

PCR protocol

RBRC 02846 Strain name 129S6/SvEvTac-Crx<tm1Clc>

Primer	primer_name	primer_seq	length	
1	02846_Fw	CTGAGTCCAGGGTCCAGGTAG	21	RIKEN BRC
2	02846_Rv	GAAACACCTGAGGCTGAATGG	21	RIKEN BRC
3	Neo10	ATCAGAGCAGCCGATTGTCTGTTG	24	Depositor's primer

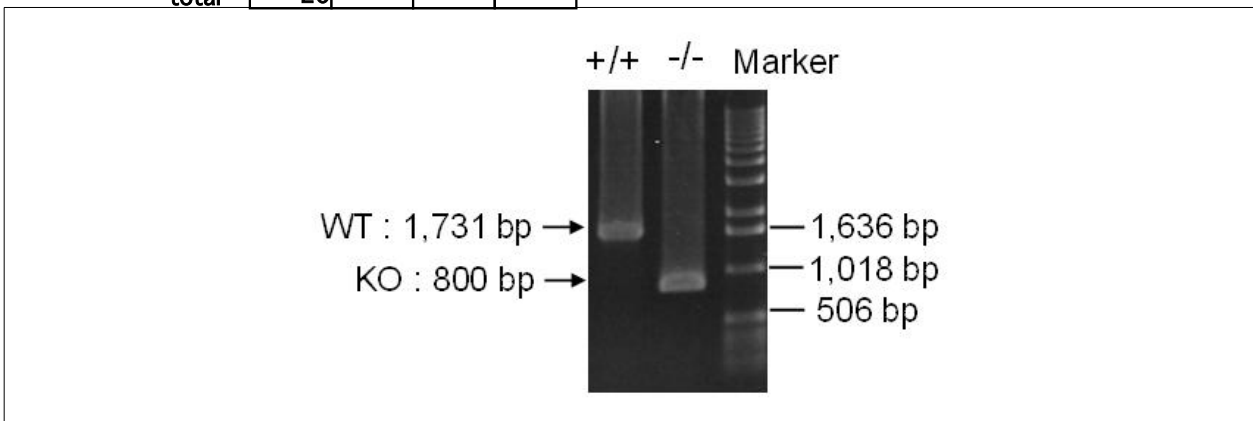
Reaction Components

Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8			
PCR buffer	2 X	10.0			
dNTP	2.0 mM	4.0			
primer 1	10 pmol/ μ l	1.0			
primer 2	10 pmol/ μ l	1.0			
primer 3	10 pmol/ μ l	1.0			
DNA		1.0			
Taq	1 U/ μ l	0.2			
total		20			

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	180*	*Repeat for 30 cycles



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	1,731 bp	WT
primer 2 primer 3	800 bp	KO

Comment Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.

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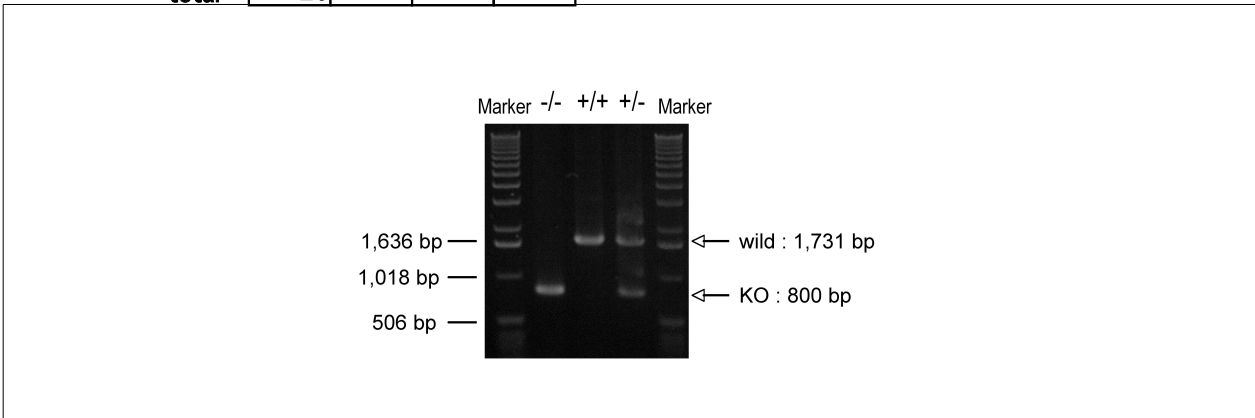
Reaction Components

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

Reaction Conditions

	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/ μ l	1.0			
primer 2	10 pmol/ μ l	1.0			
primer 3	10 pmol/ μ l	1.0			
DNA		1.0			
Taq		0.2			
total		20			

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	60	30*	
4	72	90*	*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	primer_set	product_size	product
primer 1	primer 2	1,731 bp	WT
primer 2	primer 3	800 bp	KO

Comment