PCR protocol

RBRC 02873 **Strain name** B6.CS Ngu1053-Tg(MSMG01-414M7)4Ebi

Primer	primer_name	primer_seq	length		
1	SP6arm.F	GTCTGGCACAGGGTCAGTTT	20		Depositor's primer
2	B6.SMsslp1FR	CCCCACACACACACCTCA	18		Depositor's primer
3	B6.SMsslp1F	ATTGCTGCTGATCCACACAG	20		Depositor's primer
4	SP6arm.R	CCGTCGACATTTAGGTGACA	20		Depositor's primer
5	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	Internal control (IL-2)	JAX Genotyping Protocol
6	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	Internal control (IL-2)	JAX Genotyping Protocol

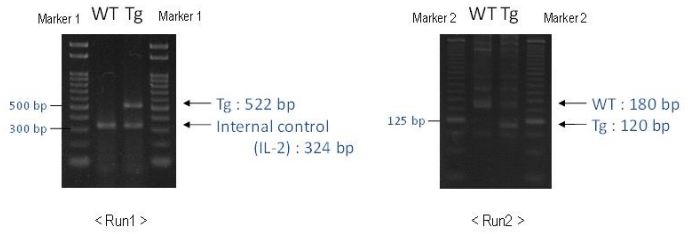
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		2.8	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	0	1.0		
primer 3	10 pmol/μl	0	1.0		
primer 4	10 pmol/μl	1.0	0		
primer 5	10 pmol/μl	1.0	0		
primer 6	10 pmol/μl	1.0	0		
DNA		1.0	1.0		
Taq		0.2	0.2		
	total	20	20		

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	150	
2	94	30	*
3	58	30	*
4	72	60	* *Repeat for 30 cycles
5	72	300	



Marker

marker Run1: Gene Ladder 100 (Wako, Cat. 316-06951), marker

Gel

Run1:2% agarose in 1 X TAE (100 V, 30 min)Run2:4% Nusieve in 0.5

PCR products

primer_set		product_size	product
primer 1	primer 4	522 bp	Tg
primer 2	primer 3	180 bp	WT
primer 2	primer 3	120 bp	Tg
primer 5	primer 6	324 bp	Internal control (IL-2)

Comment

PCR protocol

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Primer	primer_name	primer_seq	length		
1	left	TTTGTTGTTTGAGGGGTTGG	20		Depositor's primer
2	right	TTGAGTTAAAGAACAGGAGGACTACA	26		Depositor's primer
3		ATGATCAAAACATTACTCCCTTCC	24		Depositor's primer
4		ACAAAGTCAGGAGAAATGTGCA	22		Depositor's primer

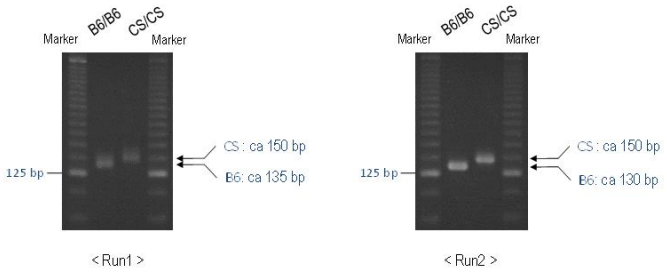
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		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		
Master Mix	2 X	5.0	5.0		
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
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	ca 135 bp	B6
primer 1	primer 2	ca 130 bp	CS
primer 3	primer 4	ca 130 bp	B6
primer 3	primer 4	ca 150 bp	CS

Comment