PCR protocol

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RBRC 02590

Strain name Jam-B(IRES-β geo) (Acc. No. CDB0413K)

Primer	primer_name	primer_seq	length		Reference
1	exon 5	TGTGGTGGAGCTACGATGCCAG	22		RIKEN BRC
2	intron 5	CCTTTTTTCTCACGATGACAGACAGAAG	28		RIKEN BRC
3	neo_F	GCAGCGCATCGCCTTCTATCGC	22		RIKEN BRC

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 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			

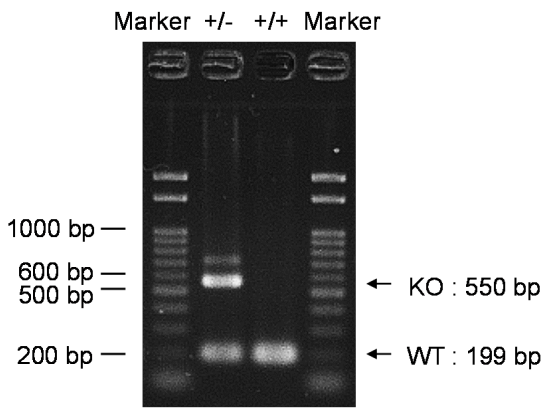
(μL)

Reaction Conditions

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

Marker Gene Ladder 100 (Wako, Cat. 316-06951)

Gel 2% agarose in 1 X TAE (100 V, 30 min)



primer_set		product_size	product (allele type)
primer 1	primer 2	199 bp	WT
primer 1	primer 3	550 bp	KO

+/+ (wild-type): 199 bp
+/- (heterozygous): 199 bp and 550 bp

Comment Standard PCR

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