PCR protocol

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RBRC 10843 **Strain name** B6.Cg-Rit1<tm1(A57G)Tumg>

Primer	primer_name	primer_seq	length		Reference
1	mRit1-Fw2	GCTGTGAATAAGATACATGACCGACC	26		EBioMedicine, 42:43-53 (2019).
2	mRit1-Rv2	CCATCTCATTGGCTCCTAAGTTG	23		EBioMedicine, 42:43-53 (2019).

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	4
H2O		2.8			
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			
DNA		1.0			
Taq	1 U/μl	0.2			
total		20			

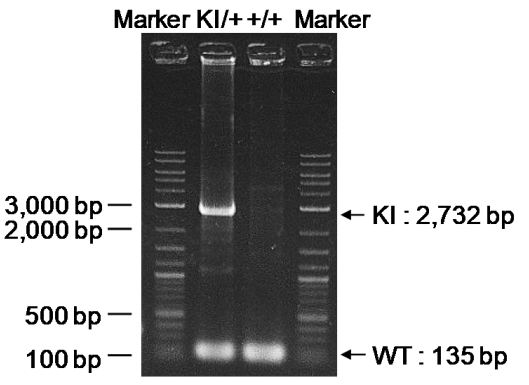
(μL)

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	135 bp	WT	+/+ (wild-type): 135 bp KI/+ (heterozygous): 135 bp and 2,732 bp KI/KI (homozygous): 2,732 bp
primer 1	primer 2	2,732 bp	KI	

Comment Standard PCR

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