PCR protocol

Sheet ID PS_07551
Release date : 8/31/2021
Print date : 8/31/2021

RBRC 01142

Strain name ICR.Cg-Tg(CAG-cat,-Notch1)1Ysa/YsaRbrc

Primer	primer_name	primer_seq	length		Reference
1	5CAG	CCTACAGCTCCTGGGCAACGTGC	23		RIKEN BRC
2	Notch1-WR1	ACAGACACACAGGGAACTTCACCC	24		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)

Reagent	Conc	Reaction 1	2	3	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			

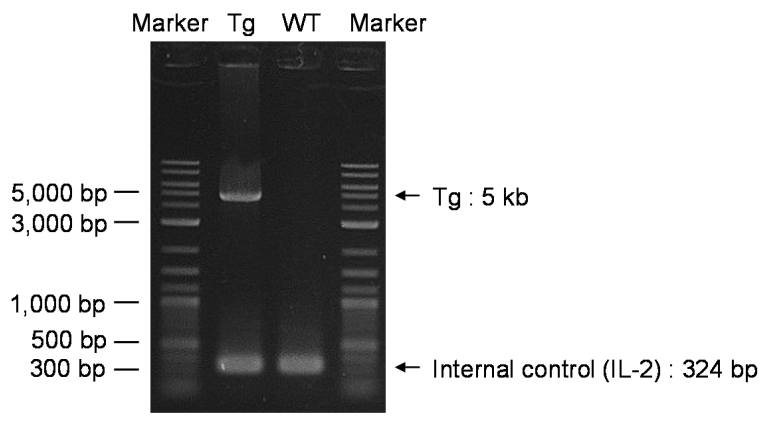
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	360*	*Repeat for 30 cycles

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

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



primer_set		product_size	product (allele type)
primer 1	primer 2	5 kb	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

Non-Tg : 324 bp
Tg : 324 bp, 5 kb

Comment

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

PCR protocol

Sheet ID PS_00003
Release date : 6/20/2006
Print date : 8/31/2021

RBRC 01142

Strain name CAG-CAT-Notch1

Primer	primer_name	primer_seq	length		Reference
1	CAT2	CAGTCAGTTGCTCAATGTACC	21		Depositor's primer
2	CAT3	ACTGGTGAAACTCACCCA	18		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)

Reagent	Conc	Reaction 1	2	3	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	5 U/μl	0.2			
	total	25			

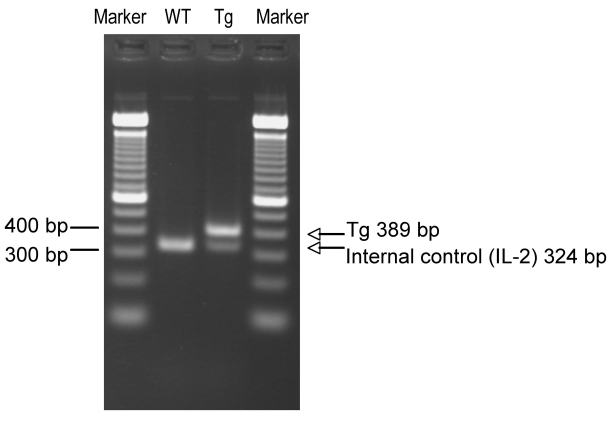
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	300	
2	94	30*	
3	55	30*	
4	72	60*	*Repeat for 35 cycles
5	72	300	

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

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



primer_set		product_size	product (allele type)
primer 1	primer 2	389 bp	Tg
primer 3	primer 4	324 bp	Internal control (IL-2)

Comment Generic PCR