

RBRC 04707

Strain name STOCK Tg(Kcnc1-EYFP)27Bsi Gad1<tm1Tama>

Primer	primer_name	primer_seq	length	
1		AGAGCAGTGAGCGGAGTGCG	20	RIKEN BRC
2	3.1-EYFP3	GCGGACTTGAAGAAGTCGTG	20	Eur. J. Neurosci., 15, 40-50 (2002).
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	JAX Genotyping Protocol
5	TR-1b	GGCACAGCTCTCCCTTCTGTTTGC	24	Depositor's primer
6	TR-3	GCTCTCCTTTCGCGTCCGACAG	23	J. Comp. Neurol., 467, 60-79 (2003).
7	TRGFP-8	CTGCTTGTGCGCCATGATATAGACG	25	Depositor's primer

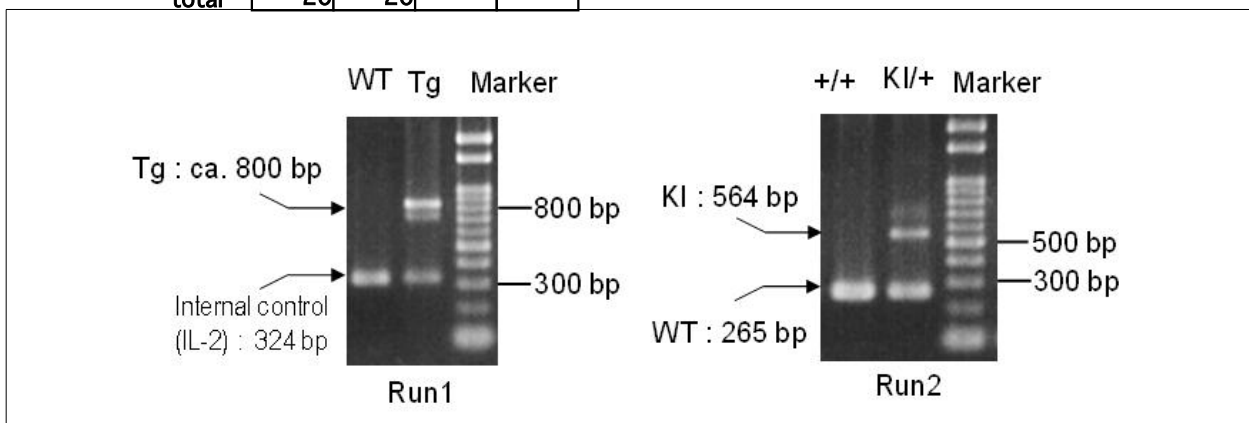
Reaction Components

Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		0.8	1.8		
PCR buffer	2 X	10.0	10.0		
dNTP	2.0 mM	4.0	4.0		
primer 1	10 pmol/μl	1.0	0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	1.0	0		
primer 4	10 pmol/μl	1.0	0		
primer 5	10 pmol/μl	0	1.0		
primer 6	10 pmol/μl	0	1.0		
primer 7	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq	1 U/μl	0.2	0.2		
total		20	20		

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	60*	*Repeat for 30 cycles



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. 800 bp	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)
primer 5	primer 6	265 bp	WT (Gad1)
primer 5	primer 7	564 bp	KI (Gad1)

Comment Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.

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Strain name STOCK Tg(Kcnc1-EYFP)27Bsi Gad1<tm1 Tama>

Primer	primer_name	primer_seq	length	
1	3.1-EYFP2	GAAGTTCATCTGCACCACCG	20	Eur. J. Neurosci., 15, 40-50 (2002).
2	3.1-EYFP3	GCGGACTTGAAGAAGTCGTG	20	Eur. J. Neurosci., 15, 40-50 (2002).
3	NR1-GT2	AGCCCTTCAAGTACCAGGGCCTGAC	25	Eur. J. Neurosci., 15, 40-50 (2002).
4	NR1-GT3	AGCGGTCCAGCAGGTACAGCATCAC	25	Eur. J. Neurosci., 15, 40-50 (2002).
5	TR-1b	GGCACAGCTCTCCCTTCTGTTTGC	24	Depositor's primer
6	TR-3	GCTCTCCTTTCGCGTTCCGACAG	23	J. Comp. Neurol., 467, 60-79 (2003).
7	TRGFP-8	CTGCTTGTGGCCATGATATAGACG	25	Depositor's primer

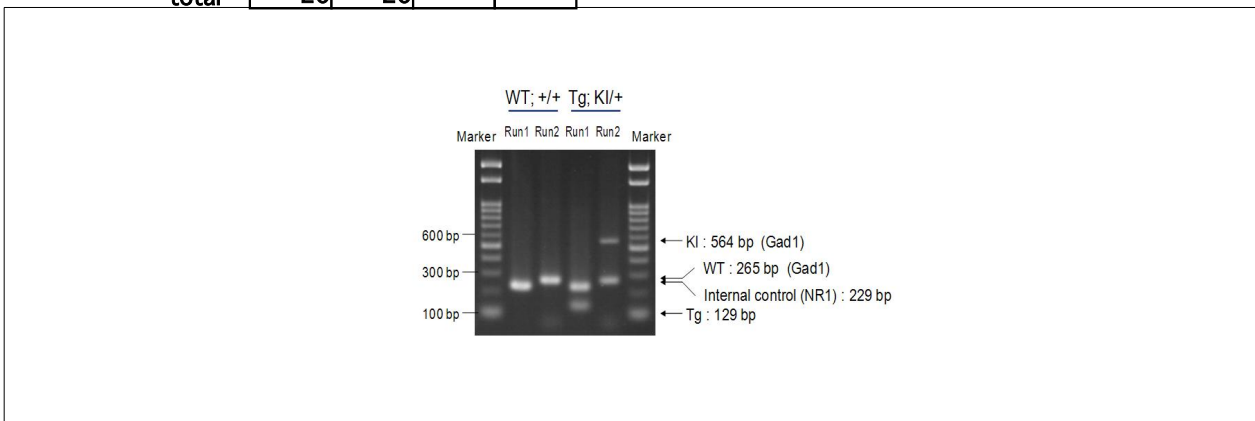
Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	65	30*	
4	72	60*	*Repeat for 30 cycles
5	72	300	

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		11.3	12.3		
PCR buffer	10 X	2.0	2.0		
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	1.0	0		
primer 4	10 pmol/μl	1.0	0		
primer 5	10 pmol/μl	0	1.0		
primer 6	10 pmol/μl	0	1.0		
primer 7	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq	5U/μl	0.1	0.1		
total		20	20		



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	129 bp	Tg
primer 3	229 bp	NR1 (Internal control)
primer 5	265 bp	WT (Gad1)
primer 5	564 bp	KI (Gad1)

Comment