

RBRC 09481

Strain name C57BL/6J-Tg(CAG-NRAS\*G12V,lacZ)

Primer	primer_name	primer_seq	length	
1	5CAG	CCTACAGCTCCTGGCAACGTGC	23	RIKEN BRC
2	exon 5	ATCTTGTTACATCACCACACAT	22	Blood, 106:1054-1062 (2005).
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	JAX Genotyping Protocol

**Reaction Components**

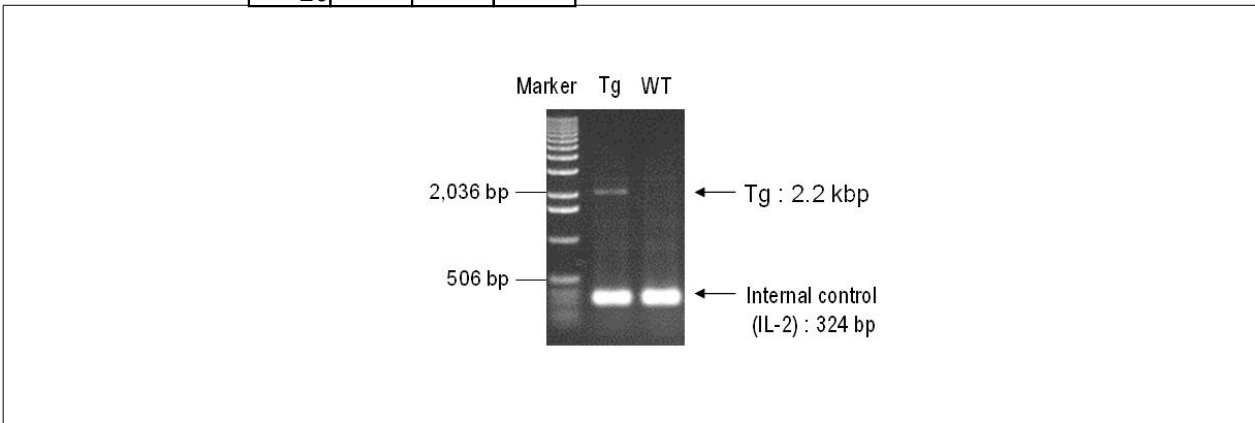
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

**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		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	240*	*Repeat for 30 cycles

Standard PCR



**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	2.2 kbp	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

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