

RBRC 06467

Strain name C57BL/6-Tg(Prnp-Sirt1,Cryaa-EGFP)1Ksh

Primer	primer_name	primer_seq	length	Reference
1	MmSirt1 S	CAAGAGGTTGTTAATGAAGC	20	Mol. Brain., 7(1):62 (2014).
2	MmSirt1 AS	TTTCCTGTTGCCTTCAATCAGCTATCG	27	Mol. Brain., 7(1):62 (2014).
3		GGTGAGCATTCCAGCTGCTGACG	23	RIKEN BRC
4	GFP-Full-R	CTTGTACAGCTCGTCCATGCCGAG	24	RIKEN BRC
5	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24	JAX Genotyping Protocol
6	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25	JAX Genotyping Protocol

Reaction Components

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

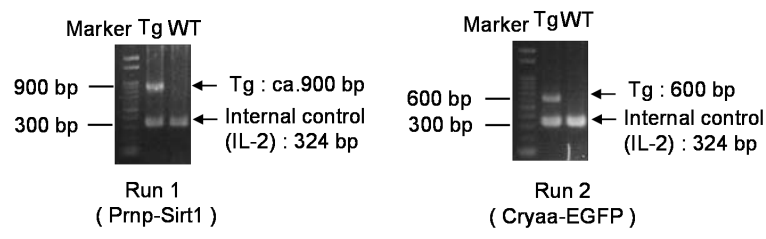
Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	90*	*Repeat for 30 cycles

Reagent	Conc	Reaction 1	2	3	4	(μL)
H2O		0.8	0.8			
PCR buffer	2 X	10.0	10.0			
dNTP	2.0 mM	4.0	4.0			
primer 1	10 pmol/μl	1.0	0			
primer 2	10 pmol/μl	1.0	0			
primer 3	10 pmol/μl	0	1.0			
primer 4	10 pmol/μl	0	1.0			
primer 5	10 pmol/μl	1.0	1.0			
primer 6	10 pmol/μl	1.0	1.0			
DNA		1.0	1.0			
Taq	1 U/μl	0.2	0.2			
total		20	20			

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	ca 900 bp	Tg(Prnp-Sirt1)
primer 3	primer 4	600 bp	Tg(Cryaa-EGFP)
primer 5	primer 6	324 bp	Internal control (IL-2)

Comment [Separated PCR](#)

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