

# PCR protocol

RBRC 06571

Strain name STOCK Tg(RARE-Hspa1b/lacZ)12Jrt

Primer	primer_name	primer_seq	length	
1	RAREb_Fw	TGCGAGTGAACCTTTTCGGTGAACCC	24	RIKEN BRC
2	3' primer	GATGGGCGCATCGTAACCGTGC	22	Depositor's primer
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	JAX Genotyping Protocol

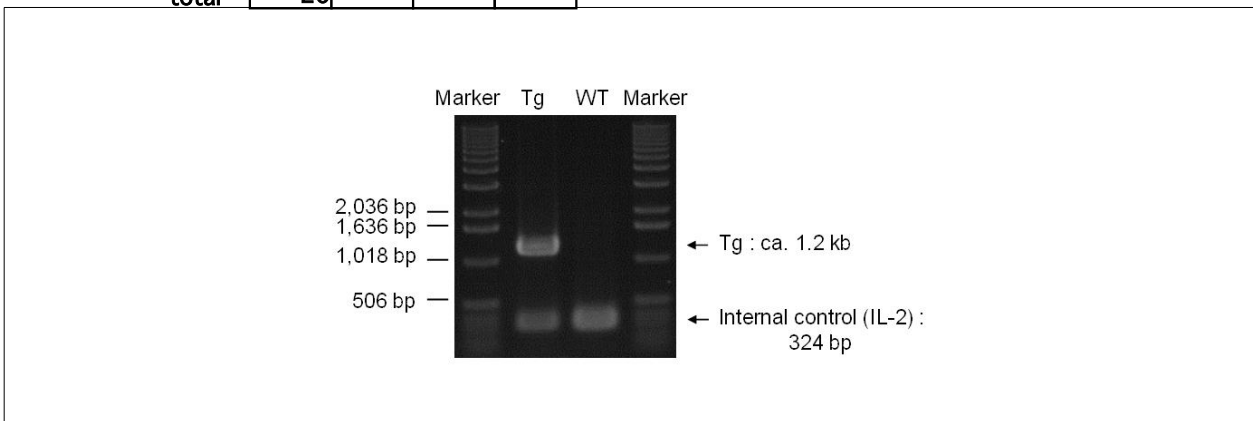
## Reaction Components

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/ $\mu$ 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>			

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



## 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. 1.2 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.