

PCR protocol

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RBRC 04222

Strain name C57BL/6N-Tg(CAG-KO,Chr2/GFP)459-1 Sasa/Rbrc

Primer	primer_name	primer_seq	length	
1	5CAG	CCTACAGCTCCTGGGCAACGTGC	23	RIKEN BRC
2	3CAG	AGCCAGAAGTCAGATGCTCAAGGGGC	26	RIKEN BRC
3	loxP-F	GCGGATCCTCGTATAATGTATGCTATACGAAGTTAT	36	RIKEN BRC
4		CTTGTACAGCTCGTCCATGCCGAG	24	RIKEN BRC
5	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	JAX Genotyping Protocol
6	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	JAX Genotyping Protocol

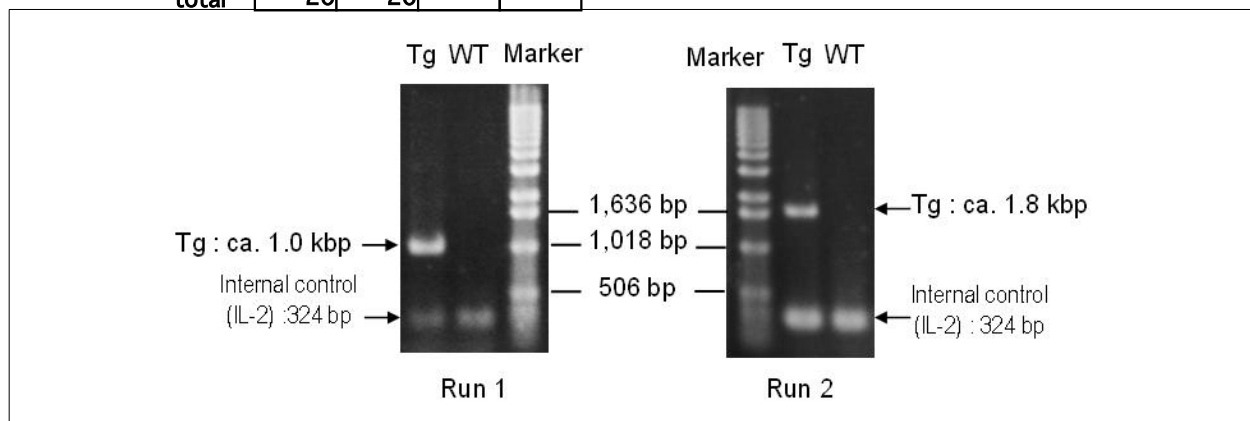
Reaction Components

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

Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		3.3	1.3		
PCR buffer	2 X GC I	10.0	10.0		
dNTP	2.5 mM	1.6	1.6		
primer 1	10 pmol/ μ l	1.0	0		
primer 2	10 pmol/ μ l	1.0	0		
primer 3	10 pmol/ μ l	0	2.0		
primer 4	10 pmol/ μ l	0	2.0		
primer 5	10 pmol/ μ l	1.0	1.0		
primer 6	10 pmol/ μ l	1.0	1.0		
DNA		1.0	1.0		
Taq	5U/ μ l	0.1	0.1		
total		20	20		

step	temp (°C)	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)

PCR products

primer_set	product_size	product
primer 1	primer 2	ca. 1.0 kbp
primer 3	primer 4	ca. 1.8 kbp
primer 5	primer 6	324 bp

Comment