

PCR protocol

RBRC 02225

Strain name ICR.Cg-Mesp1 <tm1Ysa>/YsaRbrc

Primer	primer_name	primer_seq	length	
1		GACCCAATGGTCAGGCCTCCGC	22	RIKEN BRC
2		CGTCTCGATCTTGGTCAGGTTCTG	24	RIKEN BRC
3	1-CN3-4	AGCGCGGATCATCGGTCAGACGATT	25	RIKEN BRC

Reaction Components

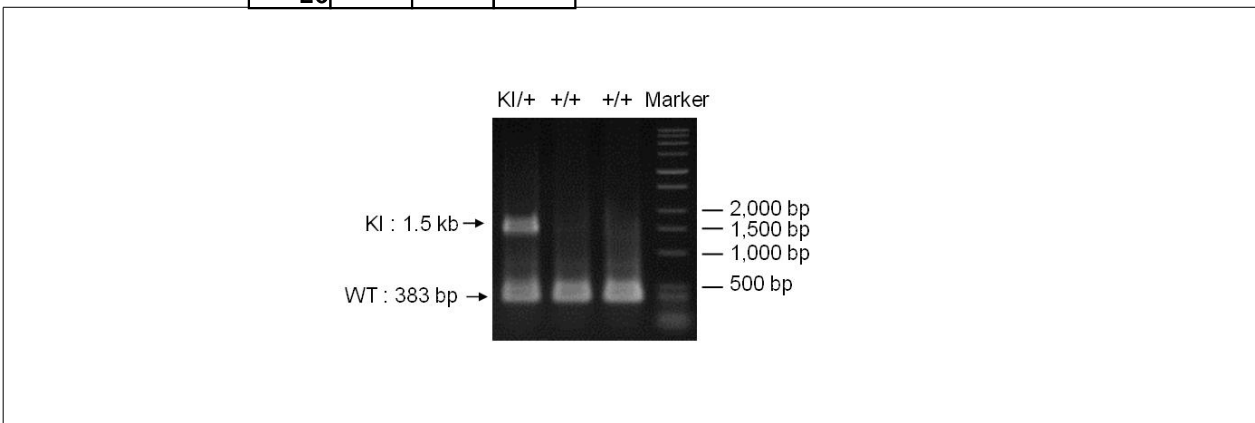
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H ₂ O		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			

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	180*	*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)

Comment

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

PCR products	primer_set	product_size	product
primer 1	primer 2	383 bp	WT
primer 1	primer 3	1.5 kb	KI

PCR protocol

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Strain name ICR.Cg-Mesp1 <tm1Ysa>/YsaRbrc

Primer	primer_name	primer_seq	length	
1	PGK-R	CTAAAGCGCATGCTCCAGACT	21	Depositor's primer
2	GR-1	ATATGCCAAGTCATTGAGGTGAGCTTTC	28	Depositor's primer
3	PB92-R5	GACAGGGTGACAATCATCCGTTGCAT	26	Depositor's primer
4	PB92-L10	AGAATCGTGGGACCCATCGTTCCAGTA	27	Depositor's primer

Reaction Components

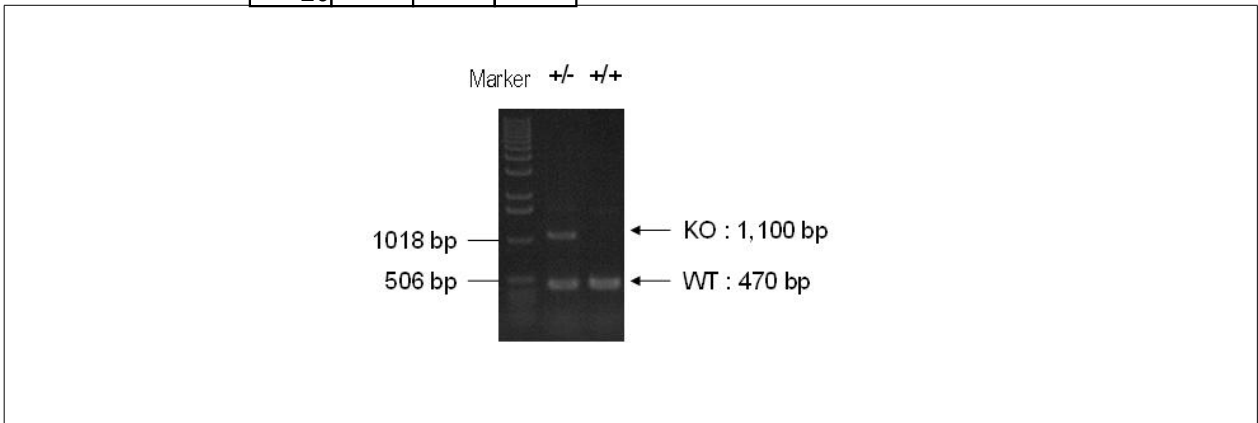
Enzyme	TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)
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Reaction Conditions

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		2.9			
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			
primer 4	10 pmol/μl	1.0			
DNA		1.0			
Taq	5 U/μl	0.1			
	total	20			

Standard PCR



Marker 1 kb DNA ladder (Invitrogen, Cat. 15615-024)	PCR products	primer_set	product_size	product
	primer 1	primer 2	1,100 bp	KO
	primer 3	primer 4	470 bp	WT
Gel 1% agarose in 1 X TAE (100 V, 30 min)				
Comment				