PCR protocol

RBRC 00157 **Strain name** C57BL/6-TgN(GFAP-nlCre)/2

Primer	primer_name	primer_seq	length		
1	LCB836 (Cre1)	GTCGATGCAACGAGTGATGA	20		
2	LCB837 (Cre2)	AGCATTGCTGTCACTTGGTC	20		
3	mLC3ex3GT	TGAGCGAGCTCATCAAGATAATCAGGT	27	Internal control	Mol. Biol. Cell., 15, 1101-1111 (2004).
4	mLC3ex4AG	GTTAGCATTGAGCTGCAAGCGCCGTCT	27	Internal control	Mol. Biol. Cell., 15, 1101-1111 (2004).

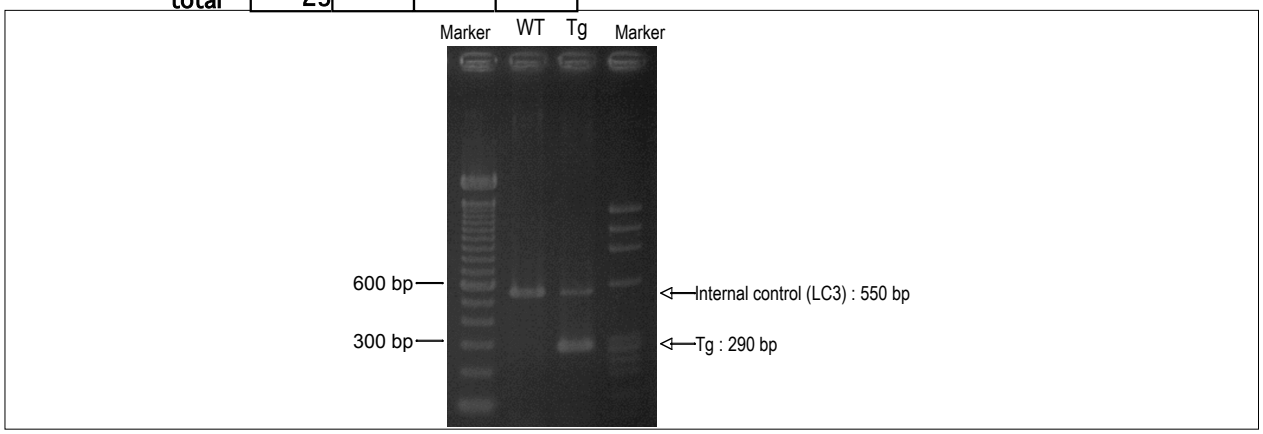
Reaction Components

Enzyme Roche Taq (Cat. No. BM1418432)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		14.3			
PCR buffer	10 X	2.5			
MgCl2	25 mM	1.0			
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			
Taq		0.2			
DNA		1.0			
	total	25			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30*	
3	60	30*	
4	72	60*	*Repeat for 35 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, 30 min)

PCR products

primer_set		product_size	product
primer 1	primer 2	290 bp	Tg
primer 3	primer 4	550 bp	Internal control (LC3)

Comment