PCR protocol

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RBRC 11516 **Strain name** C57BL/6J-Iars<em2(V79L)Nsas>

Primer	primer_name	primer_seq	length		Reference
1	mt-79L	TCCCACCCGAATCTTCTGTCATG	23		RIKEN BRC
2	Iars-F	CGTCCTGGATGTCTGCCATT	20		Depositor's primer
3		CCTTTGATGCCCAGTGTCTTATCG	24		RIKEN BRC
4	wt-79V	CGCTCACCAGAGCGGGTTTCATG	23		RIKEN BRC

Reaction Components

Enzyme QIAGEN Multiplex PCR Kit (Cat. No. 206143)

Reagent	Conc	Reaction 1	2	3	4
primer 1	10 pmol/μl	1.0			
primer 2	10 pmol/μl	1.0			
primer 3	10 pmol/μl	1.0			
primer 4	10 pmol/μl	1.0			
Master Mix	2 X	5.0			
DNA		1.0			
	total	10			

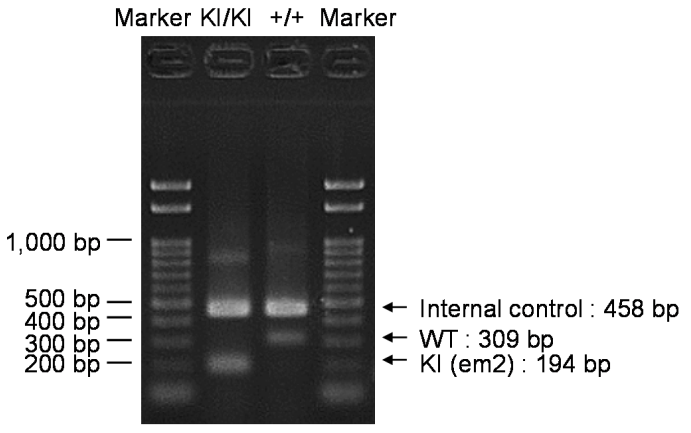
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	95	900	
2	94	30	*
3	60	90	*
4	72	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 2	primer 3	458 bp	Internal control	+/+ (wild-type): 458 bp and 309 bp KI/KI (homozygous): 458 bp and 194 bp
primer 1	primer 2	194 bp	KI	
primer 3	primer 4	309 bp	WT	

Comment Allele specific PCR (ASP)