PCR protocol

Sheet ID PS_05326
Release date : 3/31/2014
Print date : 3/31/2014

RBRC 04791 Strain name B6.129P2-Dapk1<tm1Yuk>

Primer	primer_name	primer_seq	length		
1	009	GTGTATGTATGTGTGAGTGTATGTGTAGG	29		Depositor's primer
2	015	TACTGTGCATGGTGCAGCTTTACTGAG	27		Depositor's primer
3	neo1500	ATCGCCTTCTATCGCCTTCTTGACGAG	27		Depositor's primer

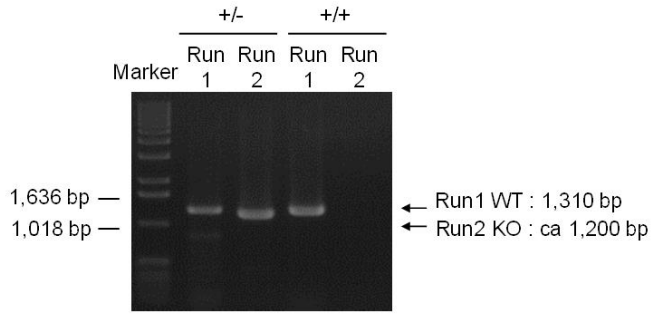
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		13.3	13.3		
PCR buffer	10 X	2.0	2.0		
dNTP	2.5 mM	1.6	1.6		
primer 1	10 pmol/μl	1.0	1.0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq	5U/μl	0.1	0.1		
	total	20	20		

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30	*
3	60	30	*
4	72	90	* *Repeat for 30 cycles
5	72	300	



Marker

1 kb DNA ladder (Invitrogen, Cat. 15615-024)

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 2	1,310 bp	WT
primer 1	primer 3	ca 1,200 bp	KO

Comment

PCR protocol

Sheet ID PS_05772
Release date : 3/31/2014
Print date : 3/31/2014

RBRC 04791 Strain name B6.129P2-Dapk1<tm1Yuk>

Primer	primer_name	primer_seq	length		
1	009	GTGTATGTATGTGTGAGTGTATGTGTAGG	29		Depositor's primer
2	015	TACTGTGCATGGTGCAGCTTTACTGAG	27		Depositor's primer
3	Neo	CAGGATCTCCTGTCATCTCACCTT	24		Depositor's primer

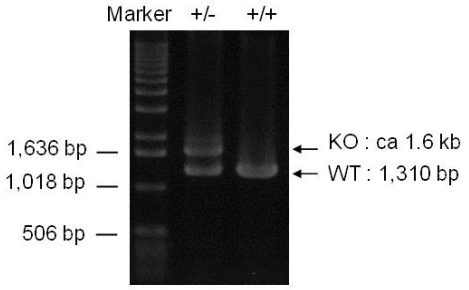
Reaction Components

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	120	* *Repeat for 30 cycles



Marker

1 kb DNA ladder (Invitrogen, Cat. 15615-024)

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 2	1,310 bp	WT
primer 1	primer 3	ca 1.6 kb	KO

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