PCR protocol

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RBRC 11515 **Strain name** C57BL/6J-Iars<em1Nsas>

Primer	primer_name	primer_seq	length		Reference
1	wt-ex2	TTACGAGATACGCTCACCAGAGC	23		RIKEN BRC
2		CCTTTGATGCCCAGTGTCTTATCG	24		RIKEN BRC
3	Iars-F	CGTCCTGGATGTCTGCCATT	20		Depositor's primer
4	mt-ex2	TTCTGTCAACGTGAAACCCGCTG	23		RIKEN BRC

Reaction Components

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

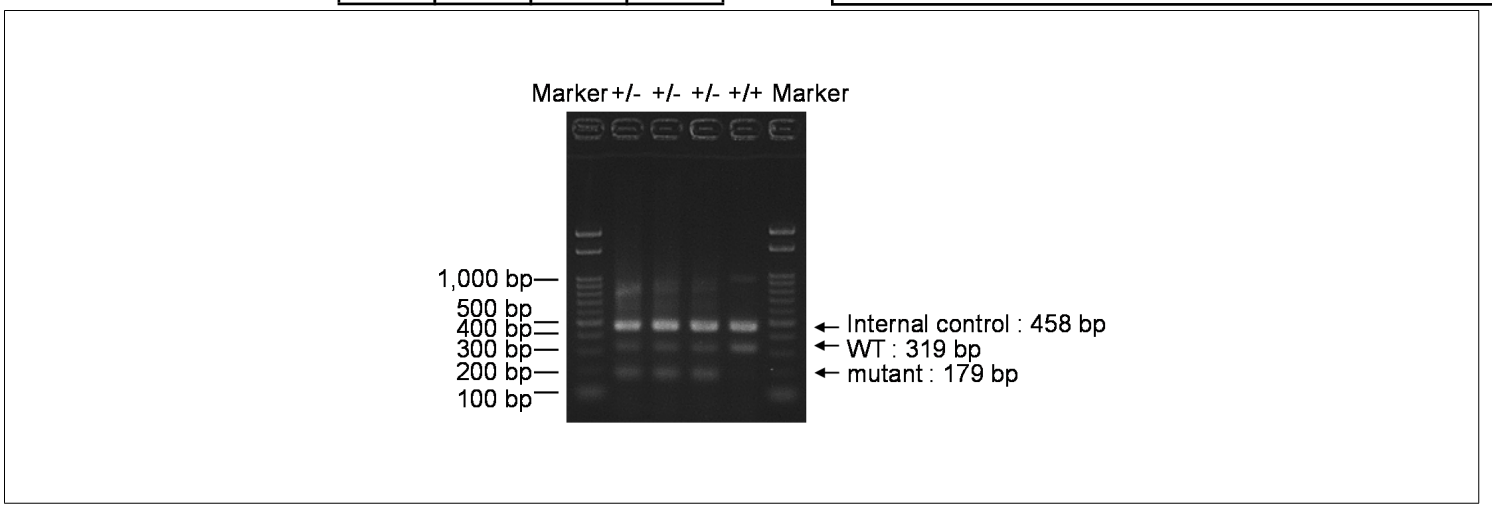
Reagent	Conc	Reaction 1	2	3	4	
primer 1	10 pmol/μl	1.0				(μL)
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
primer 4	10 pmol/μl	1.0				
Master Mix	2 X	5.0				
DNA		1.0				
	total	10				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	95	900	
2	94	30	*
3	65	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 319 bp +/- (heterozygous): 458 bp and 179 bp
primer 1	primer 2	319 bp	WT	
primer 3	primer 4	179 bp	mutant (em1)	

Comment Separated PCR