

PCR protocol

RBRC 05323 Strain name 129-Sirt1 <tm1Mcpy>

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

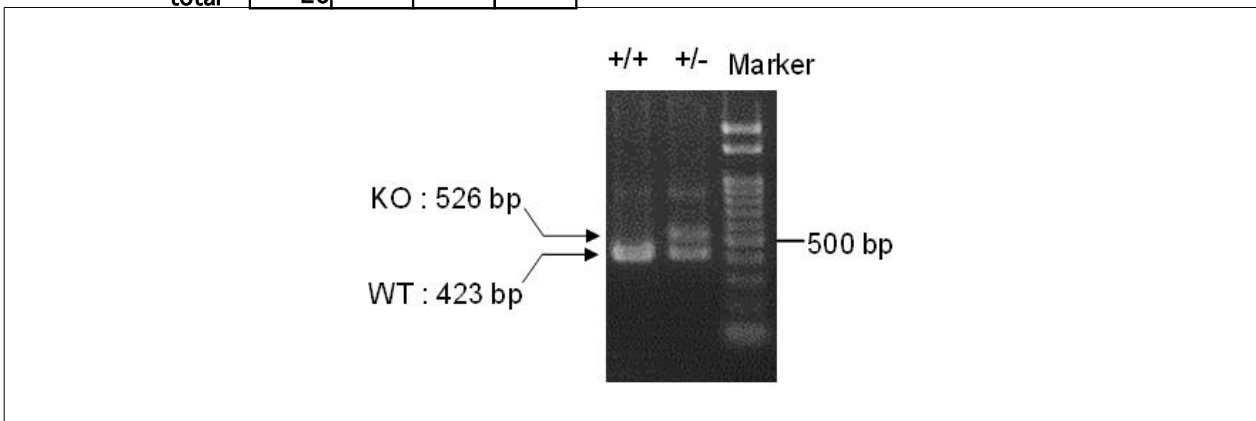
Reaction Components

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

Reaction Conditions

	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	1 U/μl	0.2			
total		20			

step	temp (°C)	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)

PCR products		product_size	product
primer 1	primer 2		
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.

