Spen Cas9-CKO Strategy

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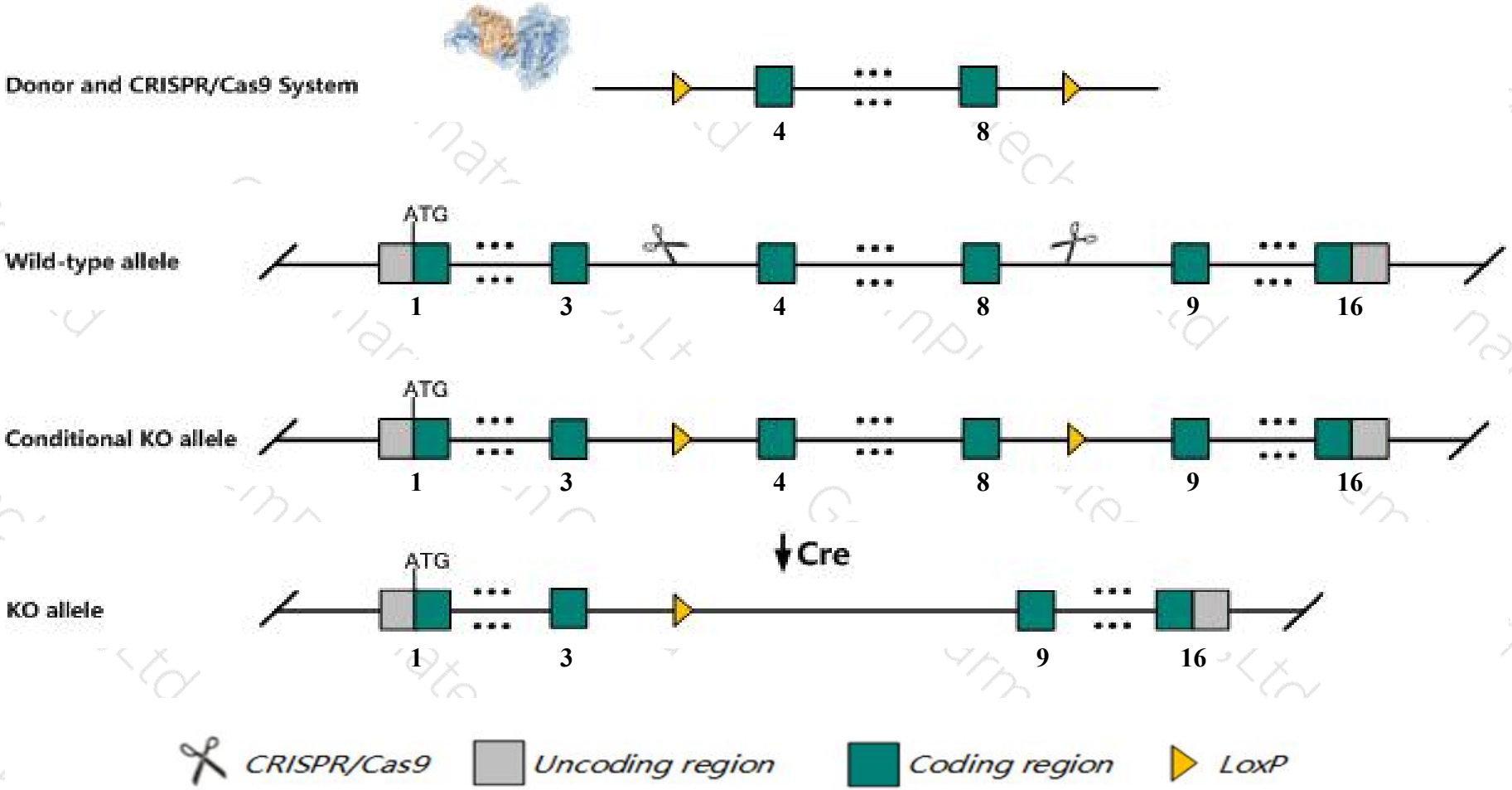
Project Overview

Project Name	***Spen***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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



Technical routes

- The *Spen* gene has 6 transcripts. According to the structure of *Spen* gene, exon4-exon8 of *Spen-202* (ENSMUST00000105786.2) transcript is recommended as the knockout region.The region contains 754bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Spen* gene. The brief process is as follows:CRISPR/Cas9 system 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 and cell types.

Notice

- According to the existing MGI data,homozygous mutant mice die during late gestation exhibiting morphological abnormalities of the heart, pancreas, and liver. Inactivation of this gene also affects the differentiation of B cells.

- The *Spen* gene is located on the Chr4. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

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Spen spen family transcription repressor [*Mus musculus* (house mouse)]

Gene ID: 56381, updated on 12-Aug-2019

Summary

Official Symbol	Spen provided by MGI
Official Full Name	spen family transcription repressor provided by MGI
Primary source	MGI:MGI:1891706
See related	Ensembl:ENSMUSG00000040761
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	Mint; mKIAA0929
Expression	Ubiquitous expression in thymus adult (RPKM 10.9), adrenal adult (RPKM 8.5) and 28 other tissues See more
Orthologs	human all

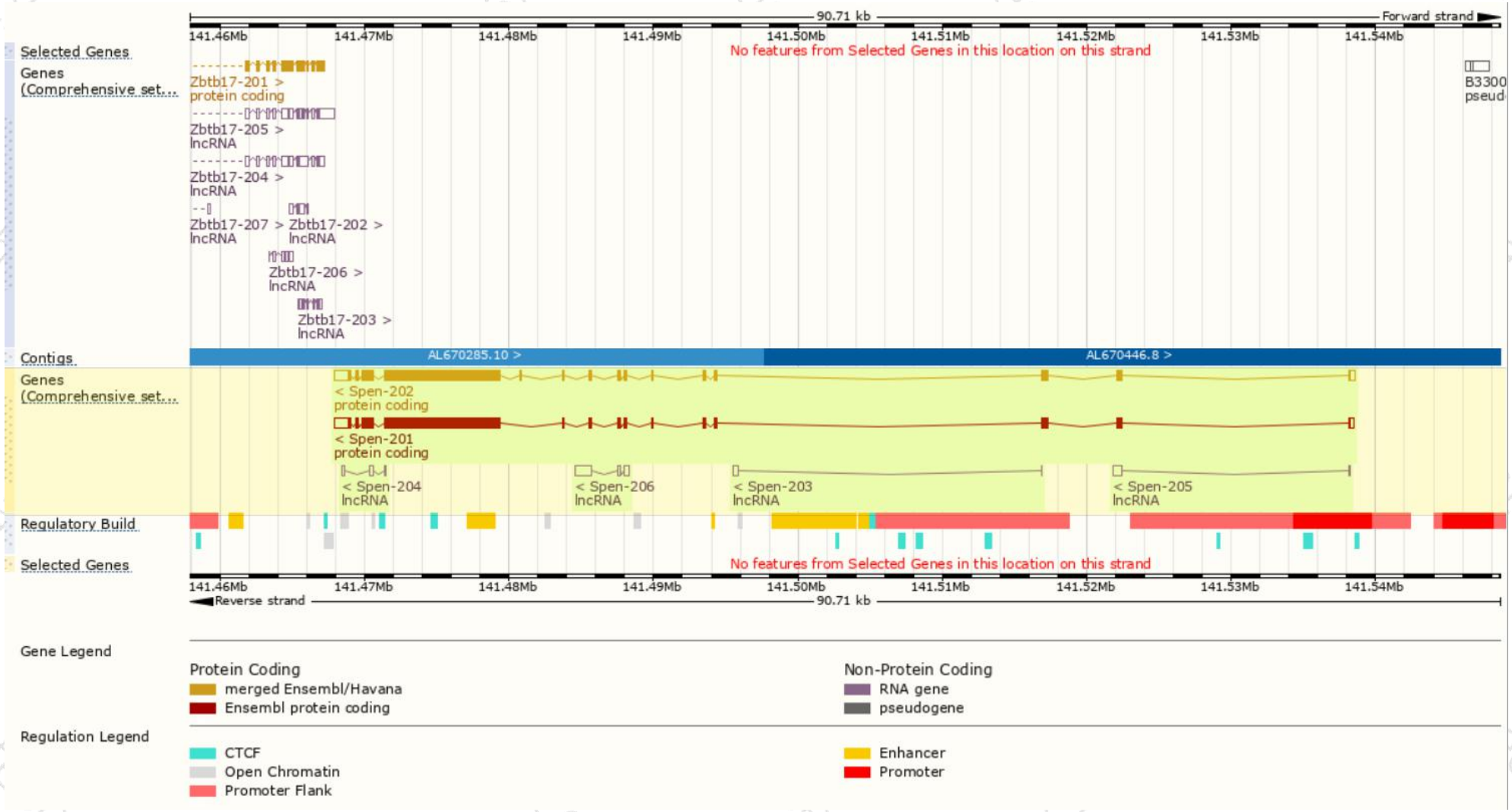
Transcript information（Ensembl）

The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Spen-201	ENSMUST00000078886.9	12082	3620aa	Protein coding	CCDS84815	A2ADB1	TSL:5 GENCODE basic APPRIS ALT2
Spen-202	ENSMUST00000105786.2	12299	3643aa	Protein coding	CCDS38940	A2ADB0	TSL:5 GENCODE basic APPRIS P3
Spen-206	ENSMUST00000152411.1	1514	No protein	lncRNA	-	-	TSL:1
Spen-205	ENSMUST00000152081.1	680	No protein	lncRNA	-	-	TSL:1
Spen-204	ENSMUST00000147227.1	481	No protein	lncRNA	-	-	TSL:3
Spen-203	ENSMUST00000144500.1	435	No protein	lncRNA	-	-	TSL:2

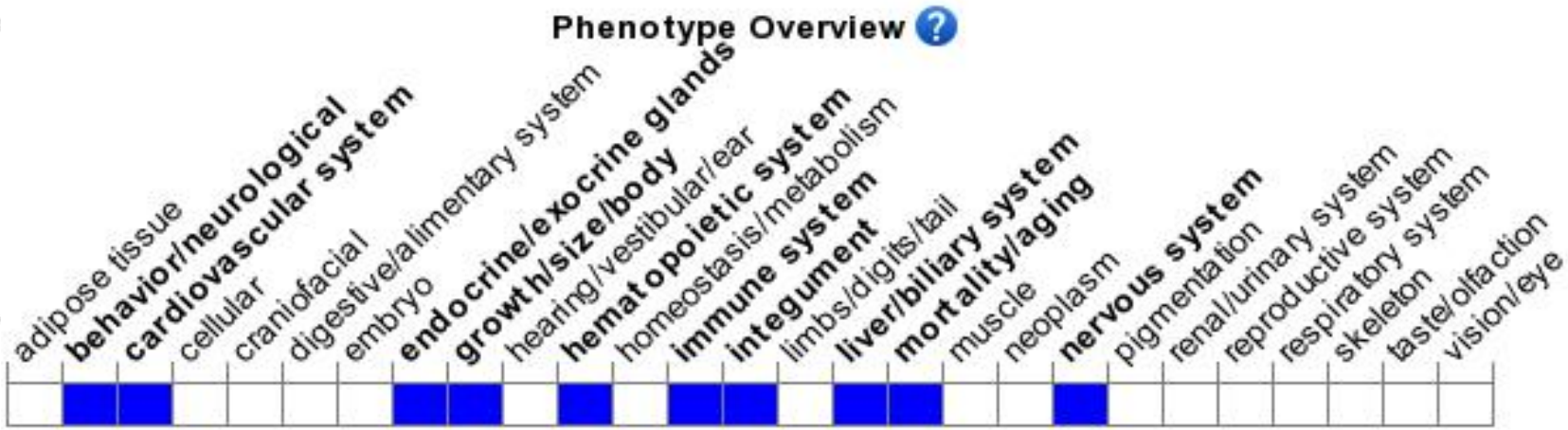
The strategy is based on the design of *Spen-202* transcript,The transcription is shown below

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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