PCR protocol

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RBRC 09256 **Strain name** B6.Cg-Gt(ROSA)26Sor<tm1.1(CAG-kikGR)Kgwa>

Primer	primer_name	primer_seq	length		
1	ROSA short	CTTCCTCTGGGGGAGTCGTTTTA	23		Depositor's primer
2	RV	GCTCACAAGACCTTAGGTCAG	21		RIKEN BRC
3	ROSA splice acceptor	TGGTCCACGAAGTGGTAGTCGGGC	24		Depositor's primer

Reaction Components

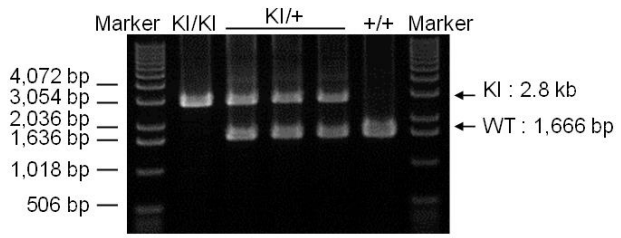
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

	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	1.5			
primer 2	10 pmol/μl	0.5			
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
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	1,666 bp	WT
primer 1	primer 3	2.8 kb	KI

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