PCR protocol

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RBRC 10695

Strain name C57BL/6J-Prkdc<em1Nsas>

Primer	primer_name	primer_seq	length		Reference
1	WT	CGTCTCTTCTTAGCCAAGCTTGTTA	25		RIKEN BRC
2	Prkdc-R	AGTTCACTCCCAATGCCAGA	20		Depositor's primer
3	Prkdc-F	TTTACTGCCACCGCCATGAT	20		Depositor's primer
4	MT	GTTAGATACCTCTTCTGTATTAATAACACA	30		RIKEN BRC

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	4
H2O		2.0	2.0		
primer 1	10 pmol/μl	1.0	0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	0	1.0		
primer 4	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Master Mix	2 X	5.0	5.0		
	total	10	10		

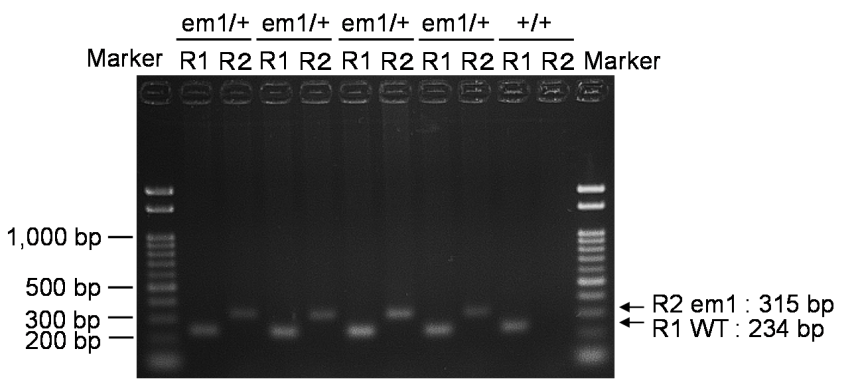
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	95	900	
2	94	30	*
3	60	90	*
4	72	60	* *Repeat for 25 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	234 bp	WT
primer 3	primer 4	315 bp	em1

Reaction 1 +/+: 234 bp
em1/+: 234 bp

Reaction 2 +/+: none
em1/+: 315 bp

Comment Allele specific PCR (ASP)