PCR protocol

Sheet ID PS_06125
Release date : 12/11/2014
Print date : 12/11/2014

RBRC 00107 Strain name C3H/HeN-TgH(p53)

Primer	primer_name	primer_seq	length		
1	p53WTKO-Rv2	ACACCCAACACCATACCATGT	21		Dr. Tadasuke TSUKIYAMA primer
2	NP4	TGCTCGACGTTGTCACTGAAGC	22		
3	LCB614(int-1)	GTTATGCATCCATACAGTACA	21		Depositor's primer

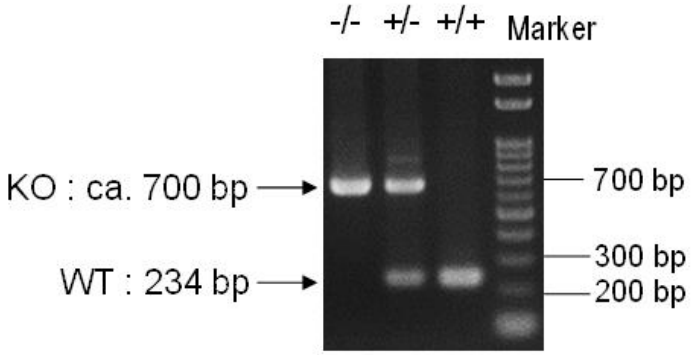
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.6			
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	0.4			
	total	20			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	57	30	*
4	68	60	* *Repeat for 30 cycles



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 3	234 bp	WT
primer 2	primer 3	ca. 700 bp	KO

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

PCR protocol

Sheet ID PS_00008
Release date : 8/6/2007
Print date : 12/11/2014

RBRC 00107 Strain name C3H/HeN-TgH(p53)

Primer	primer_name	primer_seq	length		
1	LCB614(int-1)	GTTATGCATCCATACAGTACA	21		Depositor's primer
2	LCB651(exon3)	CAGGATATCTTCTGGAGGAAG	21		Depositor's primer

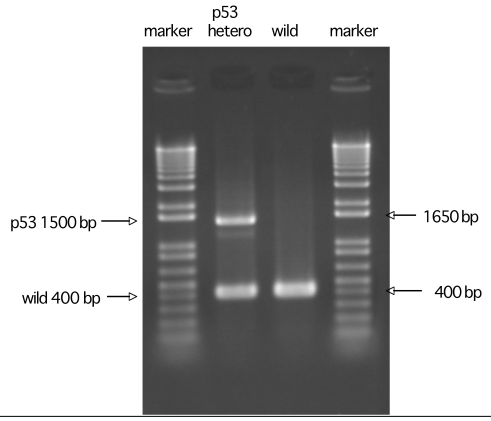
Reaction Components

Enzyme: TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		4.8			
PCR buffer	2 X GC I	10.0			
dNTP	2.5 mM	2.0			
primer 1	10 pmol/μl	1.0			
primer 2	10 pmol/μl	1.0			
Taq	5 U/μl	0.2			
DNA		1.0			
	total	20			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	300	
2	94	30	*
3	57	30	*
4	72	120	* *Repeat for 34 cycles
5	72	300	



Marker

1 kb plus DNA ladder (Invitrogen, Cat. 10787-026)

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 2	1.5 kb	KO
primer 1	primer 2	400 bp	WT

Comment