PCR protocol

Sheet ID PS_05113
Release date : 9/25/2013
Print date : 9/25/2013

RBRC 02781 Strain name B6.Cg-Cd8a<a> Tg(Tcra2C,Tcrb2C)1Dlo

Primer	primer_name	primer_seq	length		
1	NV beta8.2	GAGGCTGCAGTCACCCAAAGCCCAA	25		Depositor's primer
2	Jβ2-ANTI	TGAGAGCTGTCTCCTACTATCGATT	25		EMBO J., 12(4):1671-1680(1993).
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24		JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25		JAX Genotyping Protocol

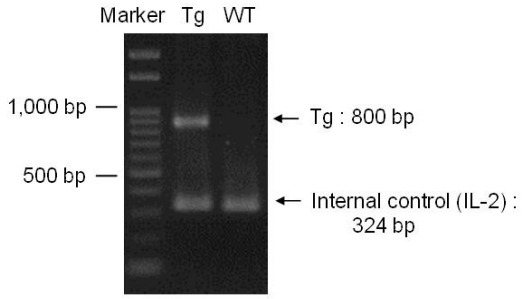
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		0.8			
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			
primer 4	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	60	* *Repeat for 30 cycles



Marker

Gene Ladder 100 (Wako, Cat. 316-06951)

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 2	800 bp	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

Comment

PCR protocol

Sheet ID PS_05463
Release date : 9/25/2013
Print date : 9/25/2013

RBRC 02781 Strain name B6.Cg-Cd8a<a> Tg(Tcra2C,Tcrb2C)1Dlo

Primer	primer_name	primer_seq	length		
1	D6Mit16-1	AGGCTTTGATGCTGTATAGG	20		
2	D6Mit16-2	CACCAGGAACGTAAGTGAGC	20		

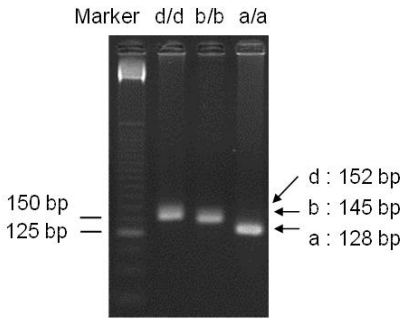
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		2.0			
Master Mix	2 X	5.0			
Primer-1	10 pmol/μl	1.0			
Primer-2	10 pmol/μl	1.0			
DNA		1.0			
	total	10			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	95	900	
2	94	30	*
3	60	90	*
4	72	60	* *Repeat for 30 cycles
5	60	1800	



Marker

25 bp DNA ladder (Invitrogen, Cat. 10597-011)

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 2	128 bp	a
primer 1	primer 2	145 bp	b
primer 1	primer 2	152 bp	d

Comment