PCR protocol

Sheet ID PS_05810
Release date : 5/21/2014
Print date : 7/11/2014

RBRC 00897 Strain name B6.Cg-Aicda<tm1Hon> (N10)/HonRbrc

Primer	primer_name	primer_seq	length		
1	227	CAACGTGGCGTCCAAACAGGCACTTCCG	28		Depositor's primer
2	AID-R1	CCAGGCTTTGAAAGTTCTTTCACG	24		Depositor's primer
3	M11	CTTGGGTGGAGAGGCTATTCGGCT	24		

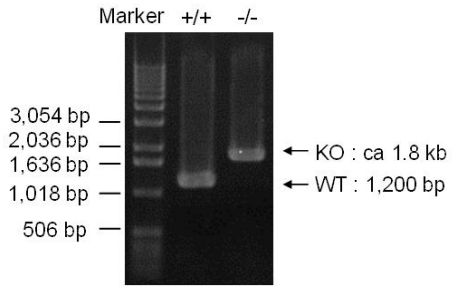
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8			
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			
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	120	* *Repeat for 30 cycles



Marker

1 kb DNA ladder (Invitrogen, Cat. 15615-024)

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 2	1,200 bp	WT
primer 2	primer 3	ca 1.8 kb	KO

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

PCR protocol

Sheet ID PS_01595
Release date : 11.19.2008
Print date : 7/11/2014

RBRC 00897 Strain name B6.Cg-Aicda<tm1Hon> (N10)/HonRbrc

Primer	primer_name	primer_seq	length		
1	227	CAACGTGGCGTCCAAACAGGCACTTCCG	28		Depositor's primer
2	AID-R1	CCAGGCTTTGAAAGTTCTTTCACG	24		Depositor's primer
3	G3	GGGCCAGCTCATTCCTCCACTC	22		Depositor's primer

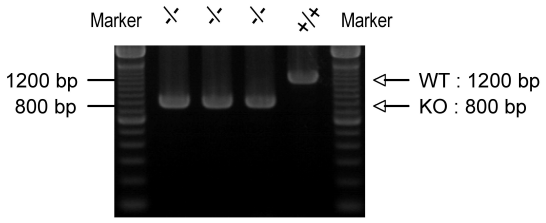
Reaction Components

Enzyme: TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		3.8			
PCR buffer	2 X GC I	10.0			
dNTP	2.5 mM	2.0			
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		0.2			
	total	20			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30	*
3	60	30	*
4	72	90	* *Repeat for 30 cycles
5	72	300	



Marker

100 bp ladder (Invitrogen, Cat. 15628-050)

Gel

2% agarose in 1 X TAE (100 V, 30 min)

PCR products

primer_set		product_size	product
primer 1	primer 2	1200 bp	WT
primer 2	primer 3	800 bp	KO

Comment

PCR protocol

Sheet ID PS_00684
Release date : 7/13/2006
Print date : 7/11/2014

RBRC 00897 Strain name B6.Cg-Aicda<tm1Hon> (N10)/HonRbrc

Primer	primer_name	primer_seq	length		
1	G3	GGGCCAGCTCATTCCTCCACTC	22		Depositor's primer
2	AID-R1	CCAGGCTTTGAAAGTTCTTTCACG	24		Depositor's primer
3	175	GGTCCCAGTCTGAGATGTAGCGTAGG	26		Depositor's primer
4	227	CAACGTGGCGTCCAAACAGGCACTTCCG	28		Depositor's primer

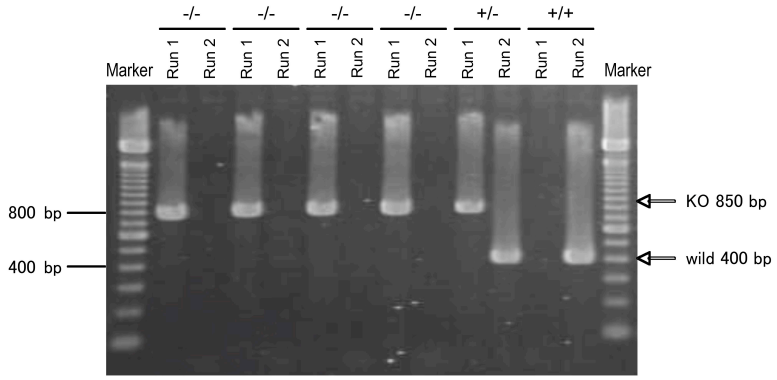
Reaction Components

Enzyme: TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		4.8	4.8		
PCR buffer	2 X GC I	10.0	10.0		
dNTP	2.5 mM	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		
Taq		0.2	0.2		
	total	20	20		

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	300	
2	94	30	*
3	60	30	*
4	72	90	* *Repeat for 32 cycles
5	72	300	



Marker

100 bp ladder (Invitrogen, Cat. 15628-050)

Gel

2% agarose in 1 X TAE (100 V, 30 min)

PCR products

primer_set		product_size	product
primer 1	primer 2	850 bp	KO
primer 3	primer 4	400 bp	WT

Comment