

RBRC 09256

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

Primer	primer_name	primer_seq	length	Reference
1	ROSA short	CTTCCTCTGGGGGAGTCGTTTAA	23	Depositor's primer
2	ROSA splice acceptor	CATCAAGGAAACCCTGGACTACTG	24	Development, 136, 4011-4020 (2009).
3	olMR0316	GGAGCGGGAGAAATGGATATG	21	JAX genotyping protocol/Nature Genetics 21, 70 - 71 (1999).

Reaction Components

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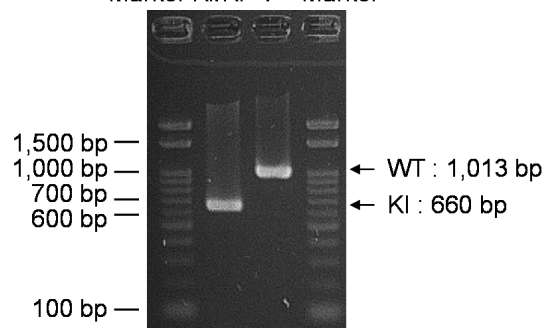
Reaction Conditions

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	(μ L)
H2O		1.8				
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/ μ l	1.0				
primer 2	10 pmol/ μ l	1.0				
primer 3	10 pmol/ μ l	1.0				
DNA		1.0				
Taq	1 U/ μ l	0.2				
total		20				

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

Marker KI/KI +/- Marker



primer_set	product_size	product (allele type)
primer 1	primer 3	1,013 bp
primer 1	primer 2	660 bp

 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	ROSA short	CTTCCTCTGGGGGAGTCGTTTAA	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)

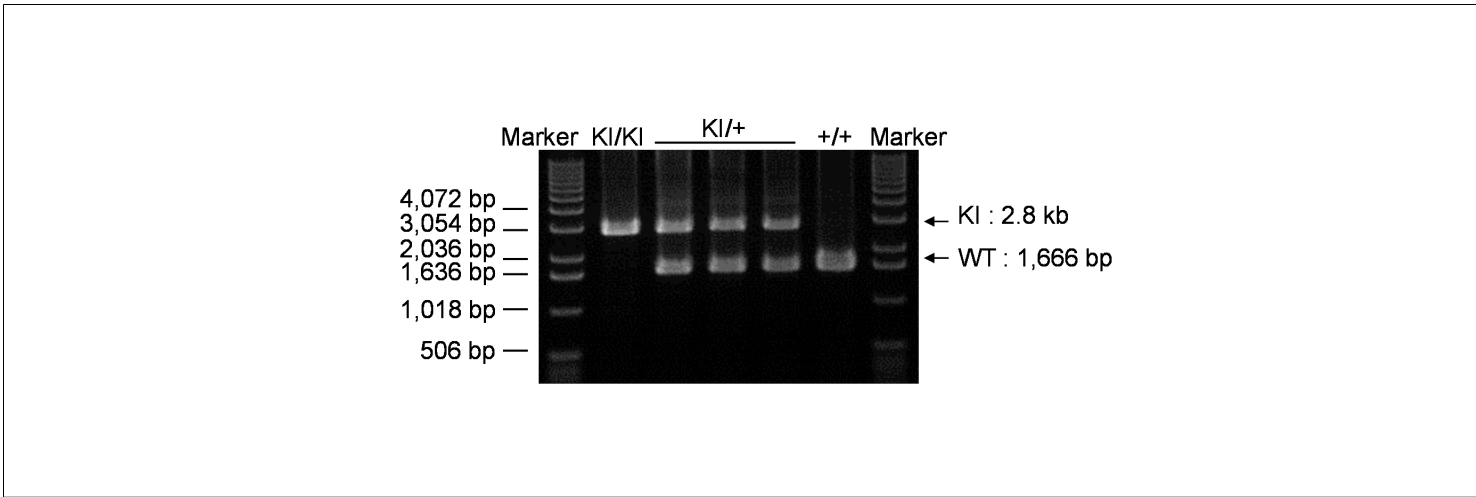
Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	240*	*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	1.5				
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				

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	1,666 bp	WT
primer 1	primer 3	2.8 kb	KI

Comment Standard PCR

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