

# PCR protocol

RBRC 09464

Strain name B6;Tg(ChAT-IL-2Ra/mVenus)997-6

Primer	primer_name	primer_seq	length	
1		GAGATGGCAGTCACAGAGGC	20	RIKEN BRC
2		GTGTCACTTGTTCGTTGTGTTCCG	25	RIKEN BRC
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	JAX Genotyping Protocol

### Reaction Components

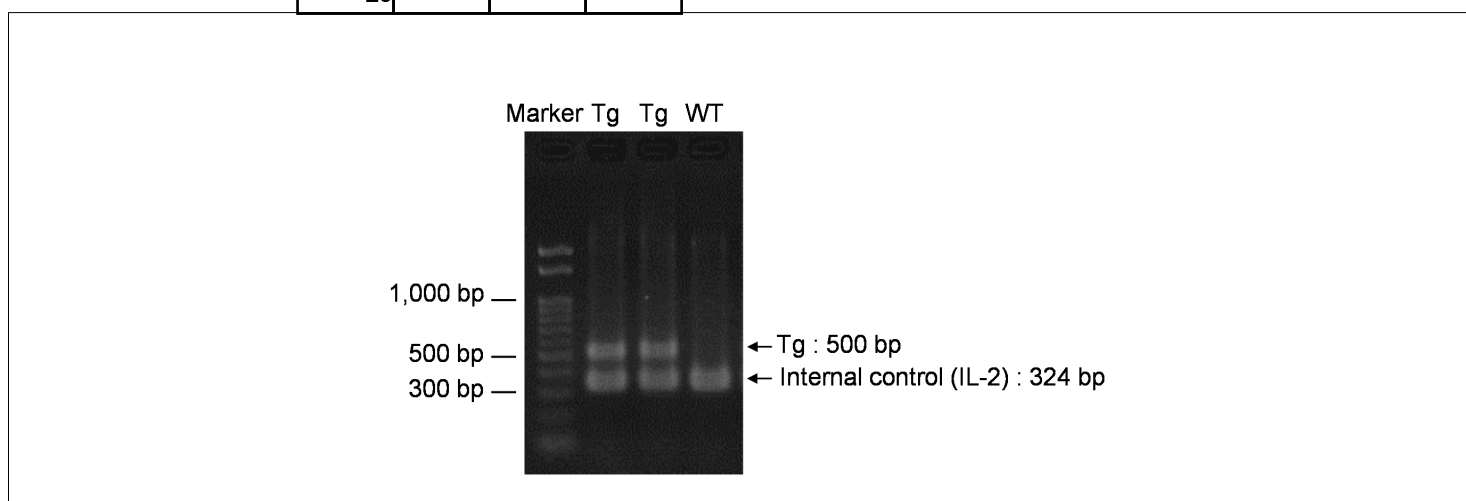
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

	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/ $\mu$ 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	60*	*Repeat for 30 cycles

### Standard PCR



Marker	PCR products	primer_set	product_size	product
Gene Ladder 100 (Wako, Cat. 316-06951)	primer 1	primer 2	500 bp	Tg
	primer 3	primer 4	324 bp	Internal control (IL-2)

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

### Comment

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