

RBRC 02657

Strain name B6. 129P2-Gt (ROSA) 26Sor<tm1 (NLS-lacZ) Ito>

Primer	primer_name	primer_seq	length		Reference
1	R26F2	AAAGTCGCTCTGAGTTGTTAT	21		Nature Genetics 21, 70-71 (1999).
2	RV	GCTCACAAAGACCTTAGGTCAG	21		RIKEN BRC
3	lacZ-R	CGCATCGTAACCGTGCATCTGCC	23		RIKEN BRC

Reaction Components

Enzyme

Reaction Conditions

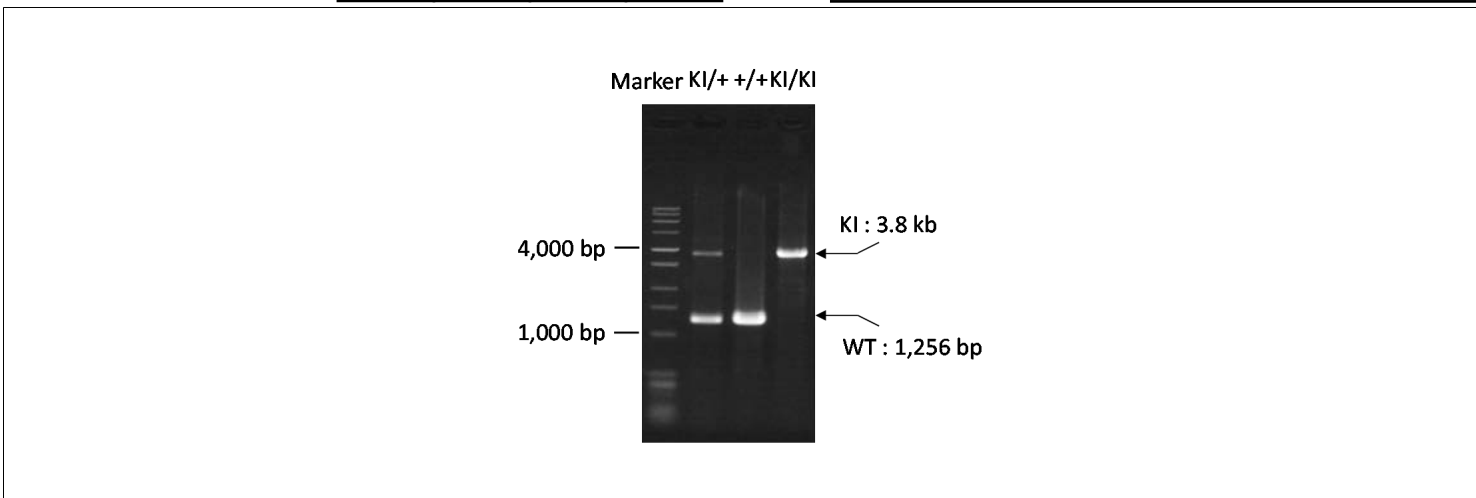
step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	300*	*Repeat for 30 cycles

Reagent	Conc	Reaction 1	2	3	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			

(μL)

Marker

Gel



primer_set	product_size	product (allele type)
primer 1	primer 2	1,256 bp WT
primer 1	primer 3	3.8 kb KI
primer 1	primer 3	1.0 kb del (After cre mediated excision)

Comment [Standard PCR](#)

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

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Primer	primer_name	primer_seq	length	Reference
1	olMR0315	GCGAAGAGTTTGTCTCAACC	21	JAX Genotyping Protocol
2	olMR0316	GGAGCGGGAGAAATGGATATG	21	JAX Genotyping Protocol
3	R26F2	AAAGTCGCTCTGAGTTGTTAT	21	Depositor's primer

Reaction Components

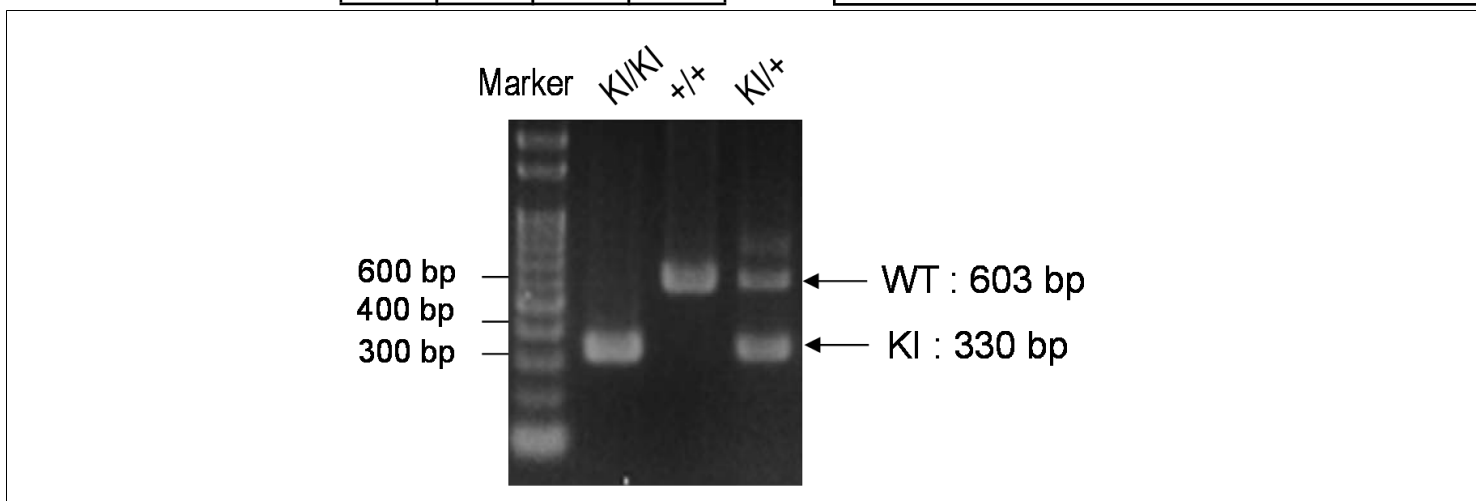
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

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

Reagent	Conc	Reaction 1	2	3	4	(μL)
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				

Marker Gene Ladder 100 (Wako, Cat. 316-06951)
Gel 2% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)
primer 1	primer 3	603 bp WT
primer 1	primer 2	330 bp KI

Comment Standard PCR

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1	olMR0315	GCGAAGAGTTTGTCTCAACC	21	JAX Genotyping Protocol
2	olMR0316	GGAGCGGGAGAAATGGATATG	21	JAX Genotyping Protocol
3	R26F2	AAAGTCGCTCTGAGTTGTTAT	21	Depositor's primer

Reaction Components

Enzyme TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

Reagent	Conc	Reaction 1	2	3	4
H2O		3.8			
PCR buffer	2 X GC I	10.0			
dNTP	2.5 mM	2.0			
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		0.2			
total		20			

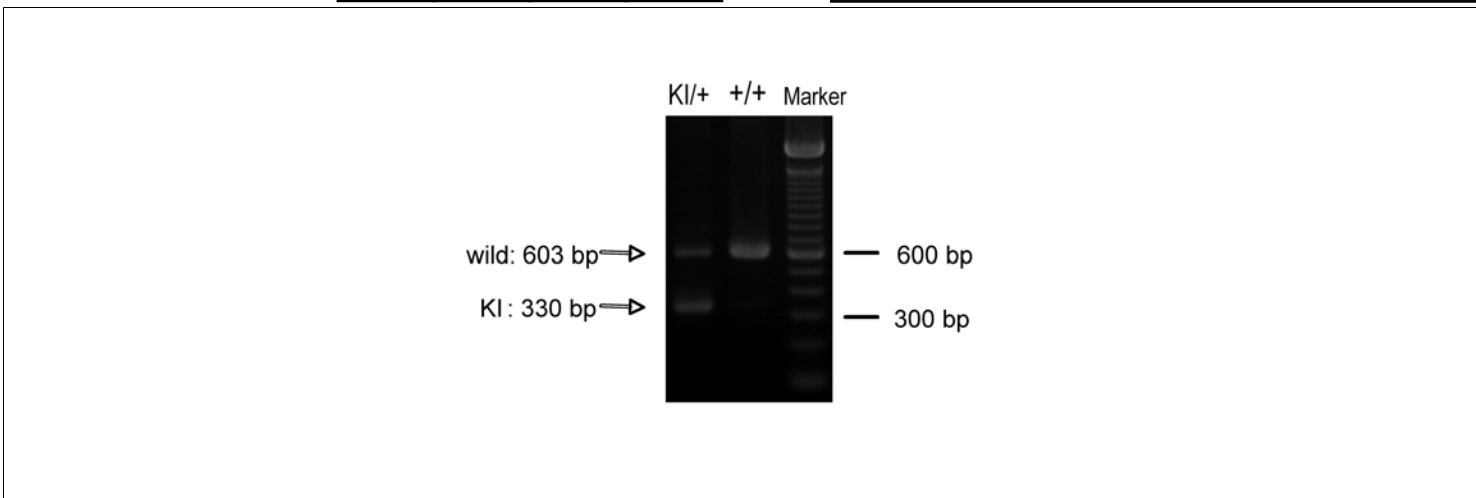
(μL)

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	60	30*	
4	72	60*	*Repeat for 30 cycles
5	72	300	

Marker 100 bp ladder (Invitrogen, Cat. 15628-050)

Gel 2% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)	
primer 1	primer 3	330 bp	KI
primer 2	primer 3	603 bp	WT

Comment Standard PCR