PCR protocol

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RBRC 05152 **Strain name** B6;129S6-Gt(ROSA)26Sor<tm1.2(CAG-eNpHR2/EYFP)Imayo>

Primer	primer_name	primer_seq	length		Reference
1	R26F2	AAAGTCGCTCTGAGTTGTTAT	21		Nature Genetics 21, 70-71 (1999).
2	oIMR0316	GGAGCGGGAGAAATGGATATG	21		JAX genotyping protocol/Nature Genetics 21, 70 - 71 (1999).
3	LFcheck-HR-rv	TGAAGGGCCACGGCACTCTCGGTC	24		Depositor's primer

Reaction Components

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

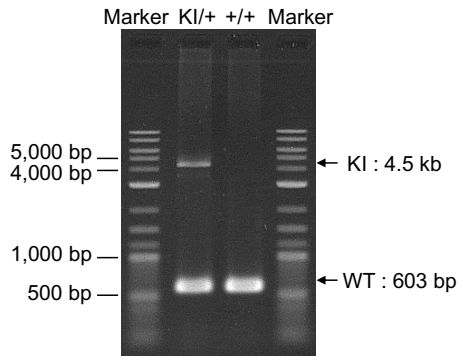
Reagent	Conc	Reaction 1	2	3	4	
H2O		1.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	30	*
4	68	360	* *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	603 bp	WT	+/+ (wild-type): 603 bp KI/+ (heterozygous): 603 bp and 4.5 kb
primer 1	primer 3	4.5 kb	KI	

Comment Standard PCR

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