

RBRC 02237

Strain name C57BL/6Cr-Tg(Dmp1-HBEGF)

Primer	primer_name	primer_seq	length	
1		CCTGTATGATGCTGACAAGGCTC	23	RIKEN BRC
2	PCR-R	GGGTCCCTCTTCTCCCTAGC	21	Depositor's primer
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	JAX Genotyping Protocol

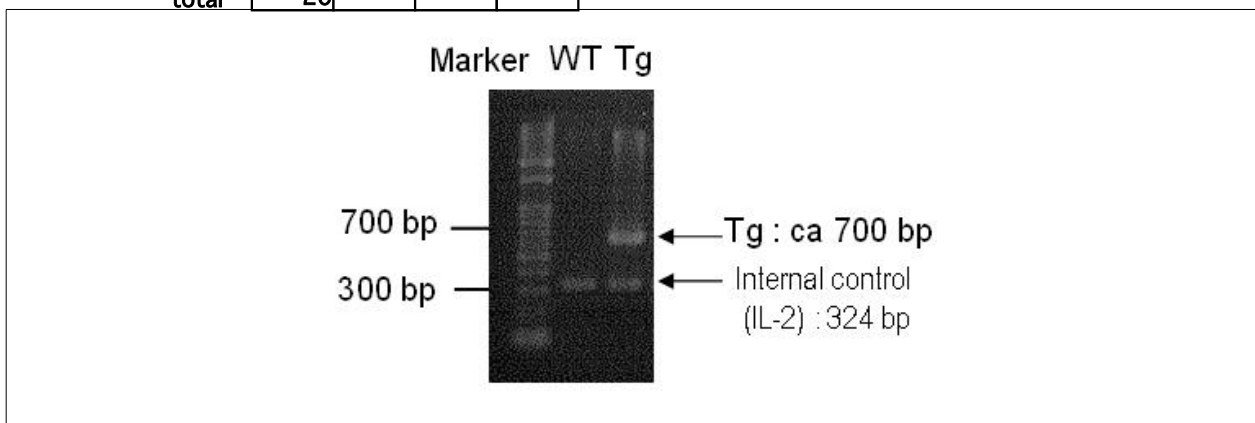
**Reaction Components**

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

**Reaction Conditions**

	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>			

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 700 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

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Strain name C57BL/6Cr-Tg(Dmp1-HBEGF)

Primer	primer_name	primer_seq	length		
1	PCR-R	GGGTCCCTCTTCTCCCTAGC	21		Depositor's primer
2	PCR-F	GTATCCACGGACCAGCTGCTAC	22		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

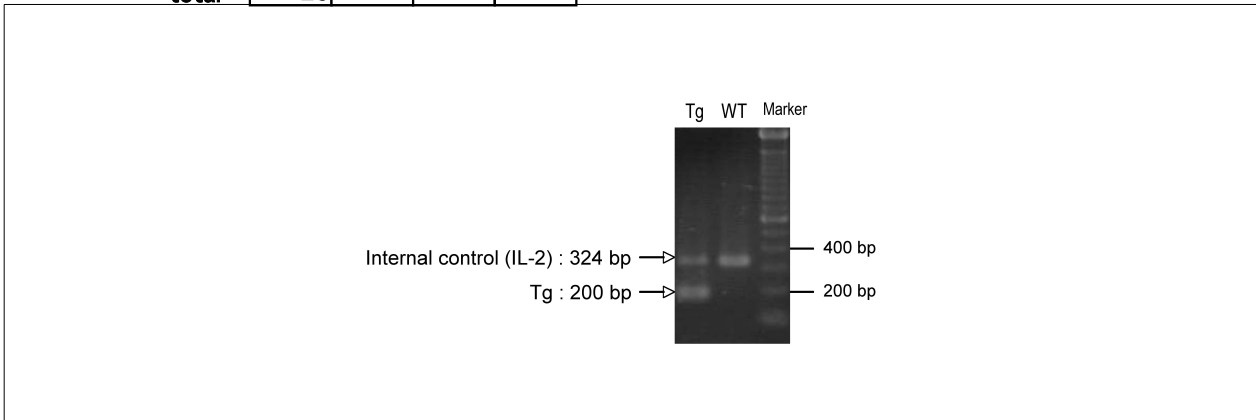
### Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

### Reaction Conditions

	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>			

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

Comment