PCR protocol

Sheet ID PS_04889
Release date : 8/01/2012
Print date : 10/6/2015

RBRC 02892 **Strain name** B6.Cg-Tg(Fucci)504Bsi Tg(Fucci)596Bsi

Primer	primer_name	primer_seq	length		
1	Gem110R	GCATCTAGATTACAGCGCCTTTCTCCGTTTTTCTGC	36		Depositor's primer
2		GGATATCCATCACACTGGCGGC	22		RIKEN BRC
3	Cdt100R	GCATCTAGATTATTTCTTTATCTTCTGGCCCGGAGA	36		Depositor's primer
4	mLC3ex4AG	GTTAGCATTGAGCTGCAAGCGCCGTCT	27		Mol. Biol. Cell., 15, 1101-1111 (2004).
5	mLC3ex3GT	TGAGCGAGCTCATCAAGATAATCAGGT	27		Mol. Biol. Cell., 15, 1101-1111 (2004).

Reaction Components

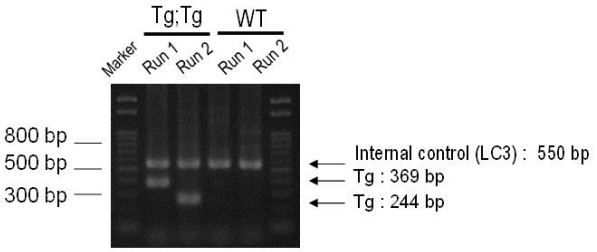
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		0.6	0.6		
PCR buffer	2 X	10.0	10.0		
dNTP	2.0 mM	4.0	4.0		
primer 1	10 pmol/μl	1.0	0		
primer 2	10 pmol/μl	1.0	1.0		
primer 3	10 pmol/μl	0	1.0		
primer 4	10 pmol/μl	1.0	1.0		
primer 5	10 pmol/μl	1.0	1.0		
DNA		1.0	1.0		
Taq	1 U/μl	0.4	0.4		
	total	20	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	369 bp	Tg(504)
primer 2	primer 3	244 bp	Tg(596)
primer 4	primer 5	550 bp	Internal control (LC3)

Comment We recommend to use a PCR enzyme for amplification of GC-rich. The targeted PCR region is GC rich.

PCR protocol

Sheet ID PS_04886
Release date : 8/01/2012
Print date : 10/6/2015

RBRC 02892 Strain name B6.Cg-Tg(Fucci)504Bsi Tg(Fucci)596Bsi

Primer	primer_name	primer_seq	length		
1	mAG-342F	CTTCTTCTACGACATCAGGTT	21		Depositor's primer
2		GAGCGGCCGCCAGTGTGATG	20		RIKEN BRC
3		ACGTGTTCTGTTACGGCCACAGAG	24		RIKEN BRC
4	oIMR0015	CAAATGTTGCTTGTCTGGTG	20		JAX Genotyping Protocol
5	oIMR0016	GTCAGTCGAGTGCACAGTTT	20		JAX Genotyping Protocol

Reaction Components

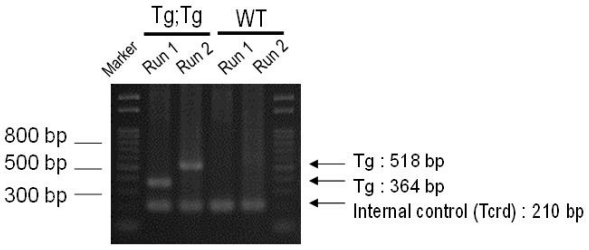
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		0.6	0.6		
PCR buffer	2 X	10.0	10.0		
dNTP	2.0 mM	4.0	4.0		
primer 1	10 pmol/μl	1.0	0		
primer 2	10 pmol/μl	1.0	1.0		
primer 3	10 pmol/μl	0	1.0		
primer 4	10 pmol/μl	1.0	1.0		
primer 5	10 pmol/μl	1.0	1.0		
DNA		1.0	1.0		
Taq	1 U/μl	0.4	0.4		
	total	20	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	364 bp	Tg(504)
primer 2	primer 3	518 bp	Tg(596)
primer 4	primer 5	210 bp	Internal control (Tcrd)

Comment

PCR protocol

Sheet ID PS_04863
Release date : 8/01/2012
Print date : 10/6/2015

RBRC 02892 Strain name B6.Cg-Tg(Fucci)504Bsi Tg(Fucci)596Bsi

Primer	primer_name	primer_seq	length		
1	mAG-342F	CTTCTTCTACGACATCAGGTT	21		Depositor's primer
2	Gem110R	GCATCTAGATTACAGCGCCTTTCTCCGTTTTTCTGC	36		Depositor's primer
3	mKO2-120F	ATGACACTACGCGTCACAAT	20		Depositor's primer
4	Cdt100R	GCATCTAGATTATTTCTTTATCTTCTGGCCCGGAGA	36		Depositor's primer
5	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24		JAX Genotyping Protocol
6	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25		JAX Genotyping Protocol

Reaction Components

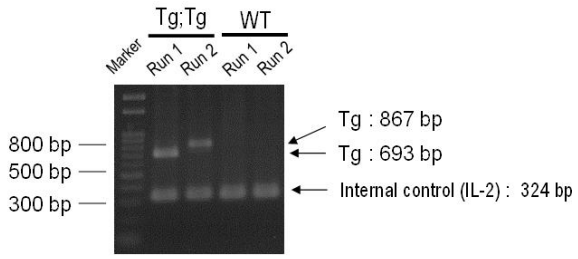
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		0.8	0.8		
PCR buffer	2 X	10.0	10.0		
dNTP	2.0 mM	4.0	4.0		
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		
primer 5	10 pmol/μl	1.0	1.0		
primer 6	10 pmol/μl	1.0	1.0		
DNA		1.0	1.0		
Taq	1 U/μl	0.2	0.2		
	total	20	20		

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	55	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	693 bp	Tg(504)
primer 3	primer 4	867 bp	Tg(596)
primer 5	primer 6	324 bp	Internal control (IL-2)

Comment

PCR protocol

Sheet ID PS_02304
Release date : 01/27/2011
Print date : 10/6/2015

RBRC 02892 Strain name B6.Cg-Tg(Fucci)504Bsi Tg(Fucci)596Bsi

Primer	primer_name	primer_seq	length		
1	mAG-342F	CTTCTTCTACGACATCAGGTT	21		Depositor's primer
2	Gem110R	GCATCTAGATTACAGCGCCTTTCTCCGTTTTTCTGC	36		Depositor's primer
3	mKO2-120F	ATGACACTACGCGTCACAAT	20		Depositor's primer
4	Cdt100R	GCATCTAGATTATTTCTTTATCTTCTGGCCCGGAGA	36		Depositor's primer
5	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	Internal control (IL-2)	JAX Genotyping Protocol
6	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	Internal control (IL-2)	JAX Genotyping Protocol

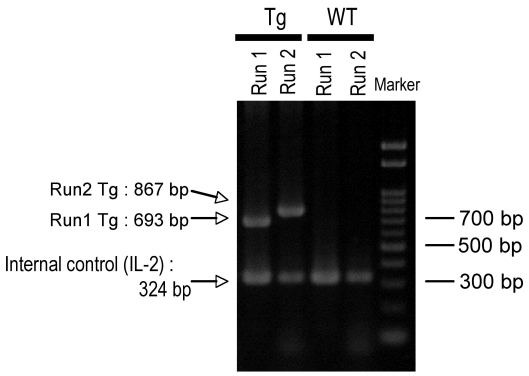
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		2.8	1.8		
PCR buffer	2 X GC I	10.0	10.0		
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	2.0		
primer 4	10 pmol/μl	0	2.0		
primer 5	10 pmol/μl	1.0	0.5		
primer 6	10 pmol/μl	1.0	0.5		
DNA		1.0	1.0		
Taq		0.2	0.2		
	total	20	20		

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30	*
3	55	30	*
4	72	60	* *Repeat for 35 cycles
5	72	300	



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	693 bp	Tg(504)
primer 3	primer 4	867 bp	Tg(596)
primer 5	primer 6	324 bp	Internal control (IL-2)

Comment