PCR protocol

Sheet ID PS_07530
Release date : 1/5/2018
Print date : 1/5/2018

RBRC 01295

Strain name CAT-RhoA DN/4-18

Primer	primer_name	primer_seq	length		
1	5CAG	CCTACAGCTCCTGGGCAACGTGC	23		RIKEN BRC
2	primer 2	TCACAAGACAAGGCAACCAGATT	23		Depositor's primer
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24		JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25		JAX Genotyping Protocol

Reaction Components

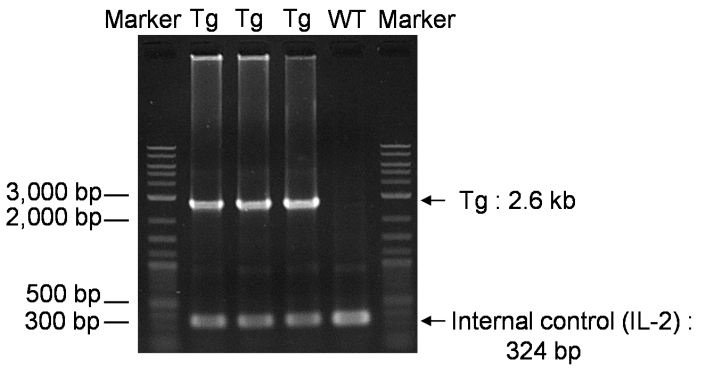
Enzyme: TOYOBO KOD FX (Code.No : KFX-101)

	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	68	240	*	*Repeat for 30 cycles

Standard PCR



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

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

PCR products	primer_set	product_size	product
primer 1	primer 2	2.6 kb	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

Comment

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

PCR protocol

Sheet ID PS_00076
Release date : 7/12/2005
Print date : 1/5/2018

RBRC 01295

Strain name CAT-RhoA DN/4-18

Primer	primer_name	primer_seq	length		
1	primer 1	ACTCATCTCAGAAGAGGATCTG	22		Depositor's primer
2	primer 2	TCACAAGACAAGGCAACCAGATT	23		Depositor's primer
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	Internal control (IL-2)	JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	Internal control (IL-2)	JAX Genotyping Protocol

Reaction Components

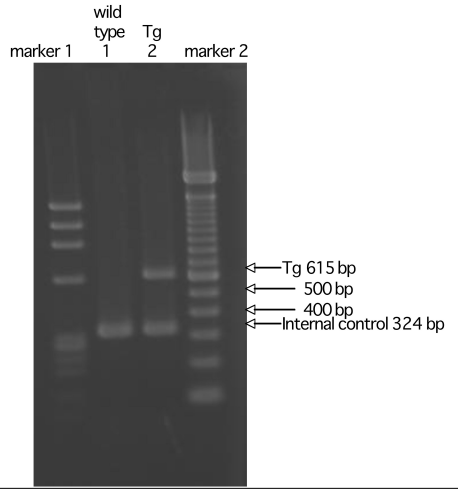
Enzyme: Roche Taq (Cat. No. BM1418432)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		15.3			
PCR buffer	10 X	2.5			
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		0.2			
	total	25			

Reaction Conditions

step	temp (℃)	time (sec)		note
1	94	300		
2	94	20	*	
3	58	30	*	
4	72	120	*	*Repeat for 30 cycles
5	72	300		

Standard PCR



Marker

marker 1: ϕX174 Hae III, marker2: 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	615 bp	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

Comment