

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

PCR protocol

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RBRC 03101

Strain name B6;129P2-Lefty2<tm1Hmd>

Primer	primer_name	primer_seq	length	
1	neo-31	ACCCAGCACTCCACTGGATA	20	Depositor's primer
2	lefty2-53	GCCTGACCTAGAGTCGTTTC	20	Depositor's primer
3	lefty2-33	GGAAGAGCTCACCTCGAAAA	20	Depositor's primer

Reaction Components

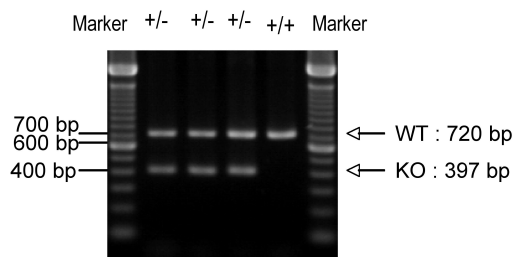
Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		16.3			
PCR buffer	10 X	2.5			
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		25			

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	57	30*	
4	72	60*	*Repeat for 30 cycles
5	72	300	

Separated PCR



Marker

100 bp ladder (Invitrogen, Cat. 15628-050)

Gel

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

PCR products

	primer_set	product_size	product
primer 2	primer 3	720 bp	WT
primer 1	primer 2	397 bp	KO

Comment