PCR protocol

RBRC 02416 **Strain name** C57BL/6-Tg(TRE-GFP)622Yasu

Primer	primer_name	primer_seq	length		
1		CCCTTTGATCTTACCCCCTTTGATC	25		RIKEN BRC
2	oIMR1416	TCCTTGAAGAAGATGGTGCG	20		JAX Genotyping Protocol
3	**oIMR**0042	CTAGGCCACAGAATTGAAAGATCT	24		JAX Genotyping Protocol
4	**oIMR**0043	GTAGGTGGAAATTCTAGCATCATCC	25		JAX Genotyping Protocol

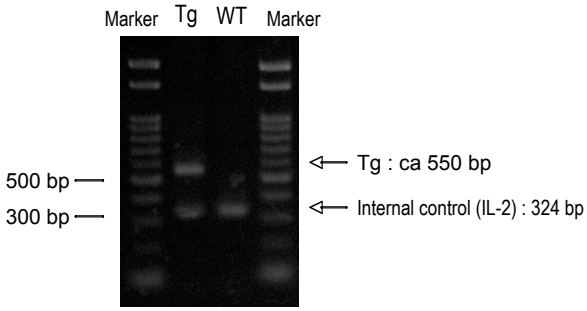
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		2.3			
PCR buffer	2 X GC I	10.0			
dNTP	2.5 mM	1.6			
primer 1	10 pmol/μl	2.0			
primer 2	10 pmol/μl	2.0			
primer 3	10 pmol/μl	0.5			
primer 4	10 pmol/μl	0.5			
DNA		1.0			
Taq	5U/μl	0.1			
	total	20			

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

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