

RBRC 00906

Strain name C57BL/6-Tg(pLck-EGFP)1Naka/Rbrc

Primer	primer_name	primer_seq	length	Reference
1	pLck-Bmil F	CTAAGGAGCTGGAACCTCTCAGC	23	Depositor's primer
2	GFP-Full-R	CTTGTACAGCTCGTCCATGCCGAG	24	RIKEN BRC
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	JAX Genotyping Protocol

**Reaction Components**

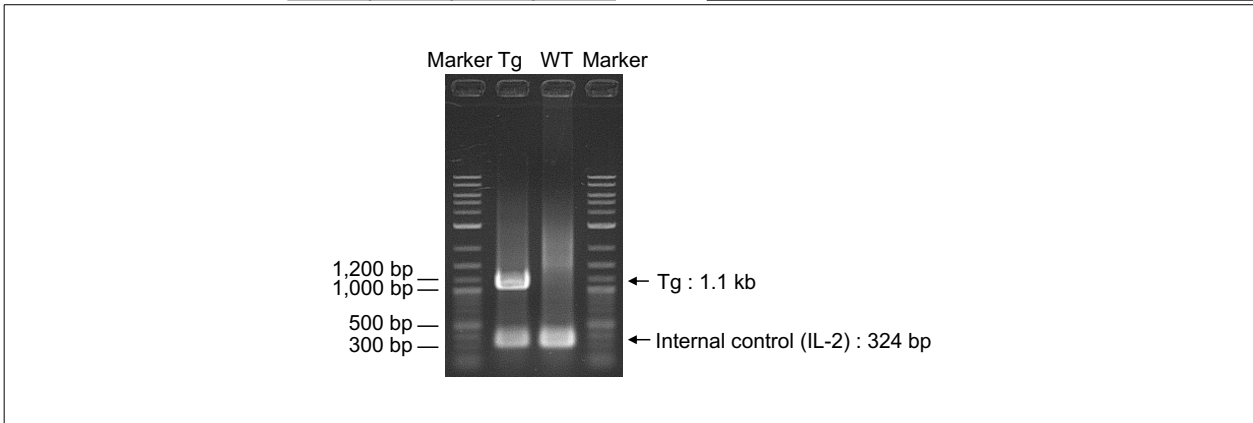
Enzyme	TOYOBO KOD FX (Code.No : KFX-101)
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Reagent	Conc	Reaction 1	2	3	4
H2O		0.8			
PCR buffer	2 X	10.0			
dNTP	2.0 mM	4.0			
primer 1	10 pmol/ $\mu$ l	1.0			
primer 2	10 pmol/ $\mu$ l	1.0			
primer 3	10 pmol/ $\mu$ l	1.0			
primer 4	10 pmol/ $\mu$ l	1.0			
DNA		1.0			
Taq	1 U/ $\mu$ l	0.2			
<b>total</b>		<b>20</b>			

**Reaction Conditions**

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	90*	*Repeat for 30 cycles

Marker	Gene Ladder 100 (Wako, Cat. 316-06951)
Gel	2% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)	
primer 1 / primer 2	1.1 kb	Tg	Non-Tg : 324 bp Tg : 324 bp, 1.1 kb
primer 3 / primer 4	324 bp	Internal control (IL-2)	

**Comment** Standard PCR

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

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Strain name plck-GFP Tg

Primer	primer_name	primer_seq	length	Reference
1	primer 1	TCTAGATCCGGATCCTTACGGGCC	24	Depositor's primer
2	primer 2	GGATCCGTGAACCGTCAGATCCGCTAGC	28	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)

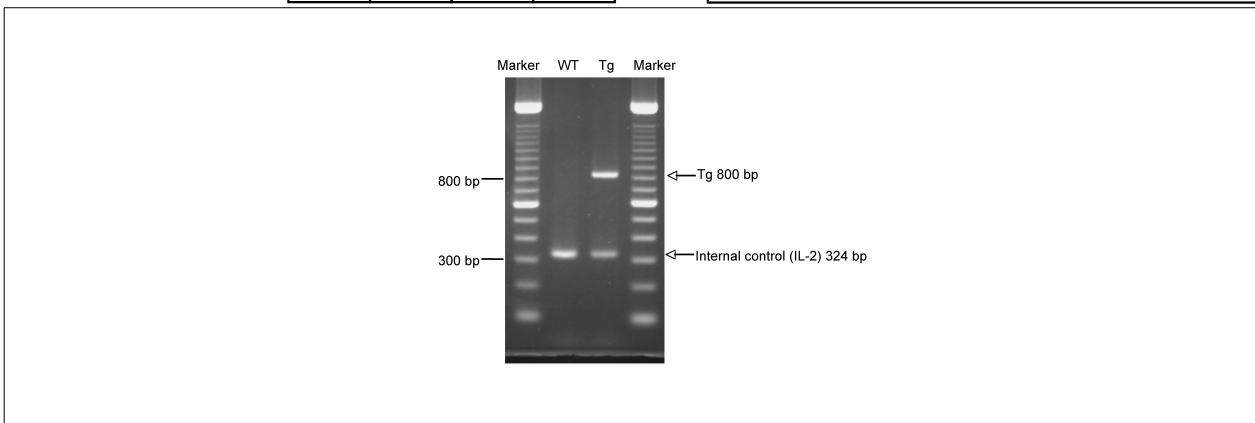
### Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	300	
2	94	30*	
3	62	60*	
4	72	60*	*Repeat for 35 cycles
5	72	300	

Reagent	Conc	Reaction 1	2	3	4	(μL)
H2O		16.3				
PCR buffer	10 X	2.5				
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	0.5				
primer 4	10 pmol/μl	0.5				
DNA		1.0				
Taq		0.2				
total		25				

Marker 1 kb plus DNA ladder (Invitrogen, Cat. 10787-026)

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



primer_set	product_size	product (allele type)
primer 1 primer 2	800 bp	Tg
primer 3 primer 4	324 bp	Internal control (IL-2)

Comment Generic PCR