PCR protocol

Sheet ID PS_06165
Release date : 1/6/2015
Print date : 1/6/2015

RBRC 02290 Strain name STOCK Tg(Nanog-GFP,Puro)1Yam

Primer	primer_name	primer_seq	length		
1	ex1_Rv	CTTACCAGCCTCTGTGCAGAGC	22		RIKEN BRC
2	olMR0872	AAGTTCATCTGCACCACCG	19		JAX Genotyping Protocol
3	mLC3ex4AG	GTTAGCATTGAGCTGCAAGCGCCGTCT	27		Mol. Biol. Cell., 15, 1101-1111(2004).
4	mLC3ex3GT	TGAGCGAGCTCATCAAGATAATCAGGT	27		Mol. Biol. Cell., 15, 1101-1111(2004).

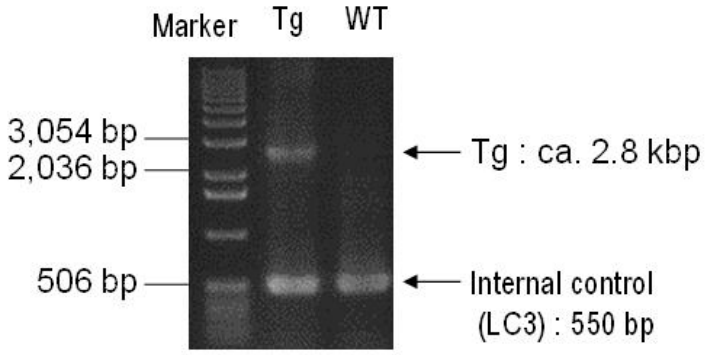
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		0.8			
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			
primer 4	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	240	* *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	ca. 2.8 kbp	Tg
primer 3	primer 4	550 bp	Internal control (LC3)

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

PCR protocol

Sheet ID PS_01306
Release date : 2/22/2008
Print date : 1/6/2015

RBRC 02290 Strain name STOCK Tg(Nanog-GFP,Puro)1Yam

Primer	primer_name	primer_seq	length		
1	PHF5' BamH1	TGGGATCCCTATGCTACTCCGTCGAAGTTC	30		Depositor's primer
2	6047AS10	CTAGGCAAACTGTGGGGACCAGGAAGAC	28		Depositor's primer
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	Internal control (IL-2)	JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	Internal control (IL-2)	JAX Genotyping Protocol

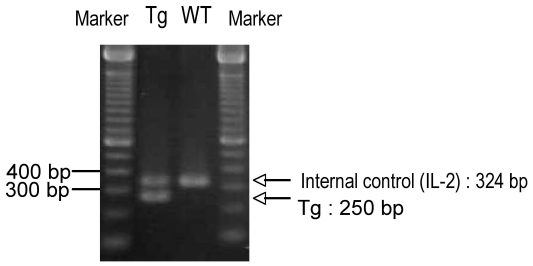
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		15.3			
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			
primer 4	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	63	30	*
4	72	60	* *Repeat for 30 cycles
5	72	300	



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	250 bp	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

Comment