PCR protocol

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RBRC 11286 **Strain name** C57BL/6J-Lta4h<em1Tyoko>

Primer	primer_name	primer_seq	length		Reference
1	F_2nd	CTCACGCTGAACCGATGAGA	20		Cell. Mol. Immunol., (2020).
2	WT	CTTCAGTGGCTCACTCCTGAATA	23	Replaced due to mistake	RIKEN BRC
3	mt	CGGATGCTGCTTCCCTGATTC	21	Replaced due to mistake	RIKEN BRC
4	R_2nd	TTTCAGGGCCATTCACTGAGTC	22	Replaced due to mistake	Cell. Mol. Immunol., (2020).

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			

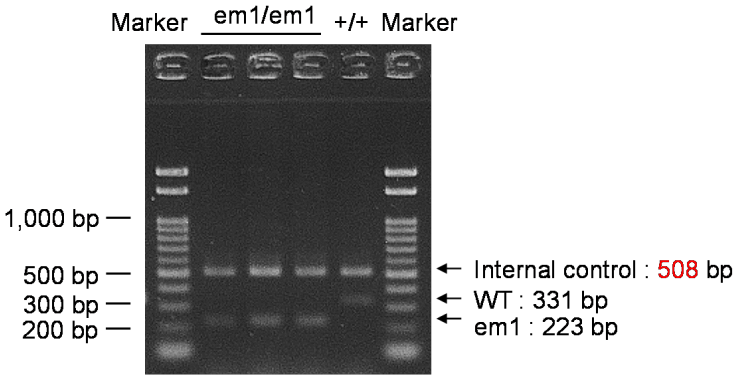
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	95	900	
2	94	30	*
3	60	90	*
4	72	60	* *Repeat for 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 4	508 bp	Internal control
primer 2	primer 4	331 bp	WT
primer 1	primer 3	223 bp	em1

+/+ (wild-type): 508 bp and 331 bp
em1/em1 (homozygous): 508 bp and 223 bp

Comment Allele specific PCR (ASP)