

PCR protocol

RBRC 04425

Strain name C57BL/6-Tg(CAG-Nup85*DN)6-1Sush

Primer	primer_name	primer_seq	length	
1	pCAGGS 3-Tg	CCAGCCACCACCTTCTGATAGGCAGCCTGCAC	32	Depositor's primer
2	pCAGGS 5-Tg	TGGGCAACGTGCTGGTTA	18	Depositor's primer
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	JAX Genotyping Protocol

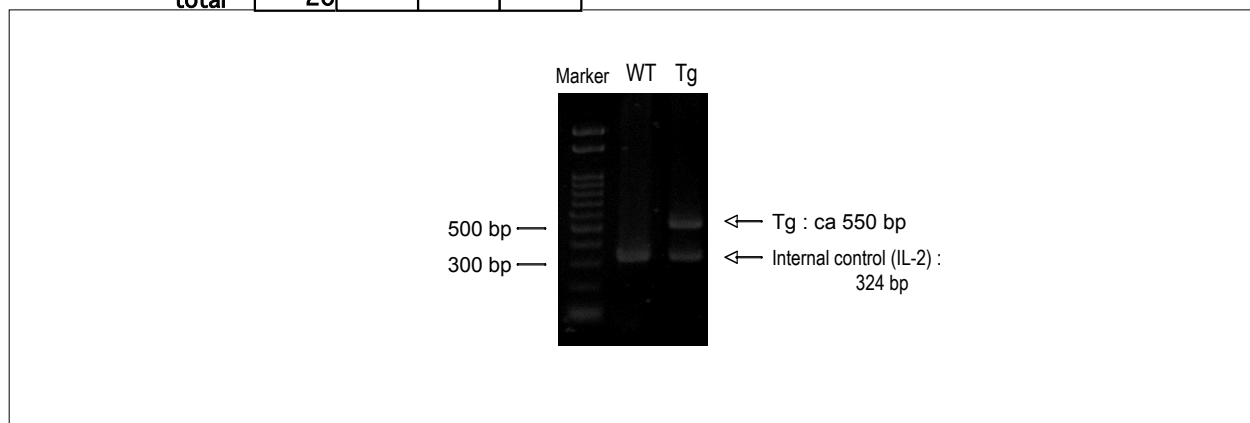
Reaction Components

Enzyme	TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)
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Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		3.3			
PCR buffer	2 X GC I	10.0			
dNTP	2.5 mM	1.6			
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	5U/ μ l	0.1			
total		20			

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



Marker

Gene Ladder 100 (Wako, Cat. 316-06951)

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 2	ca 550 bp	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

Comment