PCR protocol

RBRC 02030 **Strain name** B6D2F1-Tg(CAG/Acr-EGFP)C5-FM137Osb

Primer

	primer_name	primer_seq	length	
1	acro	gtggagctttgtgaggtcacag	22	Acro
2	gfp	CAGCTTGCCGGTGGTGCAGATG	22	GFP
3	mLC3ex3GT	TGAGCGAGCTCATCAAGATAATCAGGT	27	intron3 of the LC3 genome as an internal control
4	mLC3ex4AG	GTTAGCATTGAGCTGCAAGCGCCGTCT	27	intron3 of the LC3 genome as an internal control

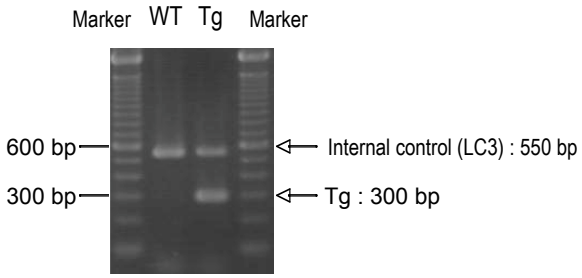
Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

	Conc	Run 1	Run 2	Run 3
H2O		15.3		
PCR buffer	10 X	2.5		
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		0.2		
	total	25		

Reaction Conditions

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



Marker

100 bp ladder (Invitrogen, Cat. 15628-050)

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 2	300 bp	Tg
primer 3	primer 4	550 bp	internal control (LC3)

Comment