

# PCR protocol

RBRC 09930

Strain name C57BL/6J-Tg(CAG-GFP/LC3/RFP/LC3<\*>deltaG)2Nmz

Primer	primer_name	primer_seq	length		
1	5CAG	CCTACAGCTCCTGGGCAACGTGC	23		RIKEN BRC
2	delta G	GTACCACCACACTGGGATCCTTAG	24		RIKEN BRC
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24		JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25		JAX Genotyping Protocol

### Reaction Components

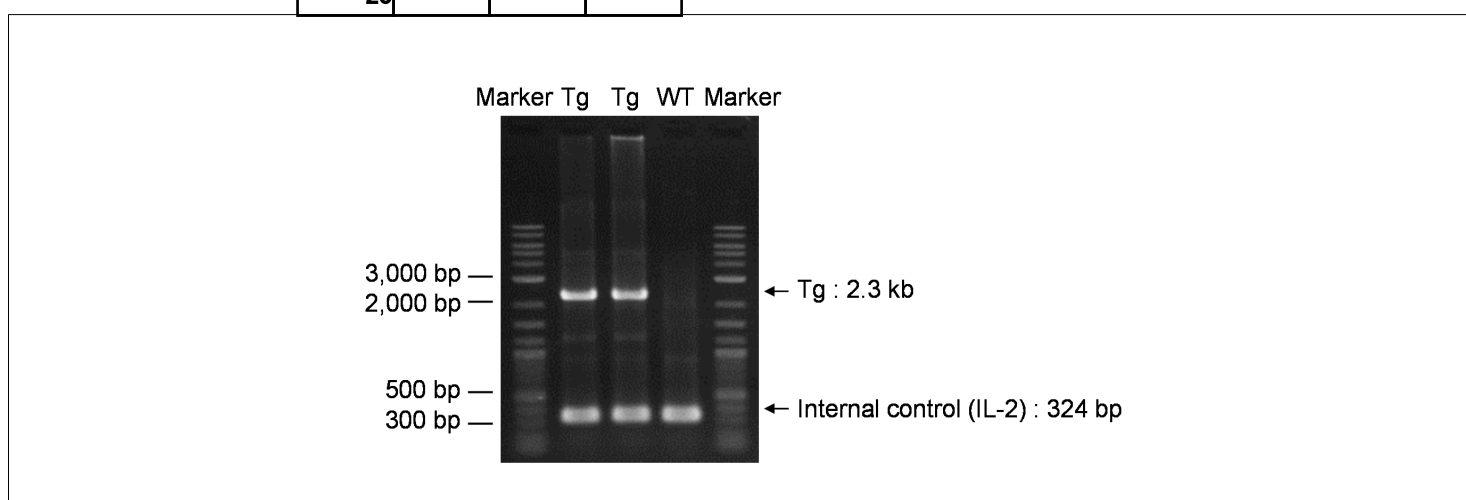
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		0.8			
PCR buffer	2 X	10.0			
dNTP	2.0 mM	4.0			
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	1 U/ μl	0.2			
total		20			

### Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	240*	*Repeat for 30 cycles

### Standard PCR



**Marker**  
2-Log DNA Ladder (NEB, Cat. N3200)

PCR products	primer_set	product_size	product
primer 1	primer 2	2.3 kb	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

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

### Comment

Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.