PCR protocol

RBRC 02889 **Strain name** D2.B6-Tg(CAG-EGFP)C14-Y01-FM131Osb/Ny

Primer

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

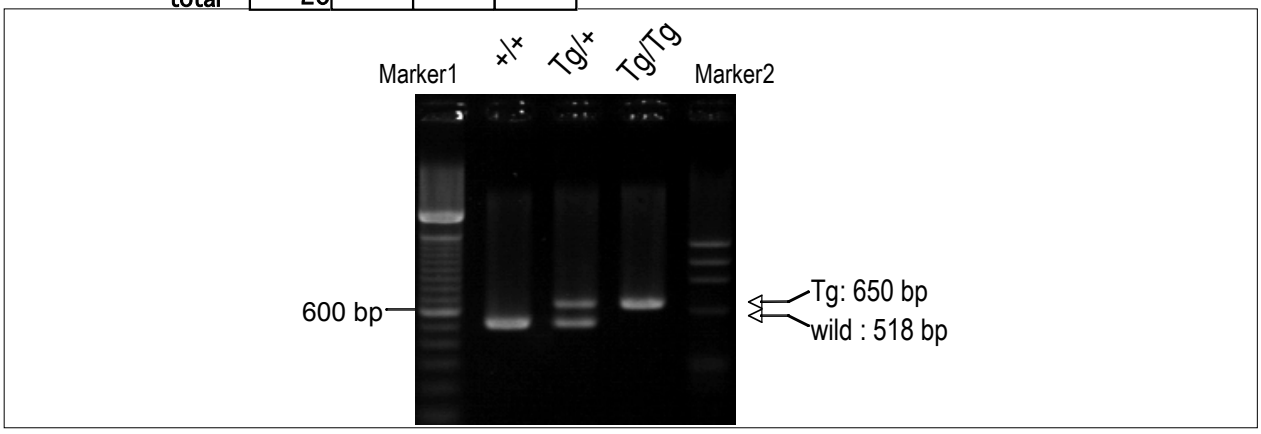
Reaction Components

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		



Marker

marker 1:100 bp ladder (Invitrogen, Cat. 15628-050), marker2: ϕX174 Hae III

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