PCR protocol

Sheet ID PS_07177
Release date : 2/1/2017
Print date : 2/1/2017

RBRC 02412 **Strain name** B6.Cg-Tg(Nes-cre)1Nogu

Primer	primer_name	primer_seq	length		
1	Rat Nes	GTTGCGTGTTGCACTGAACG	20		RIKEN BRC
2	CRE 6	TGCTGTCACTTGGTCGTGGC	20		
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24		JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25		JAX Genotyping Protocol

Reaction Components

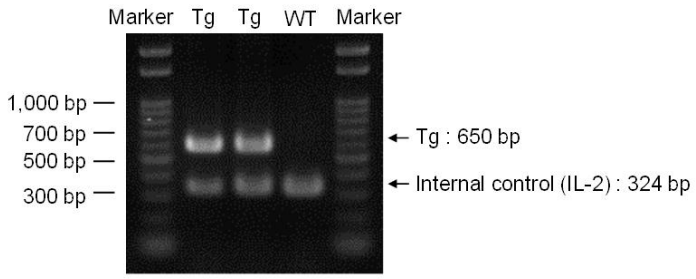
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

	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			

Reaction Conditions

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

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

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

PCR protocol

Sheet ID PS_05136
Release date : 12/11/2014
Print date : 2/1/2017

RBRC 02412 **Strain name** B6.Cg-Tg(Nestin-cre)

Primer	primer_name	primer_seq	length		
1	Nestin-check 5'	ACAGTTCACGAGGTTTCCAACACCA	25		
2	Cre	CTAATCGCCATCTTCCAGCAGG	22		
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24		JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25		JAX Genotyping Protocol

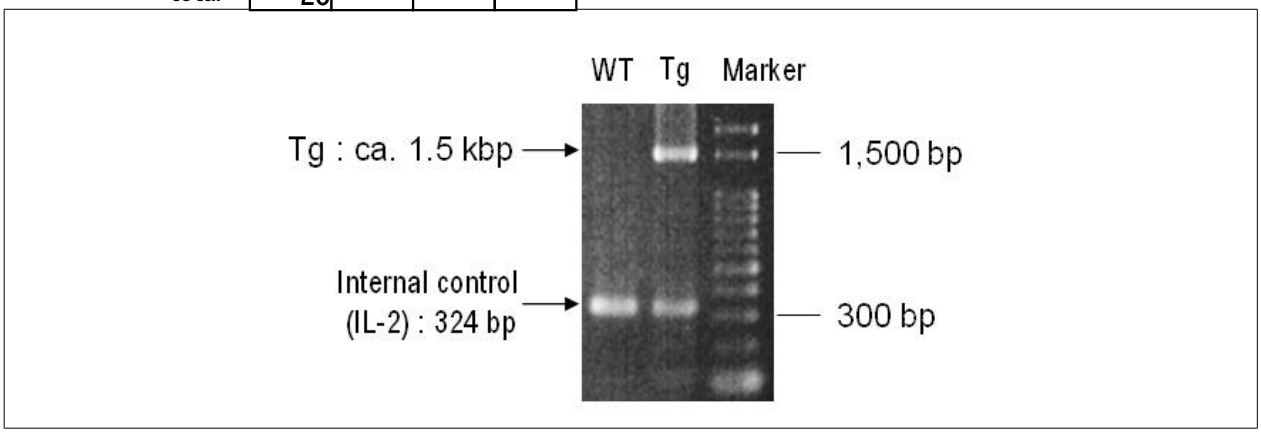
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.9			
PCR buffer	2 X GC I	10.0			
dNTP	2.5 mM	2.0			
primer 1	10 pmol/μl	2.0			
primer 2	10 pmol/μl	2.0			
primer 3	10 pmol/μl	0.5			
primer 4	10 pmol/μl	0.5			
DNA		1.0			
Taq		0.1			
	total	20			

Reaction Conditions

step	temp (℃)	time (sec)		note
1	94	180		
2	94	30	*	
3	60	30	*	
4	72	120	*	*Repeat for 35 cycles
5	72	300		



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. 1.5 kbp	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

Comment

PCR protocol

Sheet ID PS_04007
Release date : 4/26/2011
Print date : 2/1/2017

RBRC 02412 **Strain name** B6.Cg-Tg(Nestin-cre)

Primer	primer_name	primer_seq	length		
1	Nestin-check 5'	ACAGTTCACGAGGTTTCCAACACCA	25		
2	Cre	CTAATCGCCATCTTCCAGCAGG	22		
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24		JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25		JAX Genotyping Protocol

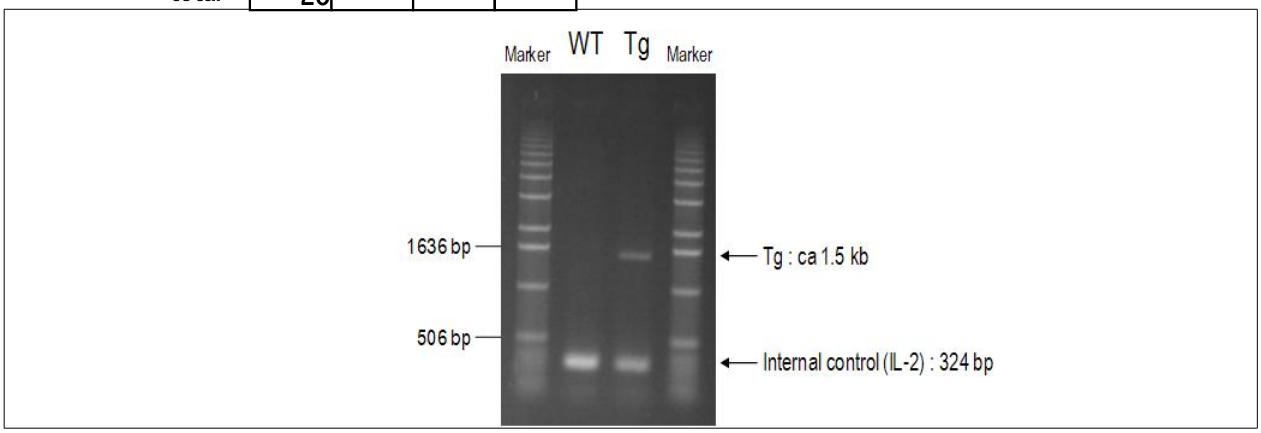
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		2.9			
PCR buffer	2 X GC I	10.0			
dNTP	2.5 mM	2.0			
primer 1	10 pmol/μl	1.5			
primer 2	10 pmol/μl	1.5			
primer 3	10 pmol/μl	0.5			
primer 4	10 pmol/μl	0.5			
DNA		1.0			
Taq		0.1			
	total	20			

Reaction Conditions

step	temp (℃)	time (sec)		note
1	94	180		
2	94	30	*	
3	60	30	*	
4	72	90	*	*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 1.5 kb	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

Comment

PCR protocol

Sheet ID PS_02187
Release date : 06/03/2009
Print date : 2/1/2017

RBRC 02412 **Strain name** B6.Cg-Tg(Nestin-cre)

Primer	primer_name	primer_seq	length		
1	Cre5P	TCGATGCAACGAGTGATGAG	20		
2	Cre3P	TTCGGCTATACGTAACAGGG	20		
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	Internal control (IL-2)	JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	Internal control (IL-2)	JAX Genotyping Protocol

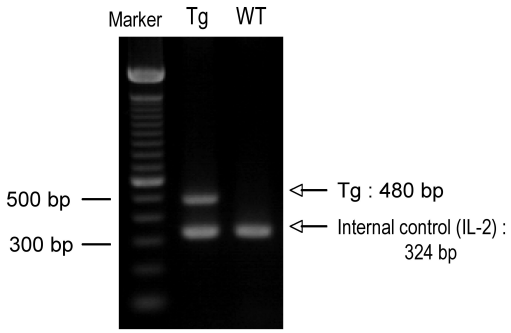
Reaction Components

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

	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			

Reaction Conditions

step	temp (℃)	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	product_size	product
primer 1	primer 2	480 bp	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

Comment