PCR protocol

Sheet ID PS_07890
Release date : 8/27/2019
Print date : 8/27/2019

RBRC 00532 Strain name C57BL/10SnSlc-H-2<aw18>/H-2<b>

Primer	primer_name	primer_seq	length		Reference
1	exon 2	CATATGCTAAATGGTAAGGGTTAGG	25		RIKEN BRC
2	intron 8_b	TACCCCCAGGGAATCACCTGCTCG	24		RIKEN BRC
3	exon 41	AACTGAGGCACTAGGCTCATCC	22		RIKEN BRC

Reaction Components

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

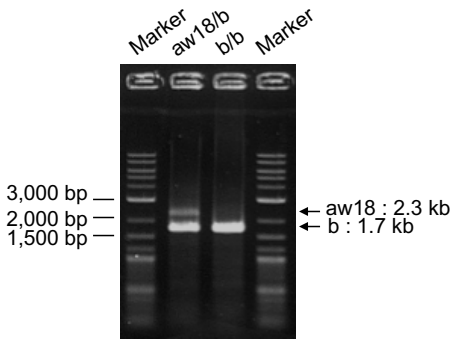
Reagent	Conc	Reaction 1	2	3	4	
H2O		1.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	30	*
4	68	180	* *Repeat for 30 cycles

Marker: 2-Log DNA Ladder (NEB, Cat. N3200)

Gel: 1% agarose in 1 X TAE (100 V, 30 min)



primer_set		product_size	product (allele type)
primer 1	primer 2	1.7 kb	H2-b
primer 1	primer 3	2.3 kb	H2-aw18

Comment Standard PCR

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

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PCR buffer	2 X	10.0	10.0			
dNTP	2.0 mM	4.0	4.0			
primer 1	10 pmol/μl	1.0	1.0			
primer 2	10 pmol/μl	1.0	0			
primer 3	10 pmol/μl	0	1.0			
DNA		1.0	1.0			
Taq	1 U/μl	0.2	0.2			
	total	20	20			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
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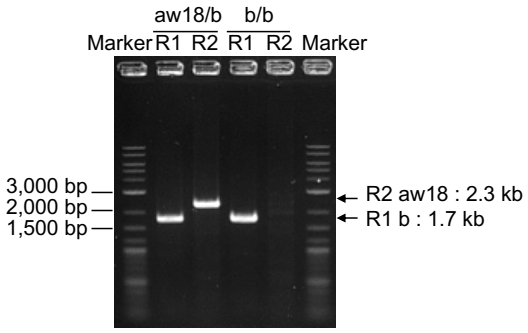

primer_set		product_size	product (allele type)
primer 1	primer 2	1.7 kb	H2-b
primer 1	primer 3	2.3 kb	H2-aw18

Comment Separated PCR

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