRC
2	Ly86-Ex1_Rv	CACAGCTCTGGTAGACTACTTC	22		RIKEN BRC
3	3Neo	CGGCCACAGTCGATGAATCC	20		Proc. Natl. Acad. Sci. USA, 94, 13069-13074 (1997).

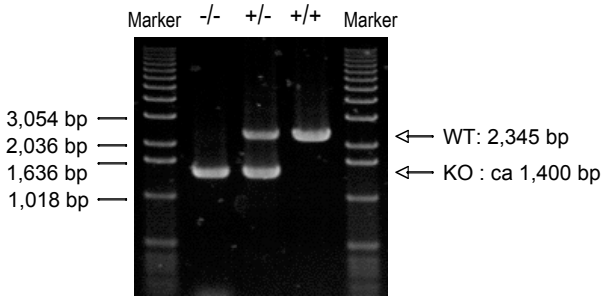
Reaction Components

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	58	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	2,345 bp	WT
primer 1	primer 3	ca 1,400 bp	KO

Comment