

RBRC 02957

Strain name C57BL/6J-Tg(Gabra6-rtTA)1Nak

Primer	primer_name	primer_seq	length	
1	ex8_F	CAGGAATCACCCTGTTTTAAACCATG	26	RIKEN BRC
2	primer Rv	CCTCGATGGTAGACCCGTAA	20	Depositor's primer
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	JAX Genotyping Protocol

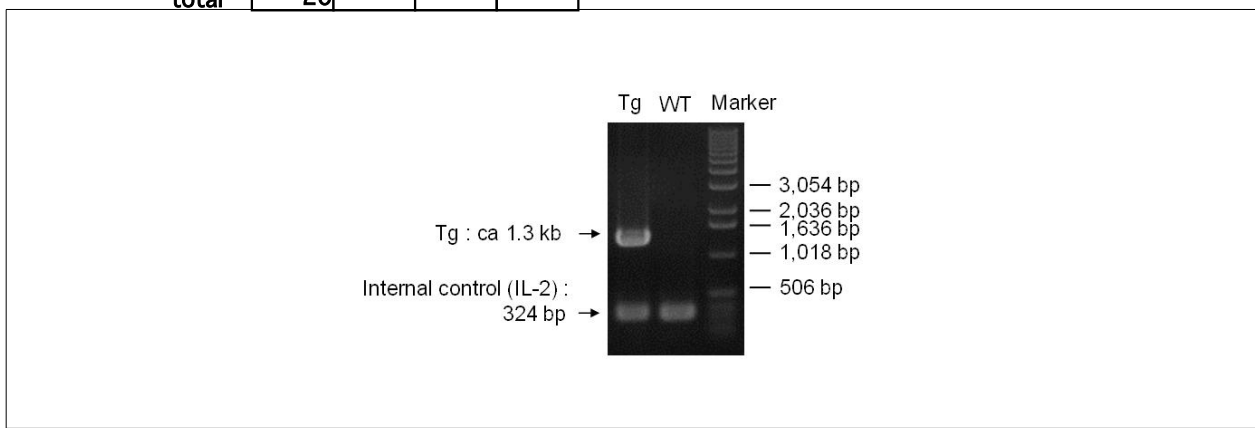
### Reaction Components

**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	120*	*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			
<b>total</b>	<b>20</b>			



### 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 1.3 kb	Tg
primer 3 - primer 4	324 bp	Internal control (IL-2)

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

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1	primer Rv	CCTCGATGGTAGACCCGTAA	20		Depositor's primer
2	primer Fw	TAACAACCCGTAAACTCGCC	20		Depositor's primer
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	Internal control (IL-2)	JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	Internal control (IL-2)	JAX Genotyping Protocol

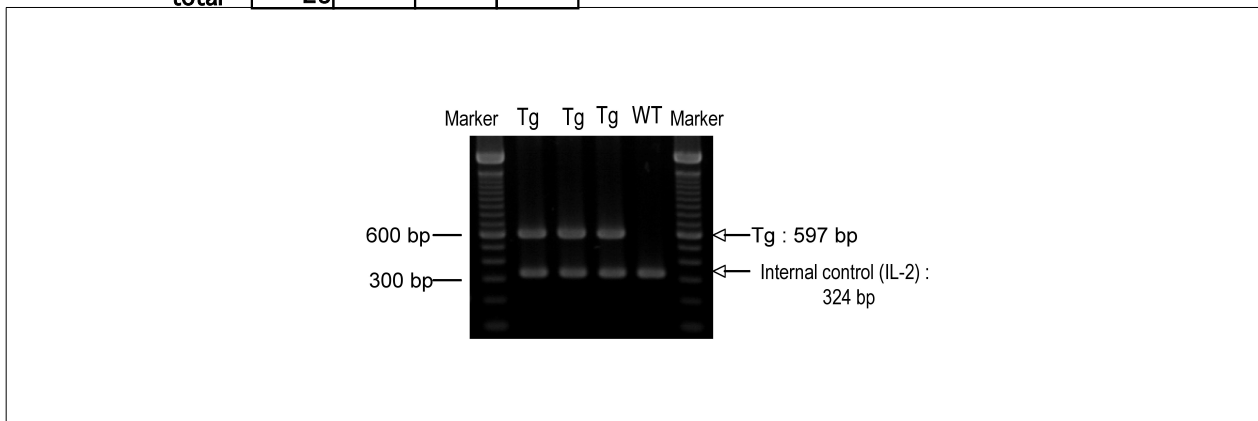
### Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		15.3			
PCR buffer	10 X	2.5			
dNTP	2.5 mM	2.0			
primer 1	10 pmol/ $\mu$ l	1.0			
primer 2	10 pmol/ $\mu$ l	1.0			
primer 3	10 pmol/ $\mu$ l	1.0			
primer 4	10 pmol/ $\mu$ l	1.0			
DNA		1.0			
Taq		0.2			
<b>total</b>		<b>25</b>			

### Reaction Conditions

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



### Marker

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

### Gel

2% agarose in 1 X TAE (100 V, 30 min)

### PCR products

primer_set	primer_set	product_size	product
primer 1	primer 2	597 bp	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

Comment