Sirt1 Cas9-KO Strategy

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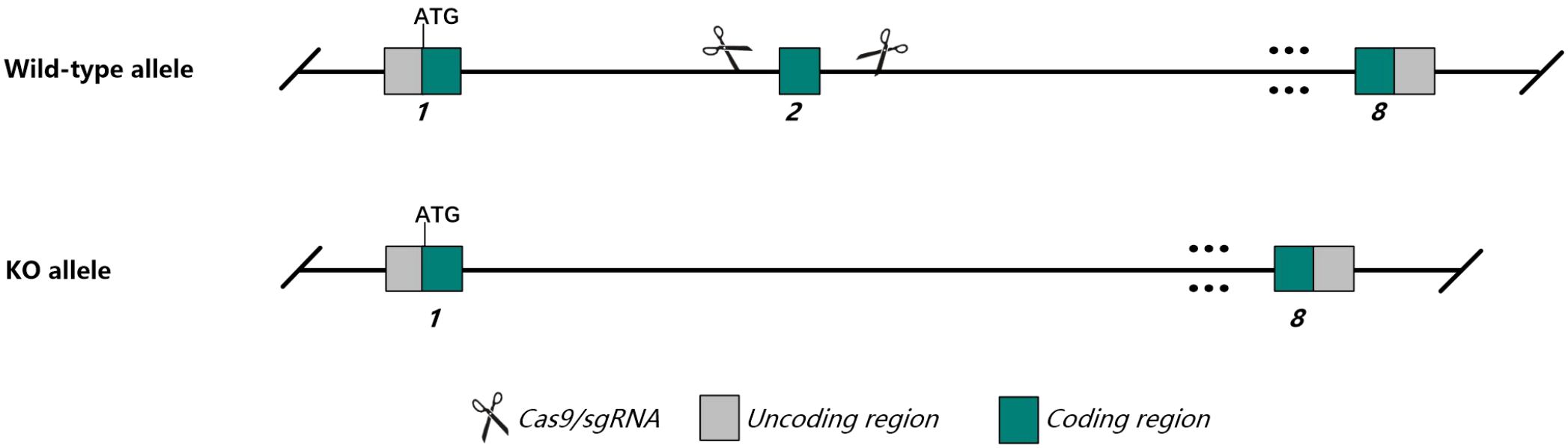
Project Overview

Project Name	***Sirt1***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Sirt1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Sirt1* gene has 7 transcripts. According to the structure of *Sirt1* gene, exon2 of *Sirt1-202* (ENSMUST00000105442.2) transcript is recommended as the knockout region.The region contains 242bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Sirt1* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6JGpt mice.Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

Notice

- According to the existing MGI data,Mice homozygous for a knock-out allele show embryonic and fetal lethality, abnormal embryogenesis, and abnormal cellular phenotypes of derived MEFs. Mice homozygous for other knock-out alleles may exhibit peri- and postnatal lethality and heart, mammary gland, eye, and reproductive system anomalies.

- The *Sirt1* gene is located on the Chr10. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.

- This Strategy is designed based on genetic information in existing databases.Due to the complexity of biological processes, all risk of the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

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Sirt1 sirtuin 1 [Mus musculus (house mouse)]

Gene ID: 93759, updated on 9-Apr-2019

Summary

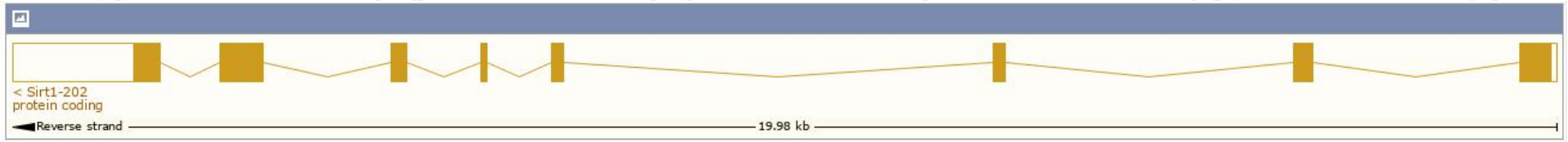
Official Symbol	Sirt1 provided by MGI
Official Full Name	sirtuin 1 provided byMGI
Primary source	MGI:MGI:2135607
See related	Ensembl:ENSMUSG00000020063
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AA673258, SIR2L1, Sir2, Sir2a, Sir2alpha
Summary	This gene encodes a member of the sirtuin family of proteins, characterized by their deacetylase activity and proposed role in longevity. The encoded protein regulates gene expression in a wide range of cell and tissue types through its NAD+-dependent deacetylation of histones, transcription factors and transcriptional coactivators. Brain-specific overexpression of this gene has been shown to result in increased median lifespan. Viability of homozygous knockout mice for this gene varies with strain background. Homozygous knockout mice of strains that do not exhibit embryonic lethality are sterile and have a reduced lifespan. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Sep 2015]
Expression	Ubiquitous expression in CNS E11.5 (RPKM 7.7), limb E14.5 (RPKM 5.8) and 26 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

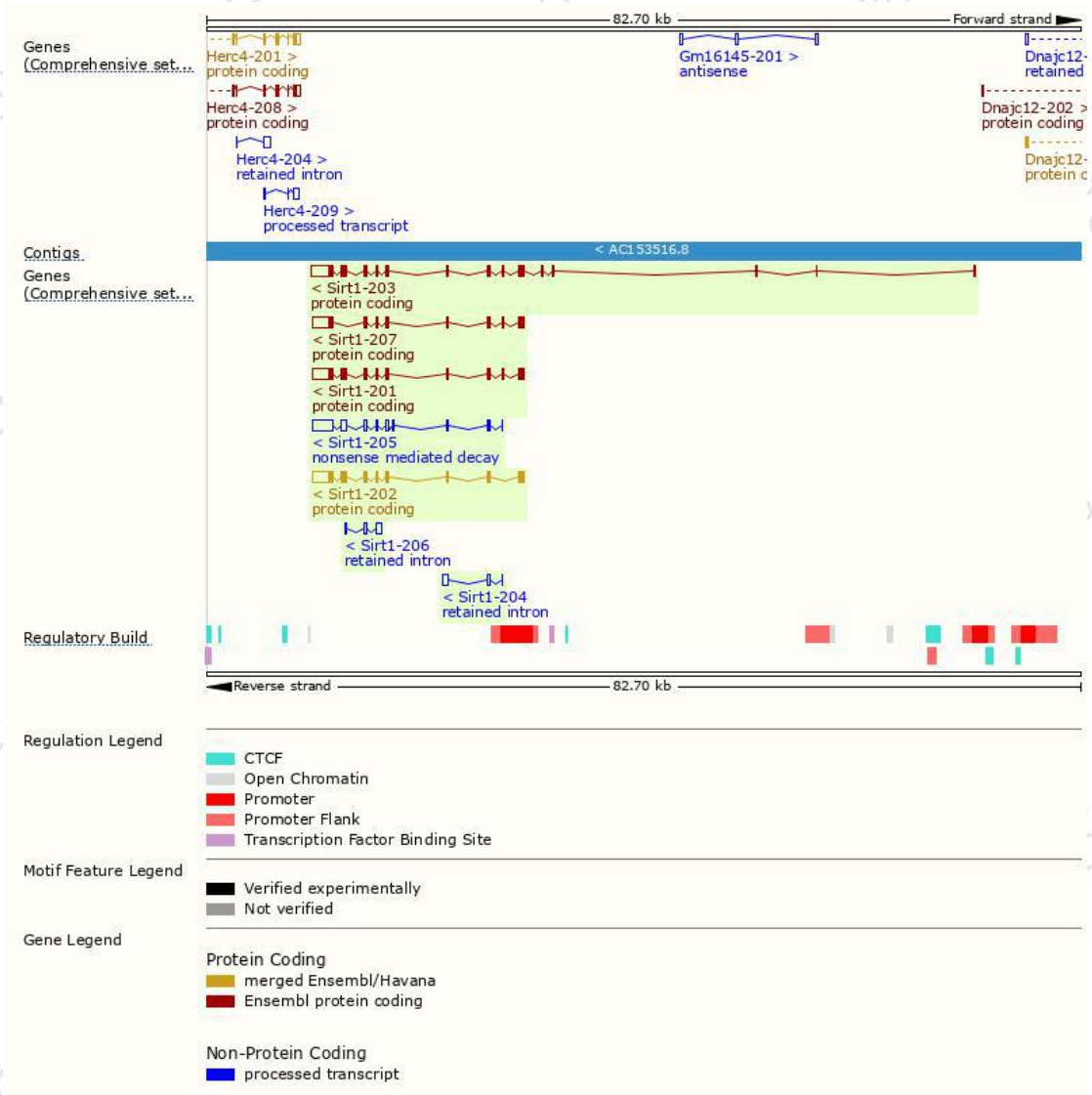
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Sirt1-203	ENSMUST00000120239.7	4430	737aa	Protein coding	CCDS23898	Q53Z05 Q923E4	TSL:5 GENCODE basic APPRIS P3
Sirt1-201	ENSMUST00000020257.12	3905	737aa	Protein coding	CCDS23898	Q53Z05 Q923E4	TSL:1 GENCODE basic APPRIS P3
Sirt1-202	ENSMUST00000105442.2	3740	698aa	Protein coding	CCDS48585	Q3UNI1	TSL:1 GENCODE basic APPRIS ALT2
Sirt1-207	ENSMUST00000177694.7	3353	553aa	Protein coding	-	Q923E4	TSL:5 GENCODE basic
Sirt1-205	ENSMUST00000146028.7	3458	140aa	Nonsense mediated decay	-	F6YAQ3	CDS 5' incomplete TSL:5
Sirt1-206	ENSMUST00000148516.1	842	No protein	Retained intron	-	-	TSL:3
Sirt1-204	ENSMUST00000131371.1	763	No protein	Retained intron	-	-	TSL:5

The strategy is based on the design of *Sirt1-202* transcript,The transcription is shown below

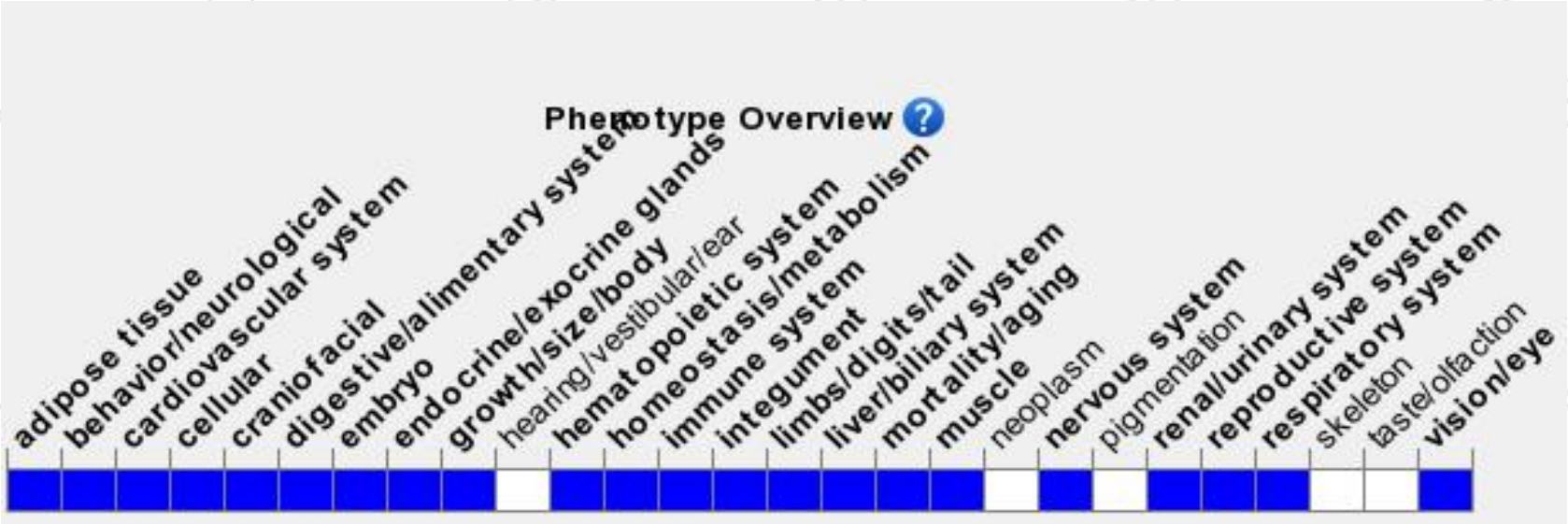


Genomic location distribution



Protein domain

Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

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If you have any questions, you are welcome to inquire.

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