PCR protocol

Sheet ID PS_04917
Release date : 11/21/2014
Print date : 11/21/2014

RBRC 02706 Strain name B6.B6D2-Tg(Fucci)504Bsi

Primer	primer_name	primer_seq	length		
1	mAG-342F	CTTCTTCTACGACATCAGGTT	21		Depositor's primer
2	Gem110R	GCATCTAGATTACAGCGCCTTTCTCCGTTTTTCTGC	36		Depositor's primer
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24		JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25		JAX Genotyping Protocol

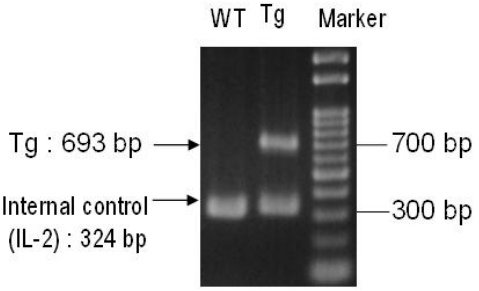
Reaction Components

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	55	30	*
4	68	60	* *Repeat for 30 cycles



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
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_02797
Release date : 08/01/2012
Print date : 11/21/2014

RBRC 02706 Strain name B6;B6D2-Tg(Fucci)504Bsi

Primer	primer_name	primer_seq	length		
1	R201	CGCGGAACTCCATATATGGGCTATG	25		RIKEN BRC
2	02706_R	GCATCCTGTGACCCCAGTCCC	21		RIKEN BRC
3	02706_F2	CTGGGAATGTTAGGGCATTCC	21		RIKEN BRC

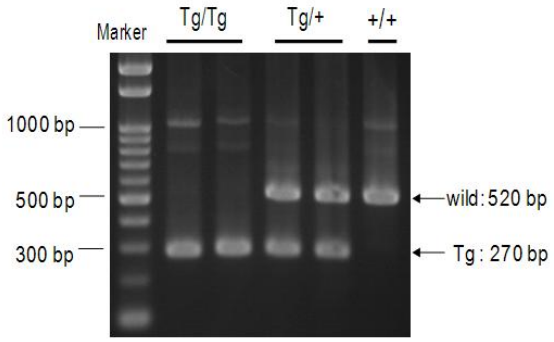
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.1			
PCR buffer	2 X	10.0			
dNTP	2.0 mM	4.0			
primer 1	10 pmol/μl	0.5			
primer 2	10 pmol/μl	2.0			
primer 3	10 pmol/μl	1.0			
DNA		1.0			
Taq		0.4			
	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



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	290 bp	Tg
primer 2	primer 3	520 bp	WT

Comment

PCR protocol

Sheet ID PS_04887
Release date : 08/01/2012
Print date : 11/21/2014

RBRC 02706 Strain name B6.B6D2-Tg(Fucci)504Bsi

Primer	primer_name	primer_seq	length		
1	mAG-342F	CTTCTTCTACGACATCAGGTT	21		Depositor's primer
2		GAGCGGCCGCCAGTGTGATG	20		RIKEN BRC
3		GGATATCCATCACACTGGCGGC	22		RIKEN BRC
4	Gem110R	GCATCTAGATTACAGCGCCTTTCTCCGTTTTTCTGC	36		Depositor's primer
5	mLC3ex4AG	GTTAGCATTGAGCTGCAAGCGCCGTCT	27		Mol. Biol. Cell., 15, 1101-1111 (2004).
6	mLC3ex3GT	TGAGCGAGCTCATCAAGATAATCAGGT	27		Mol. Biol. Cell., 15, 1101-1111 (2004).

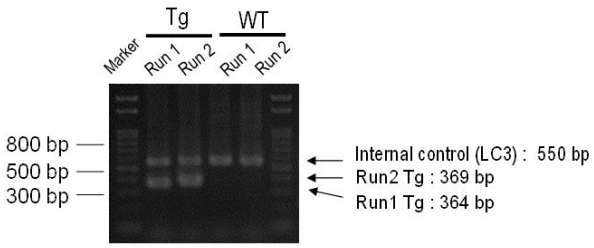
Reaction Components

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	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	0.4	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



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
primer 3	primer 4	369 bp	Tg
primer 5	primer 6	550 bp	Internal control (LC3)

Comment

PCR protocol

Sheet ID PS_01843
Release date : 06/03/2009
Print date : 11/21/2014

RBRC 02706 Strain name B6;B6D2-Tg(Fucci)504Bsi

Primer	primer_name	primer_seq	length		
1	mAG-342F	CTTCTTCTACGACATCAGGTT	21		Depositor's primer
2	Gem110R	GCATCTAGATTACAGCGCCTTTCTCCGTTTTTCTGC	36		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

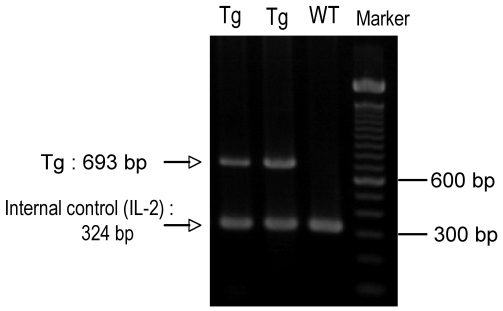
Reaction Components

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	55	30	*
4	72	60	* *Repeat for 35 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	693 bp	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

Comment