PCR protocol

Sheet ID PS_05890
Release date : 1/6/2015
Print date : 1/6/2015

RBRC 00806 Strain name GFP-LC3#53

Primer

	primer_name	primer_seq	length		
1		GCTCTAGAGCCTCTGCTAACC	21		
2	LC3*rc3	TTGCGAATTCTCAGCCGTCTTCATCTCTCTCGC	33		Depositor's primer
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24		JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25		JAX Genotyping Protocol

Reaction Components

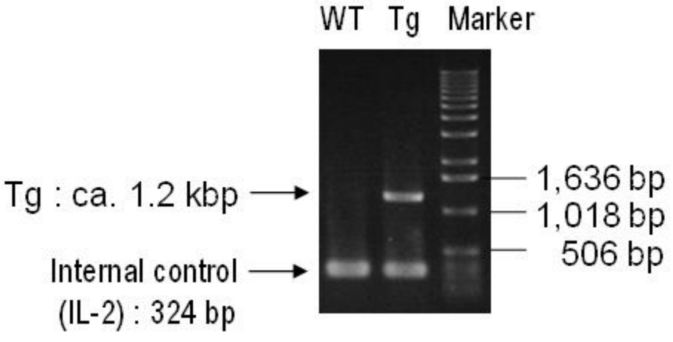
Enzyme: TOYOBO KOD FX (Code.No : KFX-101)

	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	120*	*Repeat for 30 cycles

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 2	ca. 1.2 kbp	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

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

PCR protocol

Sheet ID PS_05402
Release date : 09/13/2013
Print date : 1/6/2015

RBRC 00806 Strain name GFP-LC3#53

Primer

	primer_name	primer_seq	length		
1		ATAACTTGCTGGCCTTTCCACT	22		Autophagy, 4:61-62(2008).
2		CGGGCCATTTACCGTAAGTTAT	22		Autophagy, 4:61-62(2008).
3		GCAGCTCATTGCTGTTCCTCAA	22		Autophagy, 4:61-62(2008).

Reaction Components

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

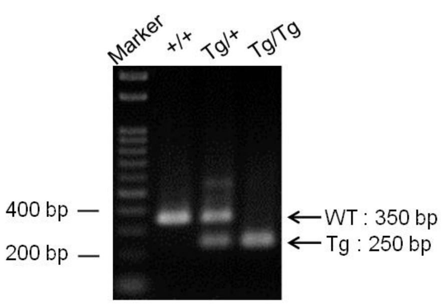
	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8			
PCR buffer	2 X	10.0			
dNTP	2 mM	4.0			
primer 1	10 pmol/μl	1.0			
primer 2	10 pmol/μl	1.0			
primer 3	10 pmol/μl	1.0			
DNA		1.0			
Taq	1 U/μl	0.2			
	total	20			

Reaction Conditions

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

Zygosity determination PCR

Standard PCR



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 2	250 bp	Tg
primer 1	primer 3	350 bp	WT

Comment: PCR to distinguish homozygotes and hemizygotes

PCR protocol

Sheet ID PS_05166
Release date : 07/04/2013
Print date : 1/6/2015

RBRC 00806 Strain name GFP-LC3#53

Primer

	primer_name	primer_seq	length		
1	GFP(LC3)1	TCCTGCTGGAGTTCGTGACCG	21		Depositor's primer
2	LC3*rc3	TTGCGAATTCTCAGCCGTCTTCATCTCTCTCGC	33		Depositor's primer
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24		JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25		JAX Genotyping Protocol

Reaction Components

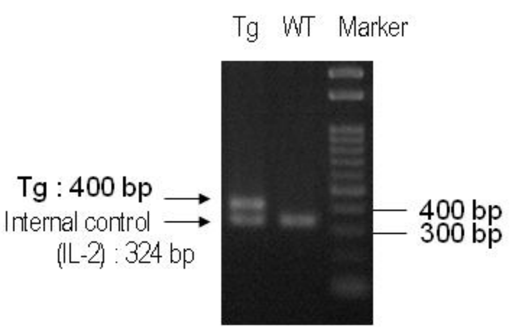
Enzyme: TOYOBO KOD FX (Code.No : KFX-101)

	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	60*	*Repeat for 30 cycles

Standard PCR



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 2	400 bp	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

Comment

PCR protocol

Sheet ID PS_02689
Release date : 6/2/2011
Print date : 1/6/2015

RBRC 00806 Strain name GFP-LC3#53

Primer

	primer_name	primer_seq	length		
1	GFP(LC3)1	TCCTGCTGGAGTTCGTGACCG	21		Depositor's primer
2	LC3*rc3	TTGCGAATTCTCAGCCGTCTTCATCTCTCTCGC	33		Depositor's primer
3	mLC3ex3GT	TGAGCGAGCTCATCAAGATAATCAGGT	27		Mol. Biol. Cell., 15, 1101-1111 (2004).
4	mLC3ex4AG	GTTAGCATTGAGCTGCAAGCGCCGTCT	27		Mol. Biol. Cell., 15, 1101-1111 (2004).

Reaction Components

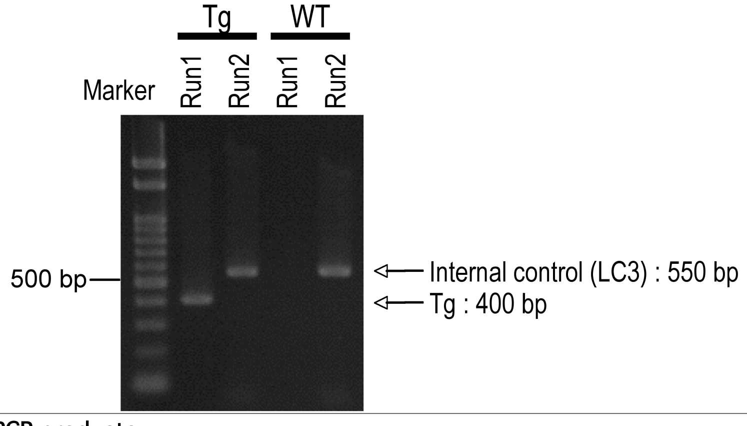
Enzyme: TaKaRa Ex Taq (Cat. No. RR001A)

	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	5U/μl	0.2	0.2		
	total	25	25		

Reaction Conditions

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

Separated PCR



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 2	400 bp	Tg
primer 3	primer 4	550 bp	Internal control

Comment

PCR protocol

Sheet ID PS_01812
Release date : 08/01/2012
Print date : 1/6/2015

RBRC 00806 Strain name GFP-LC3#53

Primer

	primer_name	primer_seq	length		
1		GGTGTTTGTTCCGTACACATCACC	24		RIKEN BRC
2		GGCAAAGCGGCAAACAACCATCAC	24		RIKEN BRC
3	C-B-ACTAT	GAGTGAAGCAGAACGTGGGGCTCACCTCGA	30		RIKEN BRC

Reaction Components

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

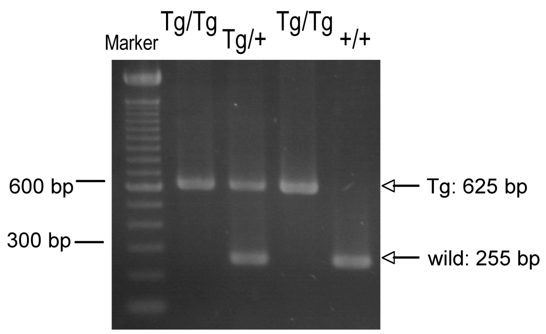
	Conc	Run 1	Run 2	Run 3	Run 4
H2O		3.8			
PCR buffer	2 X GC I	10.0			
dNTP	2.5 mM	2.0			
primer 1	10 pmol/μl	1.0			
primer 2	10 pmol/μl	1.0			
primer 3	10 pmol/μl	1.0			
DNA		1.0			
Taq		0.2			
	total	20			

Reaction Conditions

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

Zygosity determination PCR

Standard 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 3	625 bp	Tg
primer 1	primer 2	255 bp	WT

Comment PCR to distinguish homozygotes and hemizygotes

Sheet ID PS_00006
Release date : 11/16/2005
Print date : 1/6/2015

PCR protocol

RBRC 00806 Strain name GFP-LC3#53

Primer

	primer_name	primer_seq	length		
1	GFP(LC3)1	TCCTGCTGGAGTTCGTGACCG	21		Depositor's primer
2	LC3*rc3	TTGCGAATTCTCAGCCGTCTTCATCTCTCTCGC	33		Depositor's primer
3	mLC3ex3GT	TGAGCGAGCTCATCAAGATAATCAGGT	27	intron3 of the LC3 genome as an internal	Mol. Biol. Cell., 15, 1101-1111 (2004).
4	mLC3ex4AG	GTTAGCATTGAGCTGCAAGCGCCGTCT	27	intron3 of the LC3 genome as an internal	Mol. Biol. Cell., 15, 1101-1111 (2004).

Reaction Components

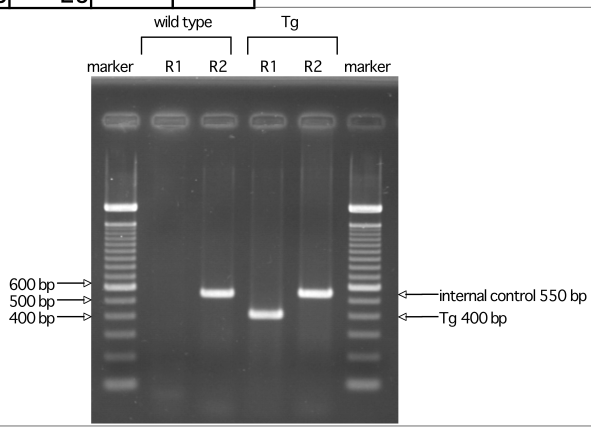
Enzyme: Roche Taq (Cat. No. BM1418432)

	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	5U/μl	0.2	0.2		
total		25	25		

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	300	
2	94	30*	
3	55	30*	
4	72	60*	*Repeat for 35 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	400 bp	Tg
primer 3	primer 4	550 bp	Internal control

Comment