PCR protocol

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RBRC 05160 **Strain name** B6;129S6-Gt(ROSA)26Sor<tm1.1(CAG-eNpHR3/EYFP)Imayo>

Primer	primer_name	primer_seq	length		Reference
1	R26F2	AAAGTCGCTCTGAGTTGTTAT	21		Depositor's primer
2	RV	GCTCACAAGACCTTAGGTCAG	21		RIKEN BRC
3	LFcheck-HR-rv	TGAAGGGCCACGGCACTCTCGGTC	24		Depositor's primer

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	4
H2O		1.6			
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	0.4			
	total	20			

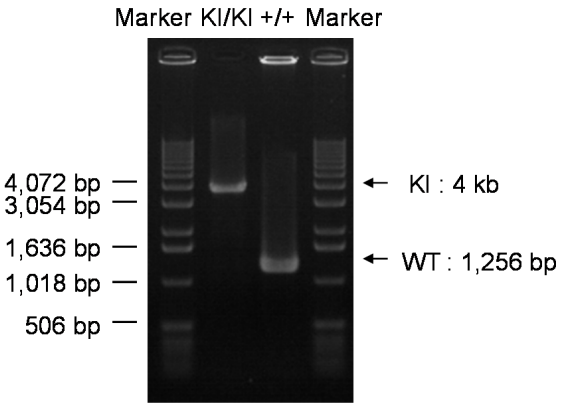
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	300*	*Repeat for 30 cycles

Marker 1 kb DNA ladder (Invitrogen, Cat. 15615-024)

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



primer_set		product_size	product (allele type)	
primer 1	primer 2	1,256 bp	WT	+/+ (wild-type):1,256 bp
primer 1	primer 3	4 kb	KI	KI/KI (homozygous): 4 kb

Comment Standard PCR

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