

# PCR protocol

RBRC 00445

Strain name C57BL/6-Tg(CAG/Acr-EGFP)C3-N01-FJ002Osb

Primer	primer_name	primer_seq	length
1	EGFP-F	GCCACCATGGTGAGC	15
2		TTACTTGTACAGCTCGTCC	19
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25
5	acro	gtggagctttgtgaggtcacag	22
6	gfp	CAGCTTGCCGGTGGTGCAGATG	22
7	oIMR0015	CAAATGTTGCTTGTCTGGTG	20
8	oIMR0016	GTCAGTCGAGTGCACAGTTT	20

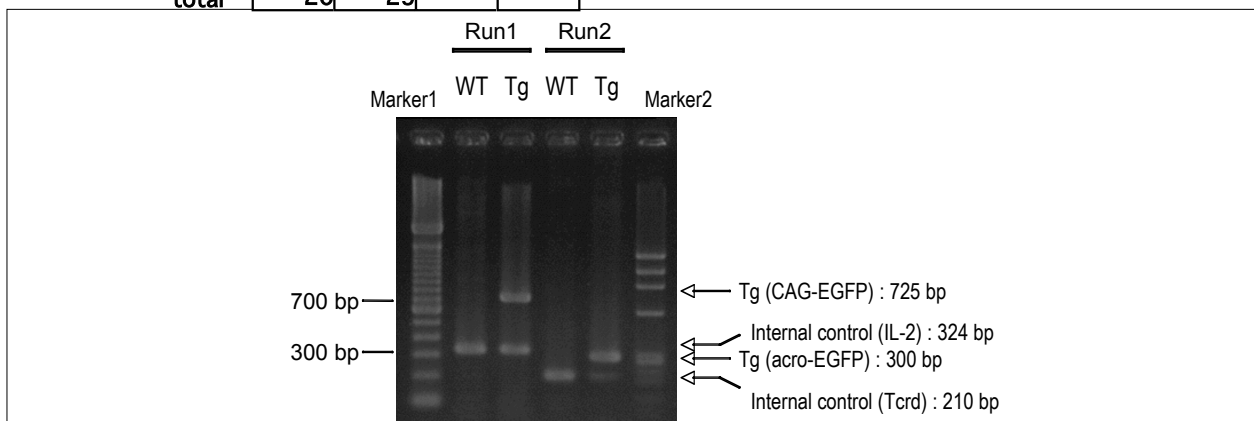
## Reaction Components

Enzyme Run1: TaKaRa LA Taq with GC buffer (Cat. No. RR02AG) Run2: Roche Taq (Cat. No. BM1418432)

## Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	55	30*	
4	72	60*	*Repeat for 30 cycles
5	72	300	

Conc	Run 1	Run 2	Run 3	Run 4
H2O	2.8	15.3		
PCR buffer	2 X	10.0	0	
PCR buffer	10 X	0	2.5	
dNTP	2.5 mM	2.0	2.0	
primer 1	10 pmol/μl	1.0	0	
primer 2	10 pmol/μl	1.0	0	
primer 3	10 pmol/μl	1.0	0	
primer 4	10 pmol/μl	1.0	0	
primer 5	10 pmol/μl	0	1.0	
primer 6	10 pmol/μl	0	1.0	
primer 7	10 pmol/μl	0	1.0	
primer 8	10 pmol/μl	0	1.0	
DNA		1.0	1.0	
Taq		0.2	0.2	
<b>total</b>		<b>20</b>	<b>25</b>	



## 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	725 bp	CAG-EGFP
primer 3	324 bp	internal control (IL-2)
primer 5	300 bp	acro-EGFP
primer 7	210 bp	internal control (Tcrd)

## Comment