

PCR protocol

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RBRC 05532

Strain name C3H/HeN-Tg(Cd4-CD4)1Yiw

Primer	primer_name	primer_seq	length	
1		GGCCTAGATTTCCTTCTGAGC	22	RIKEN BRC
2		CAGCACCCACACCGCCTTCTCCCGCTT	27	PLoS One. 2013 May 15;8(5):e63537.
3	mLC3ex4AG	GTTAGCATTGAGCTGCAAGCGCCGTCT	27	Mol. Biol. Cell., 15, 1101-1111(2004).
4	mLC3ex3GT	TGAGCGAGCTCATCAAGATAATCAGGT	27	Mol. Biol. Cell., 15, 1101-1111(2004).

Reaction Components

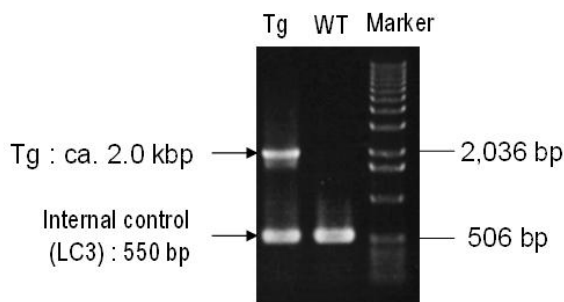
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

	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/ 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	1 U/ μ l	0.2			
total		20			

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	180*	*Repeat for 30 cycles

Standard PCR



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	ca. 2.0 kbp	Tg
primer 3	550 bp	Internal control (LC3)

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