Sheet ID PS_07535
Release date : 3/7/2018
Print date : 3/7/2018

PCR protocol

RBRC 06002 **Strain name** ICR.Cg-Gt(ROSA)26Sor<tm1(Hes1,EGFP)Imayo>

Primer	primer_name	primer_seq	length		
1	R26F2	AAAGTCGCTCTGAGTTGTTAT	21		Nature Genetics 21, 70-71 (1999).
2	oIMR0316	GGAGCGGGAGAAATGGATATG	21		JAX genotyping protocol/Nature Genetics 21, 70 – 71 (1999).
3		TGCCCTTCGCCTCTTCTCCATGATA	25		Development, 135(15):2531-2541 (2008).

Reaction Components

Enzyme

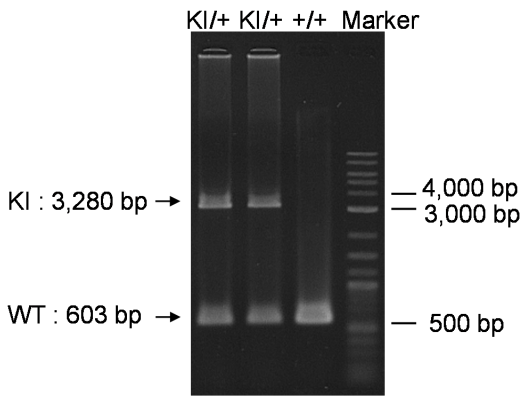
TOYOBO KOD FX (Code.No : KFX-101)

	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	1.0			
primer 2	10 pmol/μl	1.0			
primer 3	10 pmol/μl	1.0			
DNA		1.0			
Taq	1 U/μl	0.2			
	total	20			

Reaction Conditions

step	temp (℃)	time (sec)		note
1	94	120		
2	98	10	*	
3	60	30	*	
4	68	240	*	*Repeat for 30 cycles

Standard PCR



Marker

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

Gel

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

PCR products	primer_set	product_size	product
primer 1	primer 2	603 bp	WT
primer 1	primer 3	3,280 bp	KI

Comment

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