PCR protocol

Sheet ID PS_05968
Release date : 5/6/2022
Print date : 5/6/2022

RBRC 05323

Strain name 129-Sirt1<tm1Mcby>

Primer	primer_name	primer_seq	length		Reference
1	Sir2 genofwd	TTCACATTGCATGTGTGTGG	20		Depositor's primer
2		TAGCCTGCGTAGTGTTGGTG	20		Depositor's primer
3		CGATCCTGCAAGCTCCGGATGC	22		Depositor's primer

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	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	1 U/μl	0.2			
	total	20			

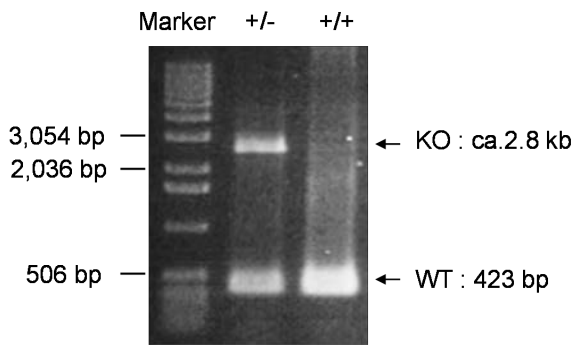
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	240*	*Repeat for 30 cycles

Marker: 1 kb DNA ladder (Invitrogen, Cat. 15615-024)

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



primer_set		product_size	product (allele type)
primer 1	primer 2	423 bp	WT
primer 1	primer 3	ca 2.8 kb	KO

Comment

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

PCR protocol

Sheet ID PS_05472
Release date : 5/22/2014
Print date : 5/6/2022

RBRC 05323

Strain name 129-Sirt1<tm1Mcby>

Primer	primer_name	primer_seq	length		Reference
1	Sir2 genofwd	TTCACATTGCATGTGTGTGG	20		Depositor's primer
2		TAGCCTGCGTAGTGTTGGTG	20		Depositor's primer
3		ATTTGGTAGGGACCCAAAGG	20		Depositor's primer

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	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	1 U/μl	0.2			
	total	20			

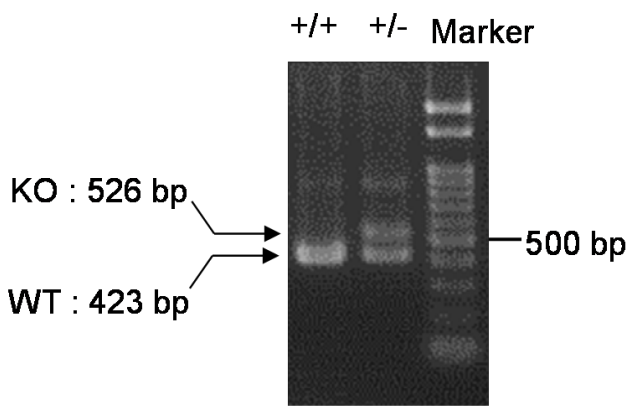
(μL)

Reaction Conditions

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

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

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



primer_set		product_size	product (allele type)
primer 1	primer 2	423 bp	WT
primer 1	primer 3	526 bp	KO

Comment

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

PCR protocol

Sheet ID PS_04623
Release date : 12/20/2012
Print date : 5/6/2022

RBRC 05323

Strain name 129-Sirt1<tm1Mcby>

Primer	primer_name	primer_seq	length		Reference
1	Sir2 genofwd	TTCACATTGCATGTGTGTGG	20		Depositor's primer
2		TAGCCTGCGTAGTGTTGGTG	20		Depositor's primer
3		ATTTGGTAGGGACCCAAAGG	20		Depositor's primer

Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

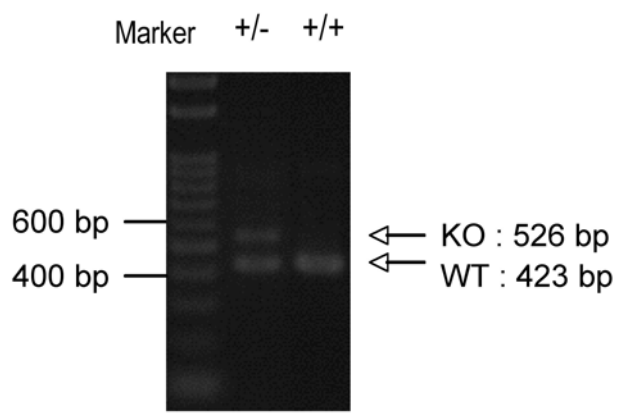
Reagent	Conc	Reaction 1	2	3	4	
H2O		12.3				(μL)
PCR buffer	10 X	2.0				
dNTP	2.5 mM	1.6				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
DNA		1.0				
Taq		0.1				
	total	20				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30	*
3	63	30	*
4	72	60	* *Repeat for 30 cycles
5	72	300	

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

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



primer_set		product_size	product (allele type)
primer 1	primer 2	423 bp	WT
primer 1	primer 3	526 bp	KO

Comment Standard PCR