

PCR protocol

RBRC 01624

Strain name C.B6-Tg(CAG-EGFP)C14-Y01-FM131Osb/Rbrc

Primer	primer_name	primer_seq	length	
1	F14-02	GCCCACTAAGATAGCTCATTGACCTTGTC	30	RIKEN BRC
2	R14-01	CCTGTCAGTGGGTTTAGGTACAGCTC	26	RIKEN BRC
3	GFP-Full-R	CTTGACAGCTCGTCCATGCCGAG	24	RIKEN BRC

Reaction Components

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

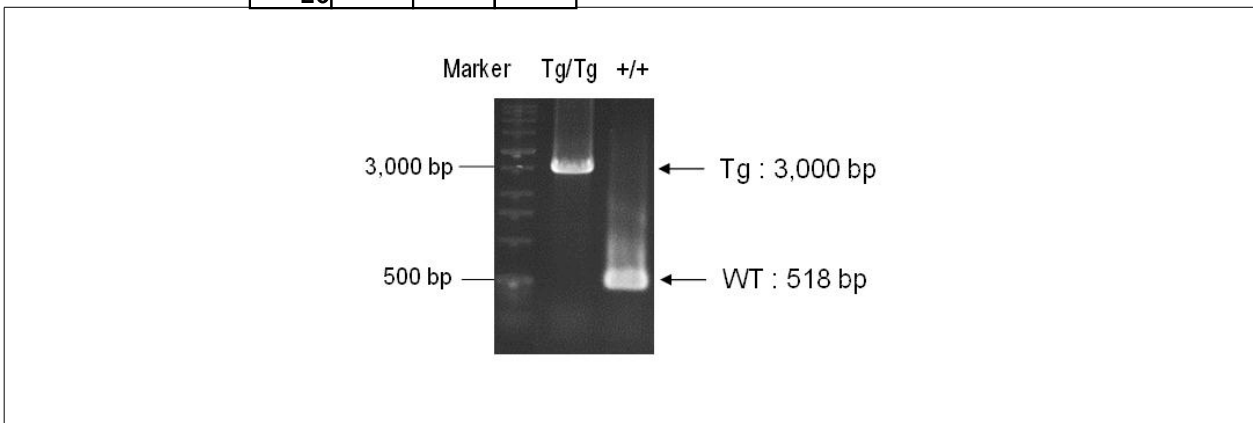
Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.6			
PCR buffer	2 X	10.0			
dNTP	2.0 mM	4.0			
primer 1	10 pmol/ μ l	1.0			
primer 2	10 pmol/ μ l	1.0			
primer 3	10 pmol/ μ l	1.0			
DNA		1.0			
Taq	1 U/ μ l	0.4			
total		20			

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

Zygosity determination PCR

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	518 bp	WT
primer 1	primer 3	3,000 bp	Tg

RBRC 01624

Strain name C;B6-Tg(CAG-EGFP)C14-Y01-FM1310sb

Primer	primer_name	primer_seq	length	
1	R201	CGCGGAACTCCATATATGGGCTATG	25	RIKEN BRC
2	F14-02	GCCCACTAAGATAGCTCATTGACCTTGTC	30	RIKEN BRC
3	R14-01	CCTGTCAGTGGGTTTAGGTACAGCTC	26	RIKEN BRC

Reaction Components

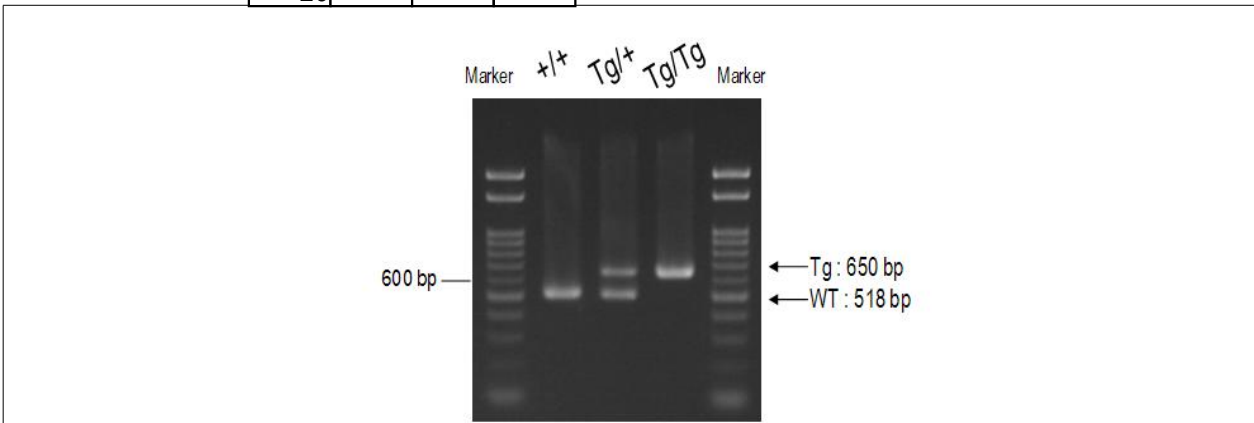
Enzyme TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

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		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			

Zygosity determination PCR



Marker
 Gene Ladder 100 (Wako, Cat. 316-06951)

Gel
 2% agarose in 1 X TAE (100 V, 35 min)

PCR products	primer_set	product_size	product
primer 1	primer 2	650 bp	Tg
primer 2	primer 3	518 bp	WT

Comment