

# PCR protocol

RBRC 09254

Strain name B6.Cg-Gt(ROSA)26Sor<tm1(CAG-kikGR)Kgwa>

Primer	primer_name	primer_seq	length	
1	ROSA short	CTTCCTCTGGGGGAGTCGTTTTA	23	Depositor's primer
2	RV	GCTCACAAGACCTTAGGTCAG	21	RIKEN BRC
3	hKikGR1-590R	TGGTCCACGAAGTGGTAGTCGGGC	24	Depositor's primer

### Reaction Components

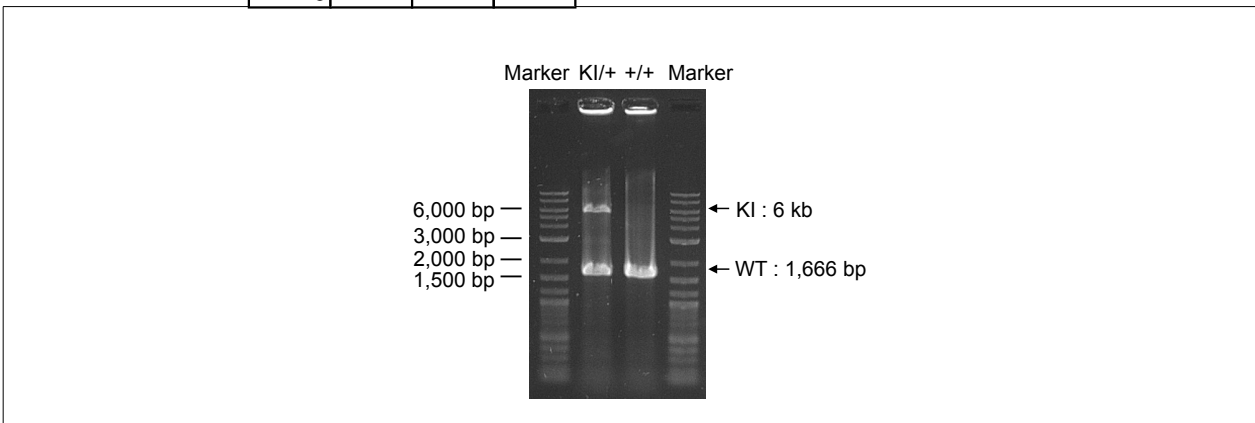
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

### Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	420*	*Repeat for 30 cycles

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8			
PCR buffer	2 X	10.0			
dNTP	2.0 mM	4.0			
primer 1	10 pmol/μl	1.5			
primer 2	10 pmol/μl	0.5			
primer 3	10 pmol/μl	1.0			
DNA		1.0			
Taq	1 U/μl	0.2			
<b>total</b>		<b>20</b>			

### Standard PCR



### Marker

2-Log DNA Ladder (NEB, Cat. N3200)

### Gel

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

### Comment

PCR products	primer_set	product_size	product
primer 1	primer 2	1,666 bp	WT
primer 1	primer 3	6.0 kb	KI
primer 1	primer 3	2.8 kb	delta stop (After cre mediated excision)

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