

PCR protocol

RBRC 05808

Strain name B6.129P2-Aldh2<tm1Kaw>

Primer	primer_name	primer_seq	length	
1	F1	CCGTACTGACTGTCCCATGCAGTGCT	26	Depositor's primer
2	int3_Rv	GTTCACTCTGGTGACCACCATAGAG	25	RIKEN BRC
3		AAGAACTCGTCAAGAAGGCGATAGAAGGCG	30	

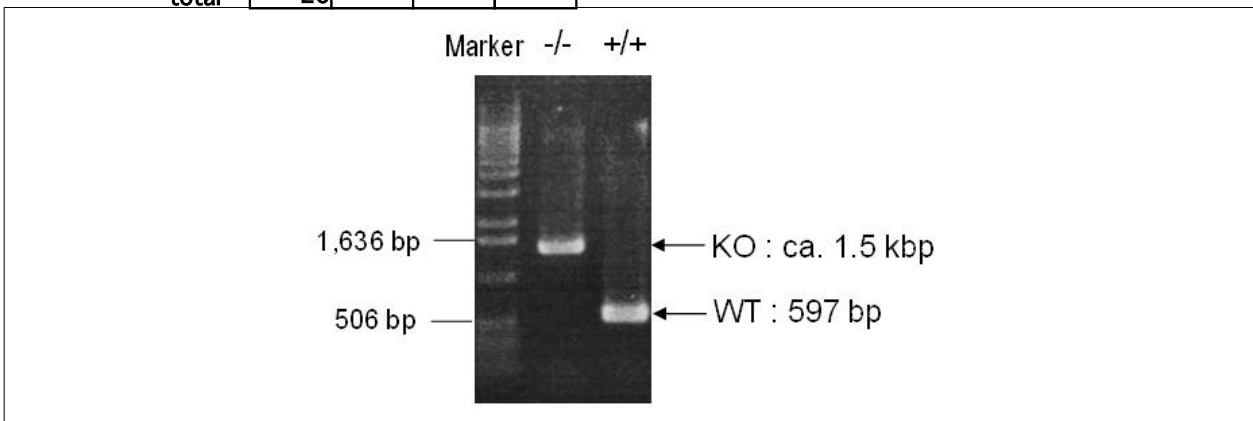
Reaction Components

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	120*	*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	597 bp	WT
primer 1 primer 3	ca. 1.5 kbp	KO

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

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3	R3	GGTGGATGTGGAATGTGTGCGAGGC	25	Depositor's primer

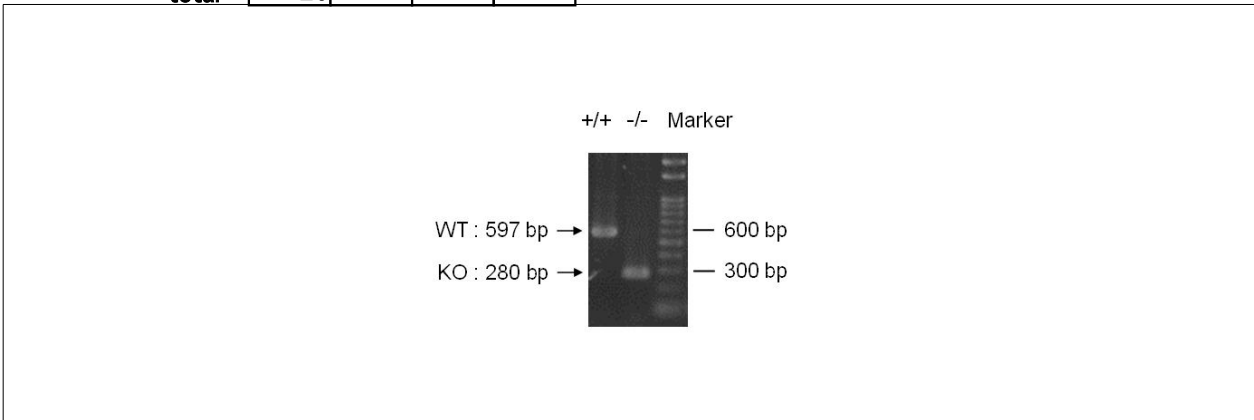
Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		12.3			
PCR buffer	10 X	2.0			
dNTP	2.5 mM	1.6			
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	5U/ μ l	0.1			
total		20			

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



Marker

Gene Ladder 100 (Wako, Cat. 316-06951)

Gel

2% agarose in 1 X TAE (100 V, 30 min)

PCR products

primer_set	product_size	product
primer 1 primer 2	597 bp	WT
primer 1 primer 3	280 bp	KO

Comment