

RBRC 09552

Strain name B6;129-Actb<tm1(tetO-GCaMP6)Kftnk>/Kftnk

Primer	primer_name	primer_seq	length	Reference
1	WT_Fw	ATTTCTGAATGGCCCAGGTCTGAG	24	RIKEN BRC
2		GGATTTGGTCATGAACCTTAGCC	23	RIKEN BRC
3	intronLow	AAGGCAGGATGATGACCAGGATGT	24	Depositor's primer

### Reaction Components

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

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			
<b>total</b>		20			

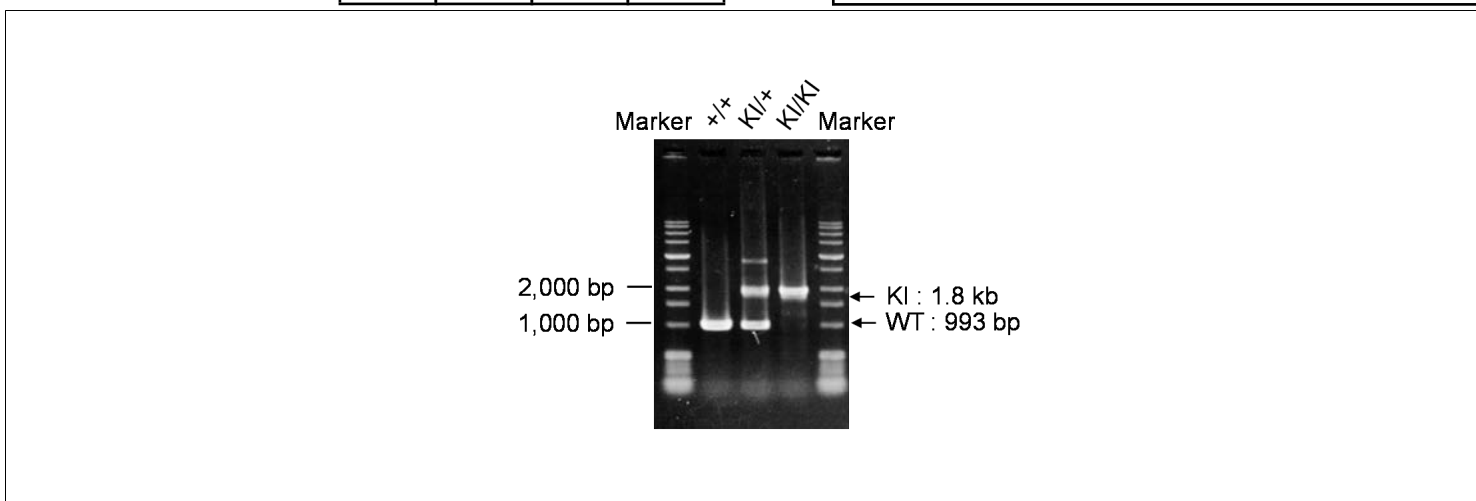
(μL)

### Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	180*	*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	993 bp WT
primer 1	primer 3	1.8 kb KI

**Comment** Standard PCR

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

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1	WT_Fw	ATTTCTGAATGGCCAGGTCTGAG	24	RIKEN BRC
2	WT_Rv	CTGCTCTGGTGTCTGTGTTACCTG	24	RIKEN BRC
3	intronLow	AAGGCAGGATGATGACCAGGATGT	24	Depositor's primer

**Reaction Components**

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

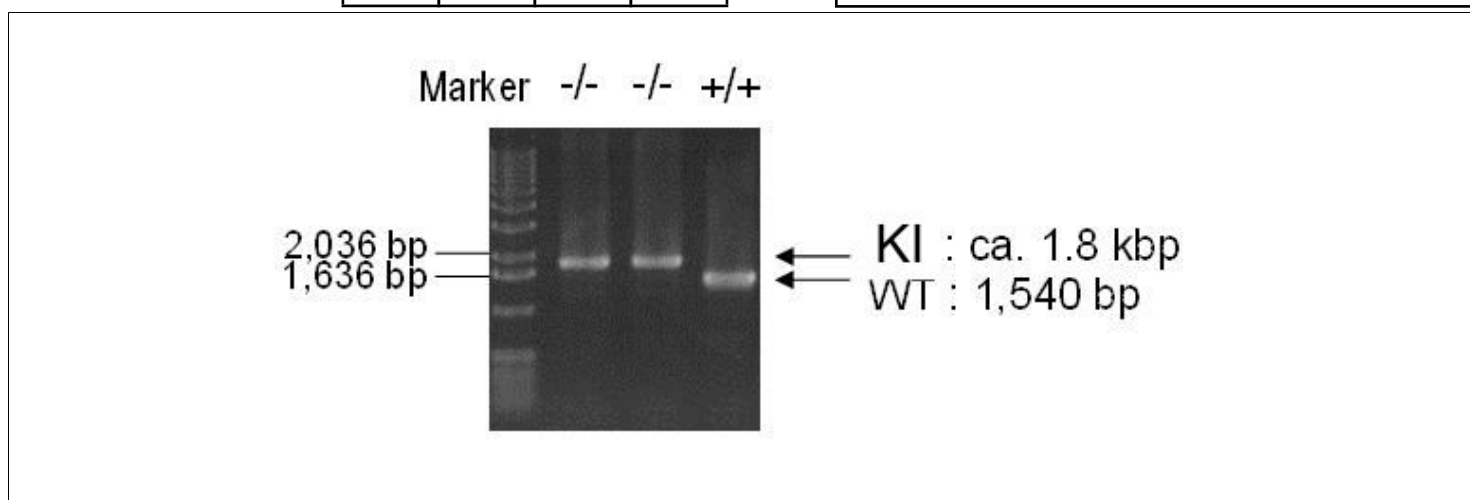
**Reaction Conditions**

step	temp (°C)	time (sec)	note
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3	60	30*	
4	68	120*	*Repeat for 30 cycles

Reagent	Conc	Reaction 1	2	3	4
H2O		1.8			
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dNTP	2.0 mM	4.0			
primer 1	10 pmol/µl	1.0			
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primer 3	10 pmol/µl	1.0			
DNA		1.0			
Taq	1 U/µl	0.2			
<b>total</b>		20			

(µL)

**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	1,540 bp WT
primer 1	primer 3	ca. 1.8 kbp KI

**Comment** Standard PCR

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