PCR protocol

Sheet ID PS_07078
Release date : 1/30/2017
Print date : 1/30/2017

RBRC 01145 Strain name Mesp1-cre

Primer	primer_name	primer_seq	length		
1		GACCCAATGGTCAGGCCTCCGC	22		RIKEN BRC
2		CGTCTCGATCTTGGTCAGGTTCTG	24		RIKEN BRC
3	1-CN3-2	TGTTTCACTATCCAGGTTACGGA	23		RIKEN BRC

Reaction Components

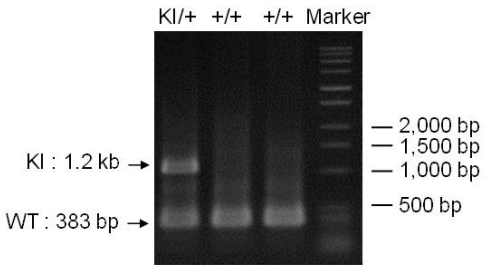
Enzyme: TOYOBO KOD FX (Code.No : KFX-101)

	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

Standard PCR



Marker
DNA Ladder,1 kb Plus (Affymetrix, Cat. 76714 500 UL)

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

PCR products	primer_set	product_size	product
primer 1	primer 2	383 bp	WT
primer 1	primer 3	1.2 kb	KI

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

PCR protocol

Sheet ID PS_01770
Release date : 06/03/2009
Print date : 1/30/2017

RBRC 01145 Strain name Mesp1-cre

Primer	primer_name	primer_seq	length		
1	GR-1	ATATGCCAAGTCATTGAGGTGAGCTTTC	28		Development, 126, 3437-3447 (1999).
2	Neo30	TTCTATCGCCTTCTTGACGAGTTCTTCTGA	30		Depositor's primer
3		GACCCAATGGTCAGGCCTCCGC	22		RIKEN BRC

Reaction Components

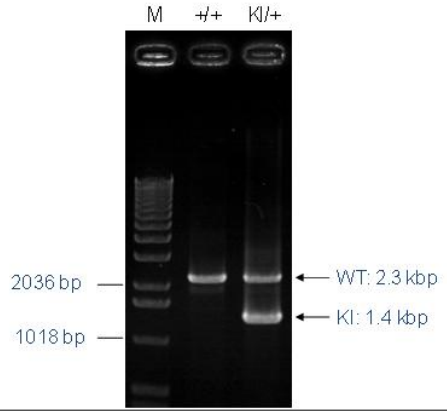
Enzyme: TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		3.8			
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			
primer 3	10 pmol/μl	1.0			
DNA		1.0			
Taq		0.2			
	total	20			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30	*
3	65	30	*
4	72	120	* *Repeat for 30 cycles
5	72	300	

Standard PCR



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 3	2.3 kb	WT
primer 1	primer 2	1.4 kb	KI

Comment

PCR protocol

Sheet ID PS_00247
Release date : 11/16/2005
Print date : 1/30/2017

RBRC 01145 Strain name Mesp1-cre

Primer	primer_name	primer_seq	length		
1	NeoAL2	GGGGATGCGGTGGGCTCTATGGCTT	25		Development, 126, 3437-3447 (1999).
2	GR-1	ATATGCCAAGTCATTGAGGTGAGCTTTC	28		Development, 126, 3437-3447 (1999).
3	PB92-R5	GACAGGGTGACAATCATCCGTTGCAT	26		Depositor's primer
4	PB92-L10	AGAATCGTGGGACCCATCGTTCCAGTA	27		Depositor's primer

Reaction Components

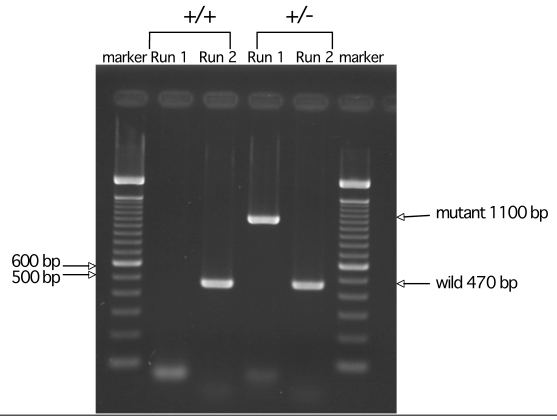
Enzyme: TaKaRa Ex Taq (Cat. No. RR001A)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		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	0		
primer 3	10 pmol/μl	0	1.0		
primer 4	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq		0.2	0.2		
	total	25	25		

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	300	
2	94	30	*
3	65	30	*
4	72	90	* *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 1	primer 2	1100 bp	KI
primer 3	primer 4	470 bp	WT

Comment