PCR protocol

RBRC 04054 **Strain name** C57BL/6N-Tg(BGLAP-PTN/CAG-EGFP)18Thg

Primer	primer_name	primer_seq	length		
1	GFP-U279	CGTCCAGGAGCGCACCATCTT	21		Depositor's primer
2	GFP-L624	CTTCTCGTTGGGGTCTTTGCTC	22		Depositor's primer
3	OSF-F1489	CTGGTTGTTGTGCTGTCTCATCA	23		Depositor's primer
4	OSF-R354	GGCTCGCTTCAGACTTCCAGTT	22		Depositor's primer
5	mLC3ex3GT	TGAGCGAGCTCATCAAGATAATCAGGT	27		Mol. Biol. Cell., 15, 1101-1111 (2004).
6	mLC3ex4AG	GTTAGCATTGAGCTGCAAGCGCCGTCT	27		Mol. Biol. Cell., 15, 1101-1111 (2004).

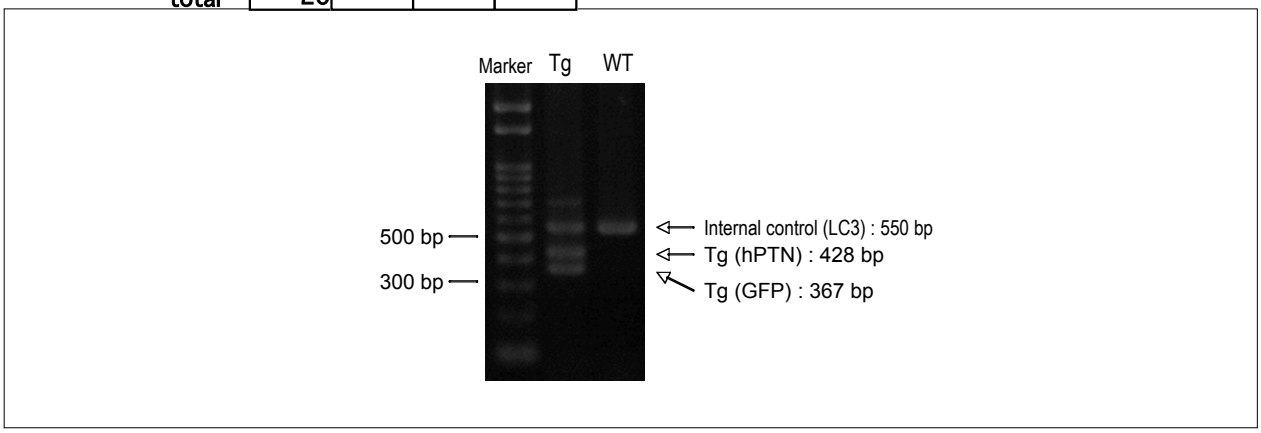
Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		9.3			
PCR buffer	10 X	2.0			
dNTP	2.5 mM	1.6			
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			
primer 5	10 pmol/μl	1.0			
primer 6	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	63	30	*
4	72	60	* *Repeat for 30 cycles
5	72	300	



Marker

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

Gel

2% agarose in 1 X TAE (100 V, 30 min)

PCR products

primer_set		product_size	product
primer 1	primer 2	367 bp	Tg (GFP)
primer 3	primer 4	428 bp	Tg (hPTN)
primer 5	primer 6	550bp	Internal control (LC3)

Comment