

RBRC 01498

Strain name C57BL/6J-Tg(Camk2-mutPolg)1Bsi

Primer	primer_name	primer_seq	length	
1		GCTCGTCAGTCAAGCCGGTTC	21	RIKEN BRC
2	mutPOLG-NewTgCheck-2R	GTGGTTGAACTGCATCAGTAGG	22	Depositor's primer
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	JAX Genotyping Protocol

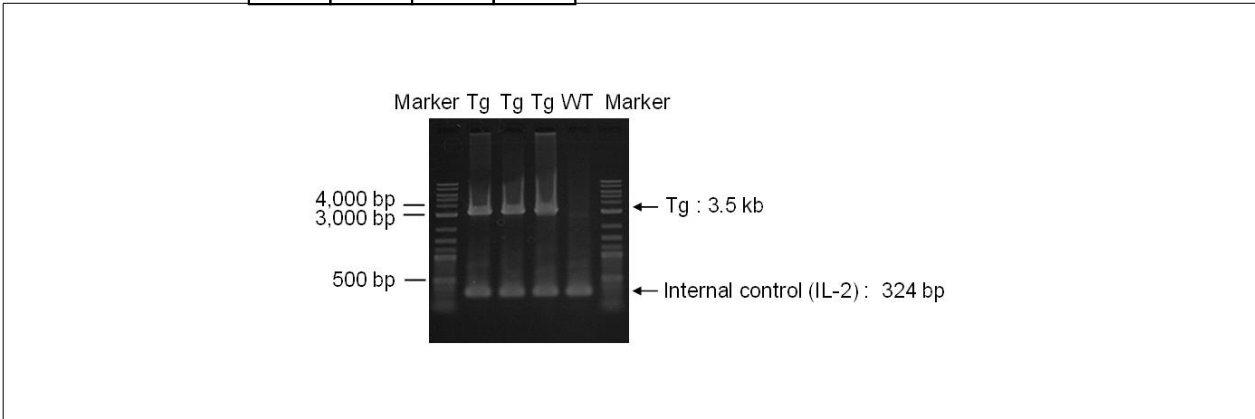
Reaction Components

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

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	240*	*Repeat for 30 cycles

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		0.8			
PCR buffer	2 X	10.0			
dNTP	2.0 mM	4.0			
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	1 U/μl	0.2			
total		20			



Marker
2-Log DNA Ladder (NEB, Cat. N3200)

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

PCR products	primer_set	product_size	product
primer 1	primer 2	3.5 kb	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

Comment

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Primer	primer_name	primer_seq	length	
1	mutPOLG-NewTgCheck-2F	AGTGAGTTGAAAGCCATGGTG	21	Depositor's primer
2	mutPOLG-NewTgCheck-1R	GTCAGGAGATTGGTGATCTGC	21	Depositor's primer
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	JAX Genotyping Protocol

Reaction Components

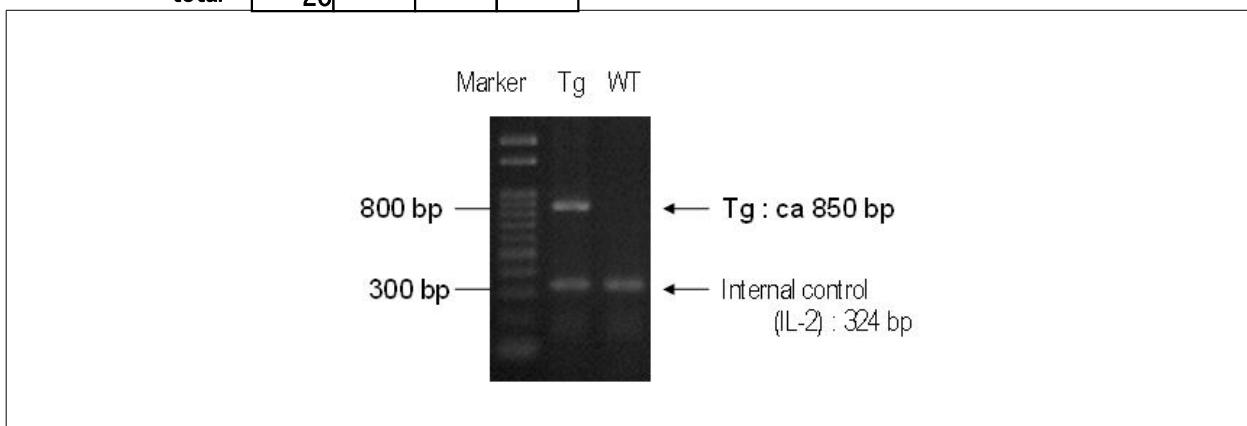
Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	57	30*	
4	72	60*	*Repeat for 30 cycles
5	72	300	

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		11.3			
PCR buffer	10 X	2.0			
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	5 U/μl	0.1			
total		20			

Standard PCR



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 850 bp	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

Comment

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Strain name C57BL/6J-Tg(Camk2-mutPolg)1Bsi

Primer	primer_name	primer_seq	length		
1	mutPOLG-NewTgCheck-1F	TGGTCAAACAGTTGAATCTTCC	22	exon 19	Depositor's primer
2	mutPOLG-NewTgCheck-1R	GTCAGGAGATTGGTGATCTGC	21	exon 21	Depositor's primer
3	mutPOLG-NewTgCheck-2F	AGTGAGTTGAAAGCCATGGTG	21		Depositor's primer
4	mutPOLG-NewTgCheck-2R	GTGGTTGAAGTGCATCAGTAGG	22		Depositor's primer

Reaction Components

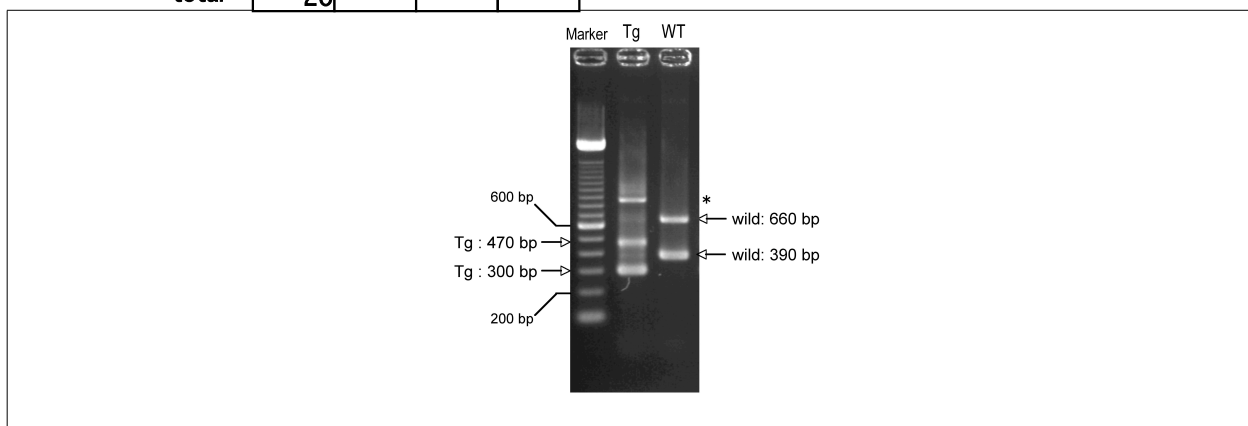
Enzyme TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	58	30*	
4	72	60*	*Repeat for 30 cycles
5	72	300	

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		2.8			
PCR buffer	2 X GC I	10.0			
dNTP	2.5 mM	2.0			
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		0.2			
total		20			

Standard PCR



Marker
100 bp ladder (Invitrogen, Cat. 15628-050)

Gel
2% agarose in 1 X TAE (100 V, 35 min)

PCR products	primer_set	product_size	product
primer 1	primer 2	470 bp	Tg
primer 3	primer 4	300 bp	Tg
primer 2	primer 3	850 bp	Tg
primer 1	primer 2	660 bp	WT
primer 3	primer 4	390 bp	WT

Comment

*heteroduplex