

PCR protocol

RBRC 01910

Strain name C57BL/6J-Tg(CMV-cre)27Rbrc

Primer	primer_name	primer_seq	length	
1		CTCTAAGCTTCACATATGCATGCAC	25	RIKEN BRC
2	Cre3	CTAATCGCCATCTTCCAGCAG	21	Depositor's primer
3	mLC3ex3GT	TGAGCGAGCTCATCAAGATAATCAGGT	27	Mol. Biol. Cell., 15, 1101-1111 (2004).
4	mLC3ex4AG	GTTAGCATTGAGCTGCAAGCGCCGTCT	27	Mol. Biol. Cell., 15, 1101-1111 (2004).

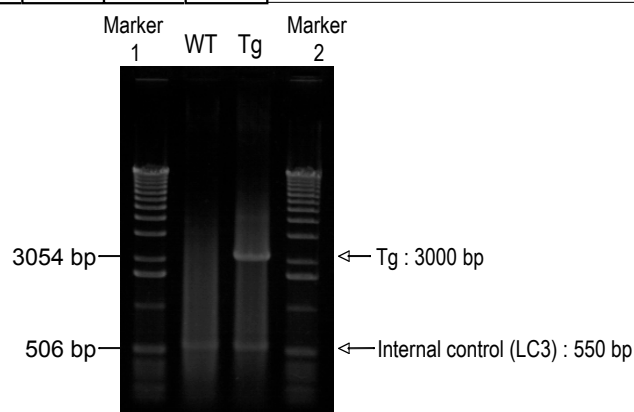
Reaction Components

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

Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		4.4			
PCR buffer	2 X GC I	10.0			
dNTP	2.5 mM	2.0			
primer 1	10 pmol/ μ l	1.0			
primer 2	10 pmol/ μ l	1.0			
primer 3	10 pmol/ μ l	0.2			
primer 4	10 pmol/ μ l	0.2			
DNA		1.0			
Taq		0.2			
total		20			

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	60	30*	
4	72	120*	*Repeat for 40 cycles
5	72	300	



Marker	PCR products		
1 kb DNA ladder (Invitrogen, Cat. 15615-024)	primer_set	product_size	product
Gel	primer 1	primer 2	ca 3000 bp
1% agarose in 1 X TAE (100 V, 30 min)	primer 3	primer 4	550 bp
			Internal control (LC3)

Comment