PCR protocol

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RBRC 04211 Strain name C57BL/6N-Tg(CAG-KO,Halo/GFP)478-11Sasa/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

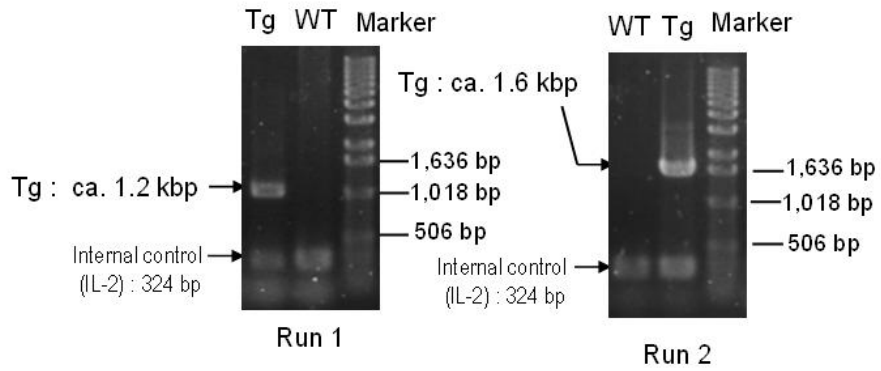
Reaction Components

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

	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/μl	1.0	0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	0	2.0		
primer 4	10 pmol/μl	0	2.0		
primer 5	10 pmol/μl	1.0	1.0		
primer 6	10 pmol/μl	1.0	1.0		
DNA		1.0	1.0		
Taq	5U/μl	0.1	0.1		
	total	20	20		

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)

PCR products

primer_set		product_size	product
primer 1	primer 2	ca. 1.2 kbp	Tg
primer 3	primer 4	ca. 1.6 kbp	Tg
primer 5	primer 6	324 bp	Internal control (IL-2)

Comment