

RBRC 01578

Strain name C57BL/6-PI3K KO

Primer	primer_name	primer_seq	length	
1	3Neo	CGGCCACAGTCGATGAATCC	20	Proc. Natl. Acad. Sci. USA, 94, 13069-13074 (1997).
2	p85aS	TGTCTTCAGTCACCCCGTTGAT	22	Depositor's primer
3	p85aAS	GGAGCCTTTATTACAGTCAGTATGT	26	Depositor's primer

Reaction Components

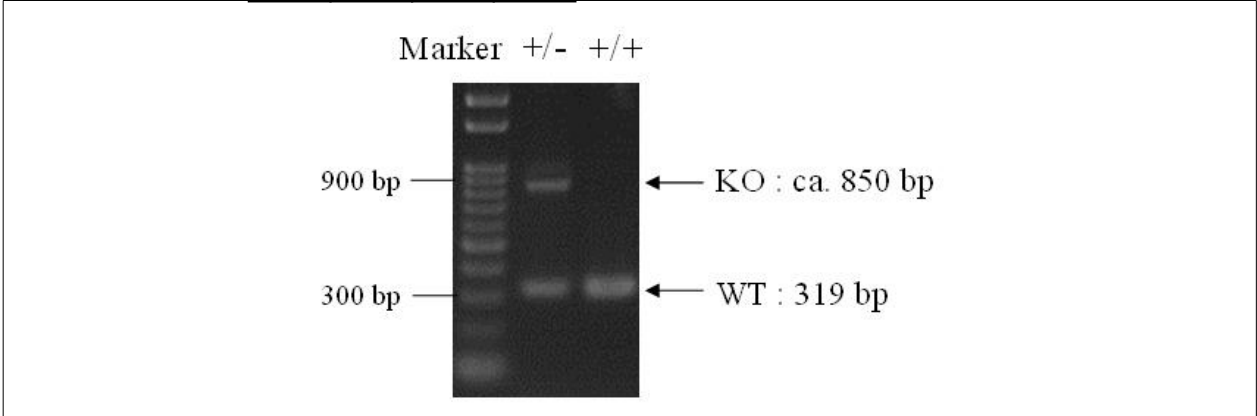
Reaction Conditions

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

Conc	Run 1	Run 2	Run 3	Run 4
H2O	1.8			
PCR buffer	2 X	10.0		
dNTP	2 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		
Taq		0.2		
DNA	1 U/ μ l	1.0		
total	20			

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

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	ca. 850 bp	KO
primer 2	319 bp	WT

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



PCR protocol

Sheet ID PS_00656
Release date : 9/6/2006
Print date : 1/13/2016

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Primer	primer_name	primer_seq	length	
1	neoAS	AGGTGAGATGACAGGAGATCCTGCCC	26	JAX Genotyping Protocol
2	p85aS	TGTCTTCAGTCACCCCGTTGAT	22	Depositor's primer
3	p85aAS	GGAGCCTTTATTCACAGTCAGTATGT	26	Depositor's primer

Reaction Components

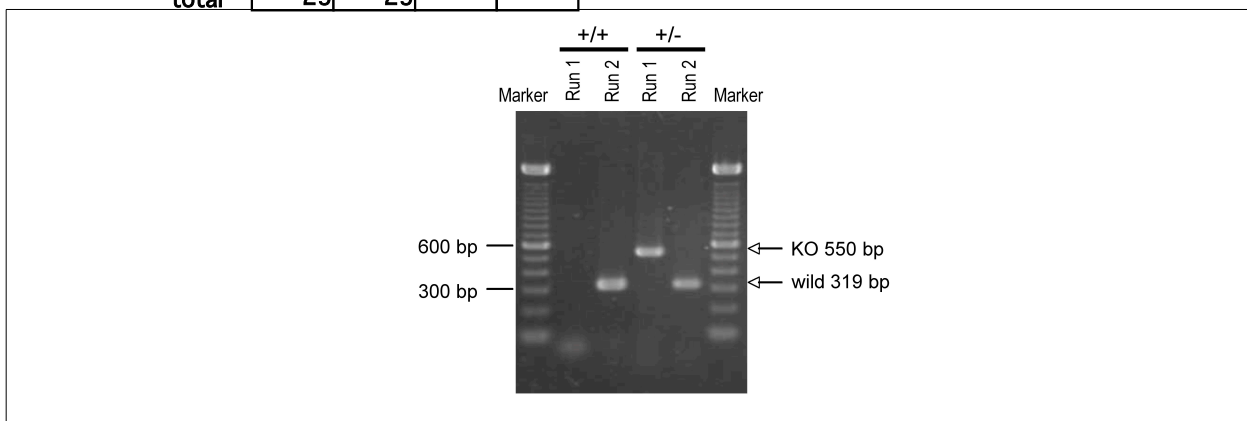
Reaction Conditions

Enzyme Roche Taq (Cat. No. BM1418432)

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	60	30*	
4	72	60*	*Repeat for 35 cycles
5	72	300	

	Conc	Run 1	Run 2	Run 3	Run 4
H ₂ O		17.3	17.3		
PCR buffer	10 X	2.5	2.5		
dNTP	2.5 mM	2.0	2.0		
primer 1	10 pmol/μl	1.0	0		
primer 2	10 pmol/μl	1.0	1.0		
primer 3	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq		0.2	0.2		
total		25	25		

Separated PCR



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

Gel
2% agarose in 1 X TAE (100 V, 35 min)

PCR products

primer_set	product_size	product
primer 1 primer 2	550 bp	KO
primer 2 primer 3	319 bp	WT

Comment