PCR protocol

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RBRC 10250 **Strain name** B6.129-Klre1<tm1.1Tgi>

Primer	primer_name	primer_seq	length		Reference
1	J03KO1	GATGGATGAAGCACCTGTAAC	21		Blood 104, 781-783 (2004).
2	J03KO3	TCAGAAACCCATCAGACCAACC	22		Blood 104, 781-783 (2004).
3		GCAGAAGAACGGCATCAAGGTG	22		RIKEN BRC

Reaction Components

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

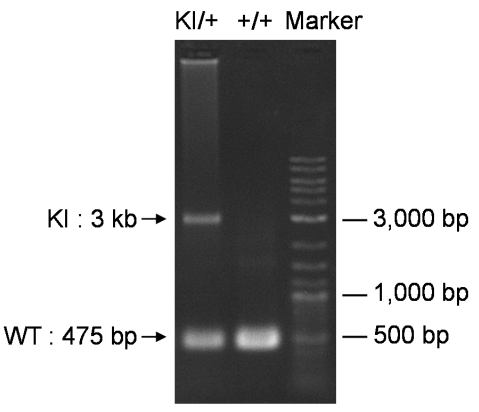
Reagent	Conc	Reaction 1	2	3	4	(μL)
H2O		1.8				
PCR buffer	2 X	10.0				
dNTP	2 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

step	temp (℃)	time (sec)		note
1	94	120		
2	98	10	*	
3	60	30	*	
4	68	240	*	*Repeat for 30 cycles

Marker 2-Log DNA Ladder (NEB, Cat. N3200)

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



primer_set		product_size	product (allele type)	
primer 1	primer 2	475 bp	WT	+/+ : 475 bp
primer 2	primer 3	3 kb	KI	KI/+ : 475 bp, 3 kb

Comment Standard PCR

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