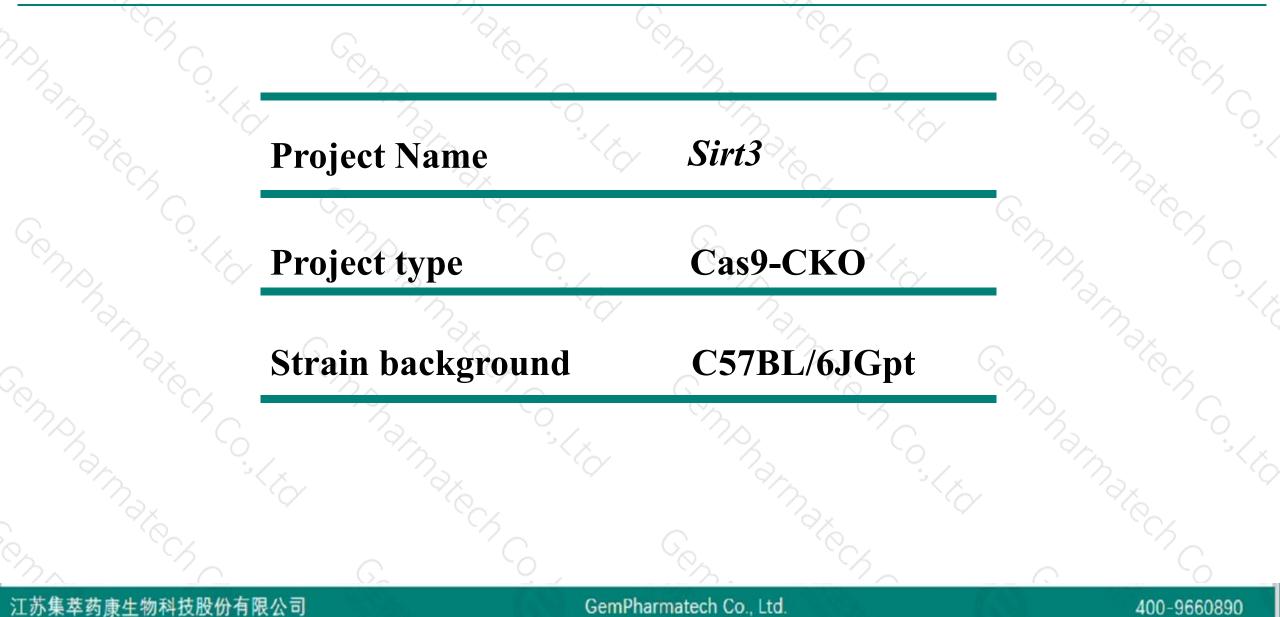
Sirt3 Cas9-CKO Strategy

Designer: Design Date: Huan Fan 2019-7-25

Project Overview

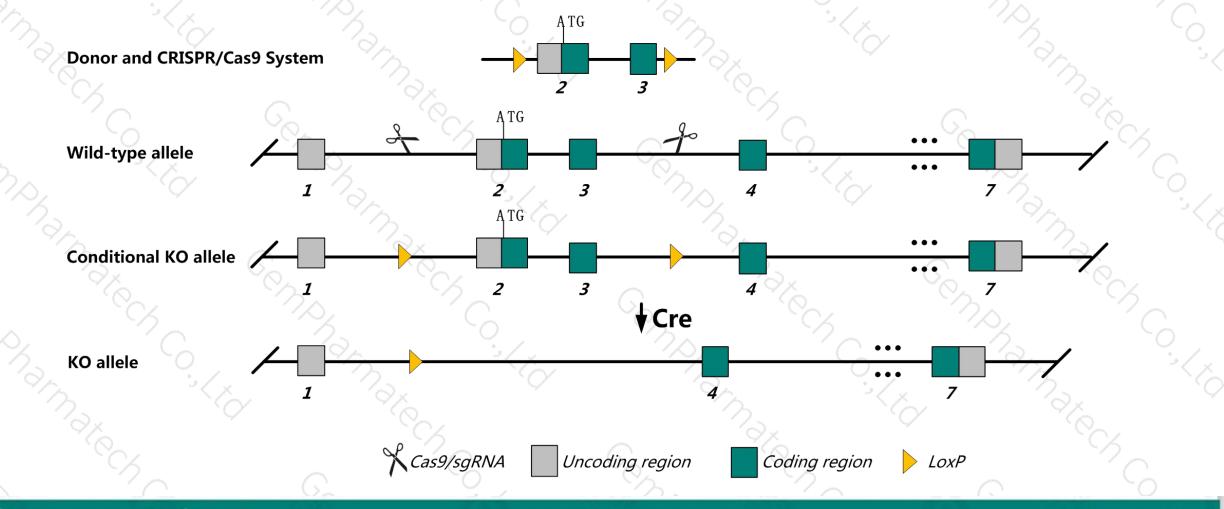




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Sirt3* gene. The schematic diagram is as follows:



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- The Sirt3 gene has 10 transcript. According to the structure of Sirt3 gene, exon2-3 of Sirt3-201 transcript is recommended as the knockout region. The region contains start codon ATG coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Sirt3* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA and Donor 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.
- The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues or cell types.



- According to the existing MGI data, Mice homozygous for one null allele exhibit increased systolic blood pressure while mice homozygous for another allele exhibit reduced ATP production.
- The Sirt3 gene is located on the Chr7. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Notice

Gene information (NCBI)



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Sirt3 sirtuin 3 [Mus musculus (house mouse)]

Gene ID: 64384, updated on 20-Jul-2019

- Summary

Official Symbol	Sirt3 provided by MGI
Official Full Name	sirtuin 3 provided by MGI
Primary source	MGI:MGI:1927665
See related	Ensembl:ENSMUSG0000025486
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae;
	Murinae; Mus; Mus
Also known as	Sir2l3; Al848213; 2310003L23Rik
Expression	Ubiquitous expression in kidney adult (RPKM 35.3), liver adult (RPKM 33.7) and 28 other tissues See more
Orthologs	human all
5	$\forall A = 3/3 = 10$

Transcript information (Ensembl)



The gene has 10 transcripts, and all transcripts are shown below:

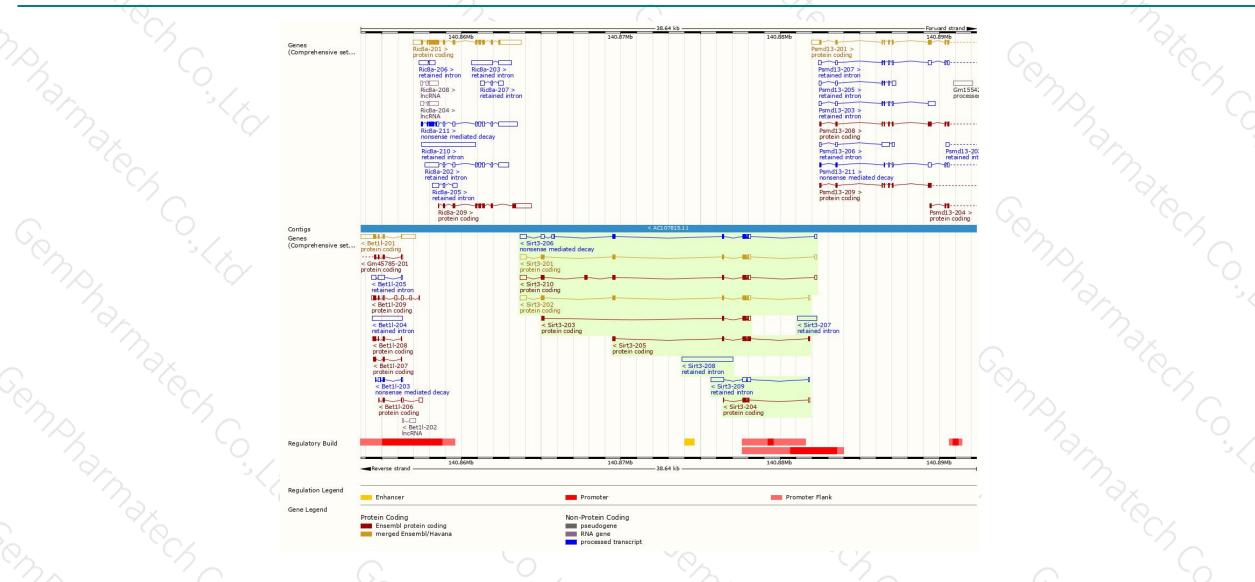
Name 💧	Transcript ID	bp 🛔	Protein 💧	Biotype 🍦	CCDS	UniProt 🔺	Flags
	ENSMUST0000026559.13	1461	<u>257aa</u>	Protein coding	<u>CCDS21989</u> &	<u>Q4FJK3</u> 률 <u>Q8R104</u> 률	TSL:1 GENCODE basic APPRIS F
Sirt3-202	ENSMUST00000106048.9	1416	<u>257aa</u>	Protein coding	<u>CCDS21989</u> &	<u>Q4FJK3</u> & <u>Q8R104</u> &	TSL:1 GENCODE basic APPRIS P
Sirt3-210	ENSMUST00000211179.1	1606	<u>309aa</u>	Protein coding		<u>A0A1B0GRY5</u> &	TSL:5 GENCODE basic
Sirt3-205	ENSMUST00000147331.8	784	<u>258aa</u>	Protein coding		D3YTK6团	CDS 3' incomplete TSL:3
Sirt3-203	ENSMUST00000137024.7	690	<u>182aa</u>	Protein coding	0	D3YVQ5₫	CDS 3' incomplete TSL:3
Sirt3-204	ENSMUST00000137710.1	456	<u>100aa</u>	Protein coding	×	<u>D3YWD6</u> 굡	CDS 3' incomplete TSL:5
Sirt3-206	ENSMUST00000210296.1	1598	<u>189aa</u>	Nonsense mediated decay		<u>A0A1B0GRF7</u> 교	TSL:5
Sirt3-208	ENSMUST00000210706.1	3206	No protein	Retained intron	÷	-	TSL:NA
Sirt3-209	ENSMUST00000210974.1	1272	No protein	Retained intron	2	2	TSL:2
Sirt3-207	ENSMUST00000210398.1	1223	No protein	Retained intron	<u></u>	1 <u>2</u>	TSL:NA

The strategy is based on the design of Sirt3-201 transcript, The transcription is shown below





Genomic location distribution



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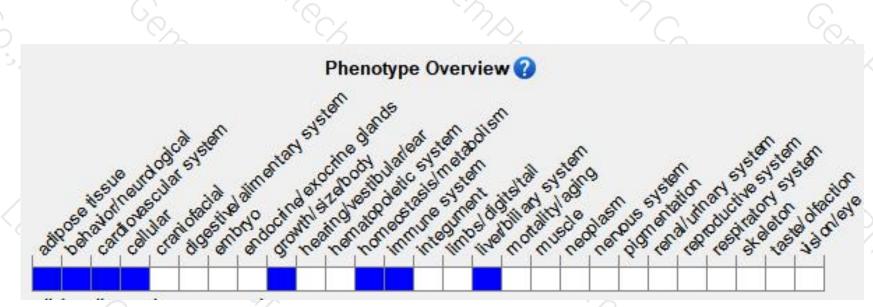
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# Mouse phenotype description(MGI)





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

According to the existing MGI data, Mutations in this locus affect cell-cycle regulation and apoptos is. Null homozygotes show high, early-onset tumor incidence; some have persistent hyaloid vasculature and cataracts. Truncated or temperature-sensitive alleles cause early aging phenotypes.

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