

PCR protocol

RBRC 04424

Strain name C57BL/6-Tg(CAG-Nup85)4-5Sush

Primer	primer_name	primer_seq	length	
1	pCAGGS 3-Tg	CCAGCCACCACCTTCTGATAGGCAGCCTGCAC	32	Depositor's primer
2	pCAGGS 5-Tg	TGGGCAACGTGCTGGTTA	18	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).

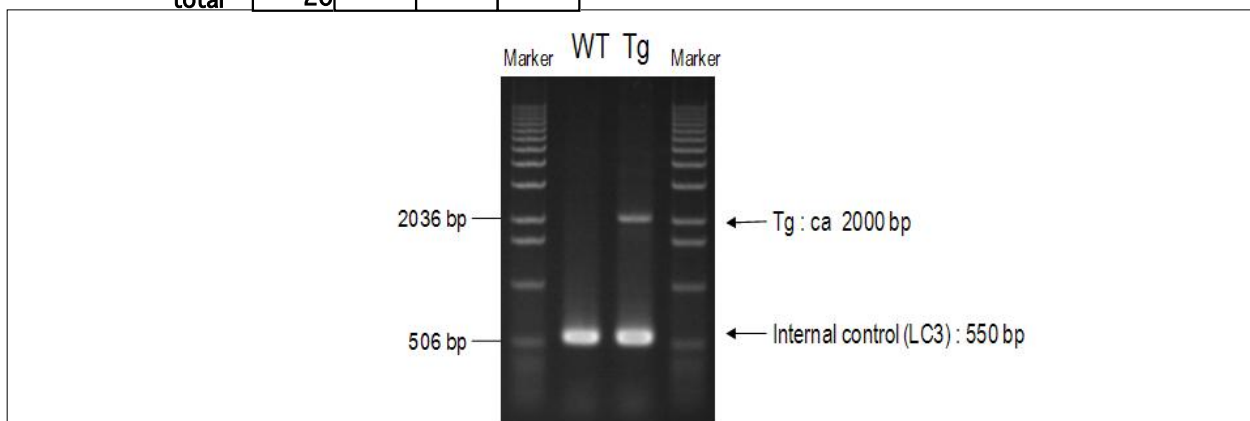
Reaction Components

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

Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		3.3			
PCR buffer	2 X GC I	10.0			
dNTP	2.5 mM	1.6			
primer 1	10 pmol/ μ l	1.0			
primer 2	10 pmol/ μ l	1.0			
primer 3	10 pmol/ μ l	1.0			
primer 4	10 pmol/ μ l	1.0			
DNA		1.0			
Taq	5U/ μ l	0.1			
total		20			

step	temp ($^{\circ}$ C)	time (sec)	note
1	94	180	
2	94	30*	
3	60	30*	
4	72	120*	*Repeat for 30 cycles
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 2000 bp	Tg
primer 3 primer 4	550 bp	Internal control (LC3)

Comment