PCR protocol

RBRC 04335 **Strain name** B6;B6C3-Tg(Thy1-ChR2/mCherry)9-7Sasa/Rbrc

Primer	primer_name	primer_seq	length		
1		GCTGGAATCAAAGGTGTGAGCTC	23		RIKEN BRC
2	mCherry-LC3 R	CTTGTACAGCTCGTCCATGCCGCC	24		Depositor's primer
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24		JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25		JAX Genotyping Protocol

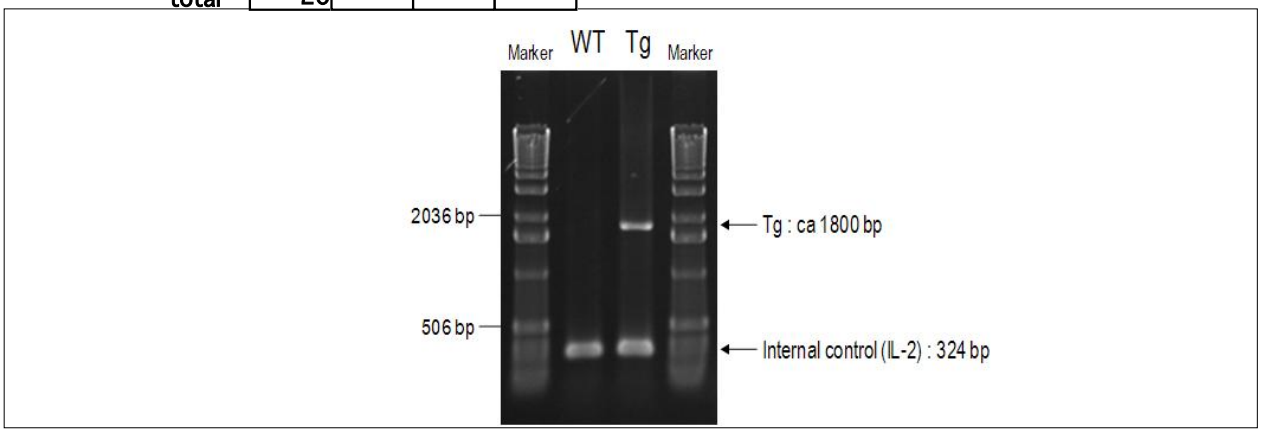
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		2.9			
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			
primer 4	10 pmol/μl	1.0			
DNA		1.0			
Taq	5U/μl	0.1			
	total	20			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30 *	
3	60	30 *	*Repeat for 30 cycles
4	72	120 *	
5	72	300	



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 1800 bp	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

Comment