PCR protocol

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RBRC 04176

Strain name C57BL/6N-Tg(Thy1-ChR2/GFP)435-1Sasa/Rbrc

Primer	primer_name	primer_seq	length		Reference
1		GCTGGAATCAAAGGTGTGAGCTC	23		RIKEN BRC
2		CTTGTACAGCTCGTCCATGCCGAG	24		RIKEN BRC
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)

Reagent	Conc	Reaction 1	2	3	4
H2O		3.3			
PCR buffer	2 X GC I	10.0			
dNTP	2.5 mM	1.6			
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		0.1			
	total	20			

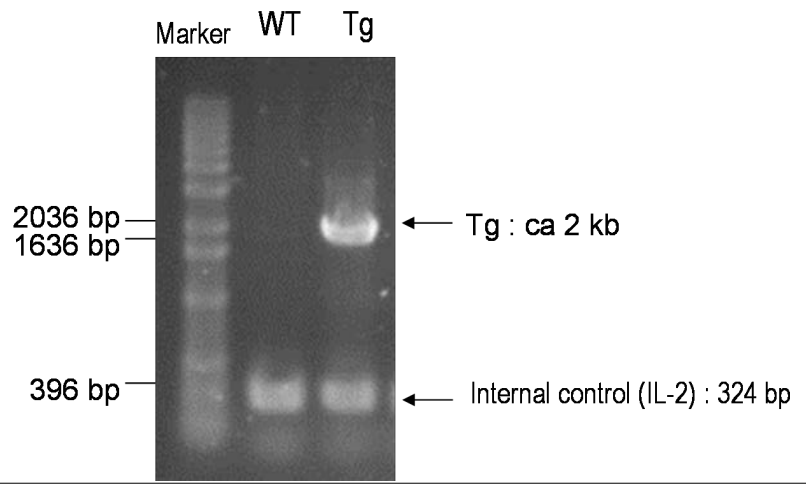
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30*	
3	60	30*	
4	72	120*	*Repeat for 30 cycles
5	72	300	

Marker: 1 kb DNA ladder (Invitrogen, Cat. 15615-024)

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



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

Comment