PCR protocol

Sheet ID PS_08071
Release date : 8/17/2022
Print date : 8/17/2022

RBRC 11120

Strain name A/J-12<SM> Iah1<em1Kobam>

Primer	primer_name	primer_seq	length		Reference
1	Iah1_short_F	TTTCTACCATGTCGCTGTGC	20		PLoS One, 15(5):e0233087 (2020).
2	Iah1_short_R	AGGCGGACCCTTTAAGCTC	19		PLoS One, 15(5):e0233087 (2020).

Reaction Components

Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reagent	Conc	Reaction 1	2	3	4
H2O		2.8			
PCR buffer	2 X	10.0			
dNTP	2.0 mM	4.0			
primer 1	10 pmol/μl	1.0			
primer 2	10 pmol/μl	1.0			
DNA		1.0			
Taq	1 U/μl	0.2			
	total	20			

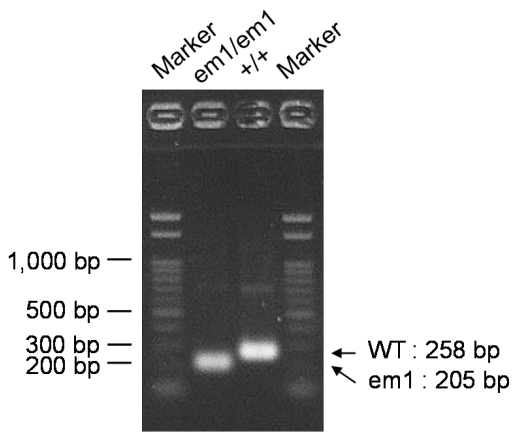
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10 *	
3	60	30 *	
4	68	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	258 bp	WT
primer 1	primer 2	205 bp	em1

Comment Standard PCR

Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.

PCR protocol

Sheet ID PS_08070
Release date : 8/17/2022
Print date : 8/17/2022

RBRC 11120

Strain name A/J-12<SM> Iah1<em1Kobam>

Primer	primer_name	primer_seq	length		Reference
1	D12Mit36-1	CATCACACCAGGTTTAGAATTTT	23		Depositor's primer
2	D12Mit36-2	AGGCACTCTTCTGACCTCCA	20		Depositor's primer
3	D12Mit6-1	ATGCTCGACATCAACCTTGG	20		Depositor's primer
4	D12Mit6-2	TATCTGTGTGGCTGGAACGA	20		Depositor's primer
5	D12Mit17-1	TCCGAGTGTTGCTTCTCCTT	20		Depositor's primer
6	D12Mit17-2	CCTTAGATGCTCAAGGCTGG	20		Depositor's primer

Reaction Components

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

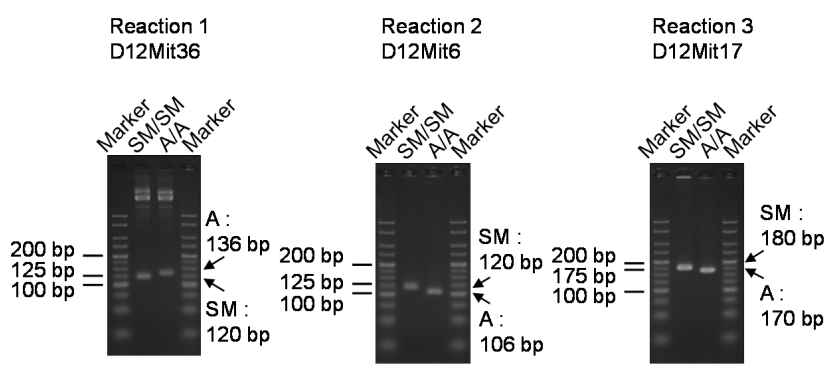
Reagent	Conc	Reaction 1	2	3	4	
H2O		2.0	2.0	2.0		(μL)
Master Mix	2 X	5.0	5.0	5.0		
primer 1	10 pmol/μl	1.0	0	0		
primer 2	10 pmol/μl	1.0	0	0		
primer 3	10 pmol/μl	0	1.0	0		
primer 4	10 pmol/μl	0	1.0	0		
primer 5	10 pmol/μl	0	0	1.0		
primer 6	10 pmol/μl	0	0	1.0		
DNA		1.0	1.0	1.0		
	total	10	10	10		

Reaction Conditions

step	temp (℃)	time (sec)	note
1	95	900	
2	94	30*	
3	60	90*	
4	72	60*	*Repeat for 30 cycles

Marker 25bp DNA Step Ladder (Wako, Cat. 292-69001)

Gel 4% Nusieve in 0.5 X TBE (100 V, 60 min)



primer_set		product_size	product (allele type)
primer 1	primer 2	120 bp, 136 bp	SM, A
primer 3	primer 4	120 bp, 106 bp	SM, A
primer 5	primer 6	180 bp, 170 bp	SM, A

Comment Separated PCR