PCR protocol

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RBRC 11394

Strain name C57BL/6J-Was<em1(I292T)Imsmt>

Primer	primer_name	primer_seq	length		Reference
1		CCATAGCCCTCTTGGATACTTG	22		Depositor's primer
2	WT	CCTGGTCCTCAATAAAATCGTAGACG	26		RIKEN BRC
3	MT	GACGCAGAGACCTCCAAGCTTGC	23		RIKEN BRC
4		GGACCAGAGAAGAAGGTGCTTA	22		Depositor's primer

Reaction Components

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

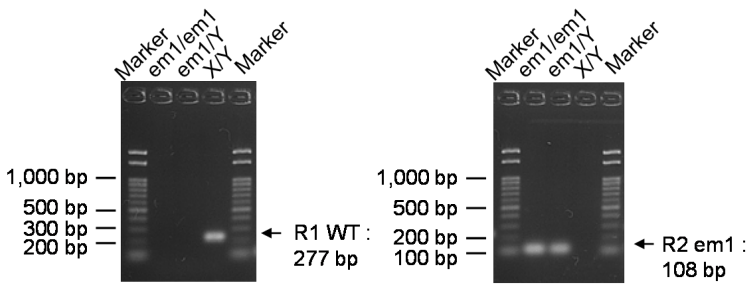
Reagent	Conc	Reaction 1	2	3	4	
H2O		2.0	2.0			(μL)
Master Mix	2 X	5.0	5.0			
primer 1	10 pmol/μl	1.0	0			
primer 2	10 pmol/μl	1.0	0			
primer 3	10 pmol/μl	0	1.0			
primer 4	10 pmol/μl	0	1.0			
DNA		1.0	1.0			
	total	10	10			

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 1	primer 2	277 bp	WT
primer 3	primer 4	108 bp	em1

Comment Allele specific PCR (ASP)