PCR protocol

Sheet ID PS_05469
Release date : 4/13/2015
Print date : 4/13/2015

RBRC 02231 Strain name B6;129-Atg5<tm1Nmz>

Primer	primer_name	primer_seq	length		
1	ATG5 check 2	ACAACGTCGAGCACAGCTGCGCAAGG	26		Nature, 441, 885-889(2006).
2	ATG5 exon3-1	GAATATGAAGGCACACCCCTGAAATG	26		Nature, 441, 885-889(2006).
3	ATG5 short 2	GTACTGCATAATGGTTTAACTCTTGC	26		Nature, 441, 885-889(2006).

Reaction Components

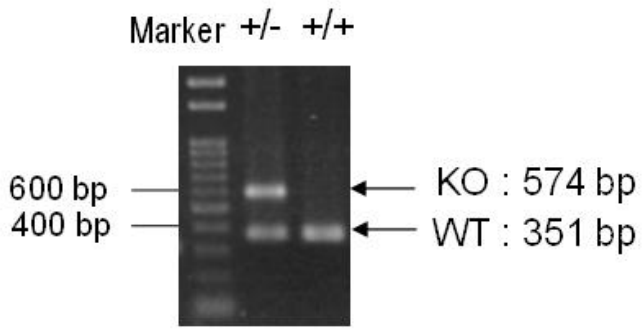
Enzyme: TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8			
PCR buffer	2 X	10.0			
dNTP	2.0 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			

Reaction Conditions

step	temp (℃)	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 2	primer 3	351 bp	WT
primer 1	primer 3	574 bp	KO

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

PCR protocol

Sheet ID PS_01269
Release date : 3/1/2008
Print date : 4/13/2015

RBRC 02231 Strain name B6;129-Atg5<tm1Nmz>

Primer	primer_name	primer_seq	length		
1	ATG5 check 2	ACAACGTCGAGCACAGCTGCGCAAGG	26		Nature, 441, 885-889(2006).
2	ATG5 exon3-1	GAATATGAAGGCACACCCCTGAAATG	26		Nature, 441, 885-889(2006).
3	ATG5 short 2	GTACTGCATAATGGTTTAACTCTTGC	26		Nature, 441, 885-889(2006).

Reaction Components

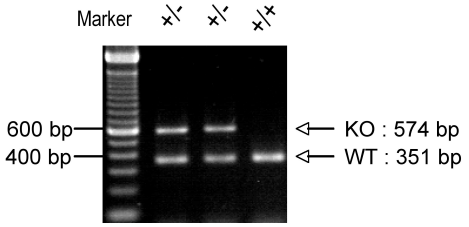
Enzyme: TaKaRa Ex Taq (Cat. No. RR001A)

	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/μl	1.0			
primer 2	10 pmol/μl	1.0			
primer 3	10 pmol/μl	1.0			
DNA		1.0			
Taq		0.2			
	total	25			

Reaction Conditions

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

Standard 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	351 bp	WT
primer 1	primer 3	574 bp	KO

Comment