

RBRC 02388

Strain name B6.129P2-Ly96<tm1Kmiy>

Primer	primer_name	primer_seq	length	Reference
1	MD-2 KO a	ATGTTGCCATTATTCTCTTTTCGA	25	Depositor's primer
2	MD-2 KO b	TGCCTTTAATTCCAGGAGGTAGAGG	25	Depositor's primer
3	3Neo	CGGCCACAGTCGATGAATCC	20	Proc. Natl. Acad. Sci. USA, 94, 13069-13074(1997).

Reaction Components

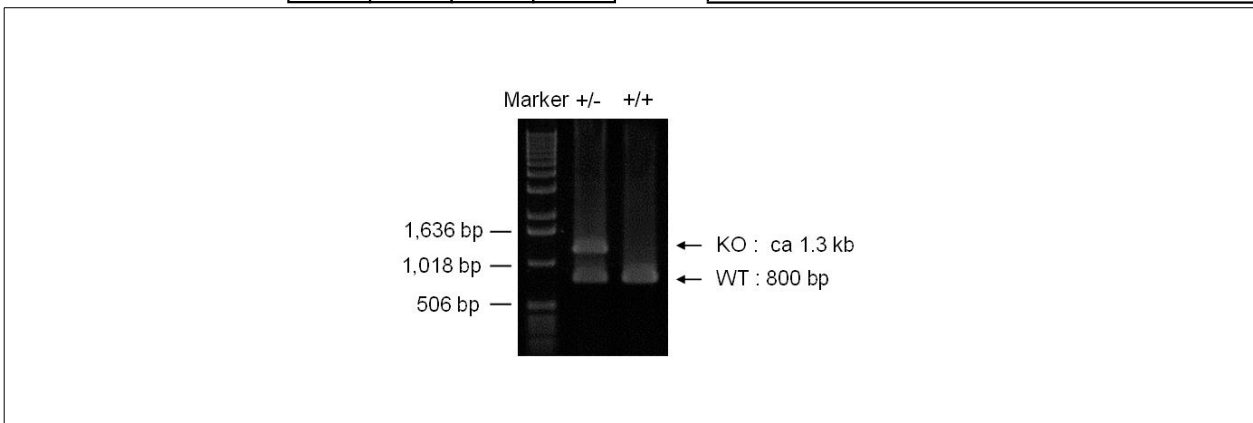
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

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

Reagent	Conc	Reaction 1	2	3	4	(µL)
H2O		1.8				
PCR buffer	10 X	10.0				
dNTP	2.0 mM	4.0				
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	1 U/µl	0.2				
total		20				

Marker 1 kb DNA ladder (Invitrogen, Cat. 15615-024)
Gel 1% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)
primer 1	800 bp	WT
primer 2	ca 1.3 kb	KO

Comment Standard PCR

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

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Primer	primer_name	primer_seq	length	Reference
1		GTCGAGTCCGATGGTCTTCCTGGC	24	RIKEN BRC
2	MD-2 KO b	TGCCTTTAATTCCAGGAGGTAGAGG	25	Depositor's primer

Reaction Components

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

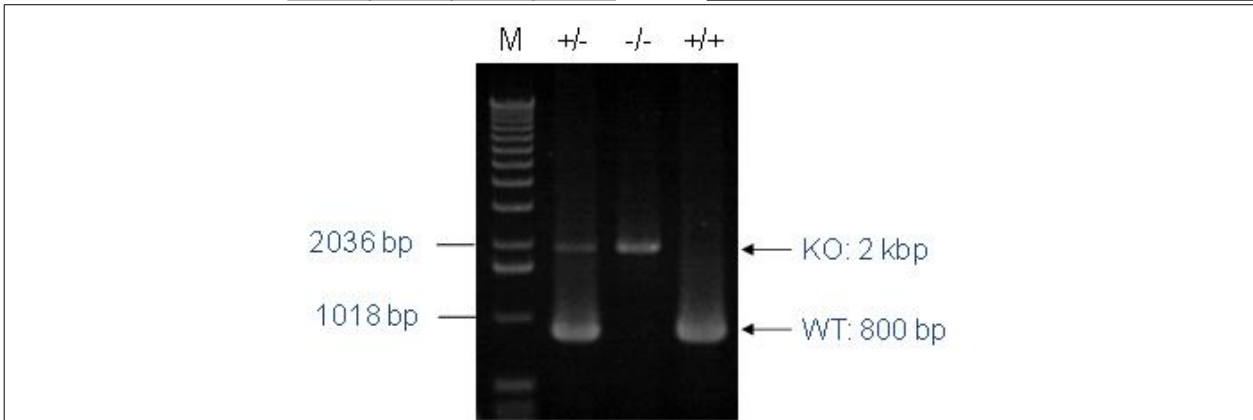
Reagent	Conc	Reaction 1	2	3	4
H2O		4.8			
PCR buffer	2 X GC I	10.0			
dNTP	2.5 mM	2.0			
primer 1	10 pmol/ μ l	1.0			
primer 2	10 pmol/ μ l	1.0			
DNA		1.0			
Taq		0.2			
total		20			

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	60	30*	
4	72	120*	*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)



primer 1	primer 2	product_size	product (allele type)
primer 1	primer 2	800 bp	WT
primer 1	primer 2	2 kbp	KO

Comment Standard PCR