PCR protocol

Sheet ID PS_05445
Release date : 1/23/2014
Print date : 1/23/2014

RBRC 05939 Strain name B6.Cg-Tg(Mt1-RET)304Ina Hr<hr>

Primer	primer_name	primer_seq	length		
1	00187_F	GTGCCTGCTGCCACATATGTGG	22		RIKEN BRC
2	00187_R	GCATCCGAGGGCATGCGTGCAC	22		RIKEN BRC
3	00187_FM	CCACACTCGGCGCGCCAGTC	20		RIKEN BRC

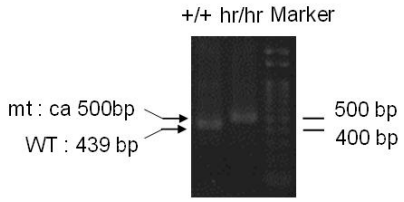
Reaction Components

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		0.2			
	total	20			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	55	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	439 bp	WT
primer 2	primer 3	ca 500 bp	mutant

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

PCR protocol

Sheet ID PS_05446
Release date : 1/23/2014
Print date : 1/23/2014

RBRC 05939 Strain name B6.Cg-Tg(Mt1-RET)304Ina Hr<hr>

Primer	primer_name	primer_seq	length		
1	mt1	GTCCTCTAAGCGTCACCACGACTTC	25		RIKEN BRC
2	RFP/RET(~1500)	ACTCGGGGAGGCGTTC	16		Depositor's primer
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24		JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25		JAX Genotyping Protocol

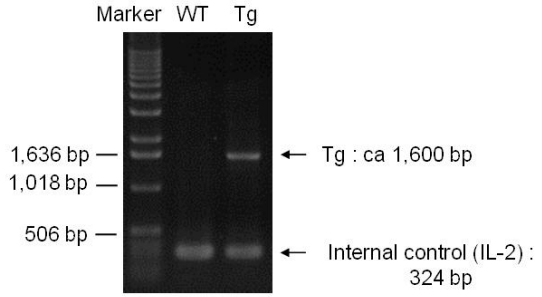
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		2.9			
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			
primer 4	10 pmol/μl	1.0			
DNA		1.0			
Taq	5 U/μl	0.1			
	total	20			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30	*
3	60	30	*
4	72	120	* *Repeat for 30 cycles
5	72	300	



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	ca 1600 bp	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

Comment