

PCR protocol

RBRC 09382

Strain name B6.Cg-Ndn<tm1Ky>

Primer	primer_name	primer_seq	length	
1	Typing F2	CGACTGTGAGATGCAGGACAGCGA	24	Depositor's primer
2	Typing wt-R2	CCTCAGGTGCAGCCCGAAC	19	Depositor's primer
3	Typing ko-R3	GTCTGTTGTGCCAGTCATAGCCGAA	26	Depositor's primer

Reaction Components

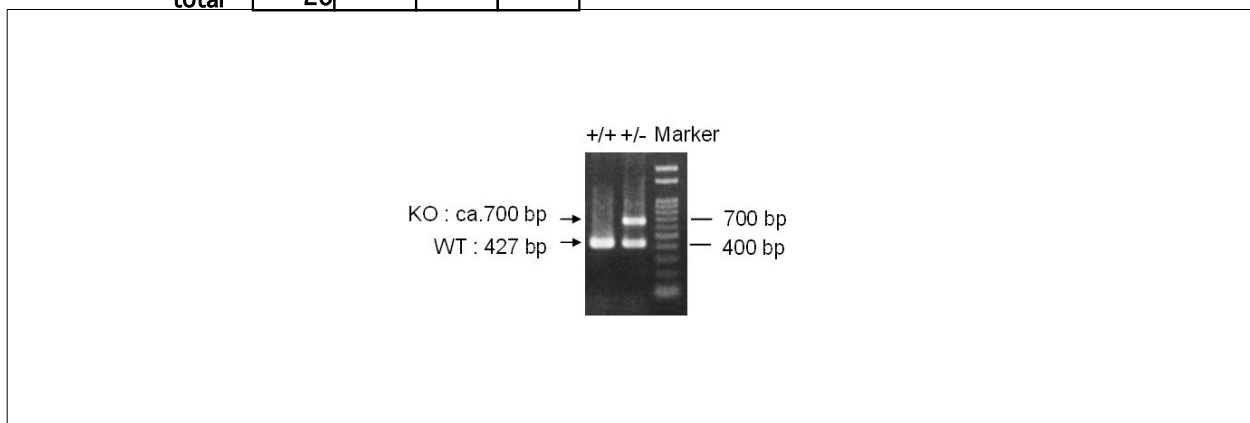
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

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

	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			

Standard PCR



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	427 bp	WT
primer 1 primer 3	ca 700 bp	KO

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