Spi1 Cas9-CKO *Strategy*

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Reviewer: **Lingyan Wu**

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Project Overview

Project Name	***Spi1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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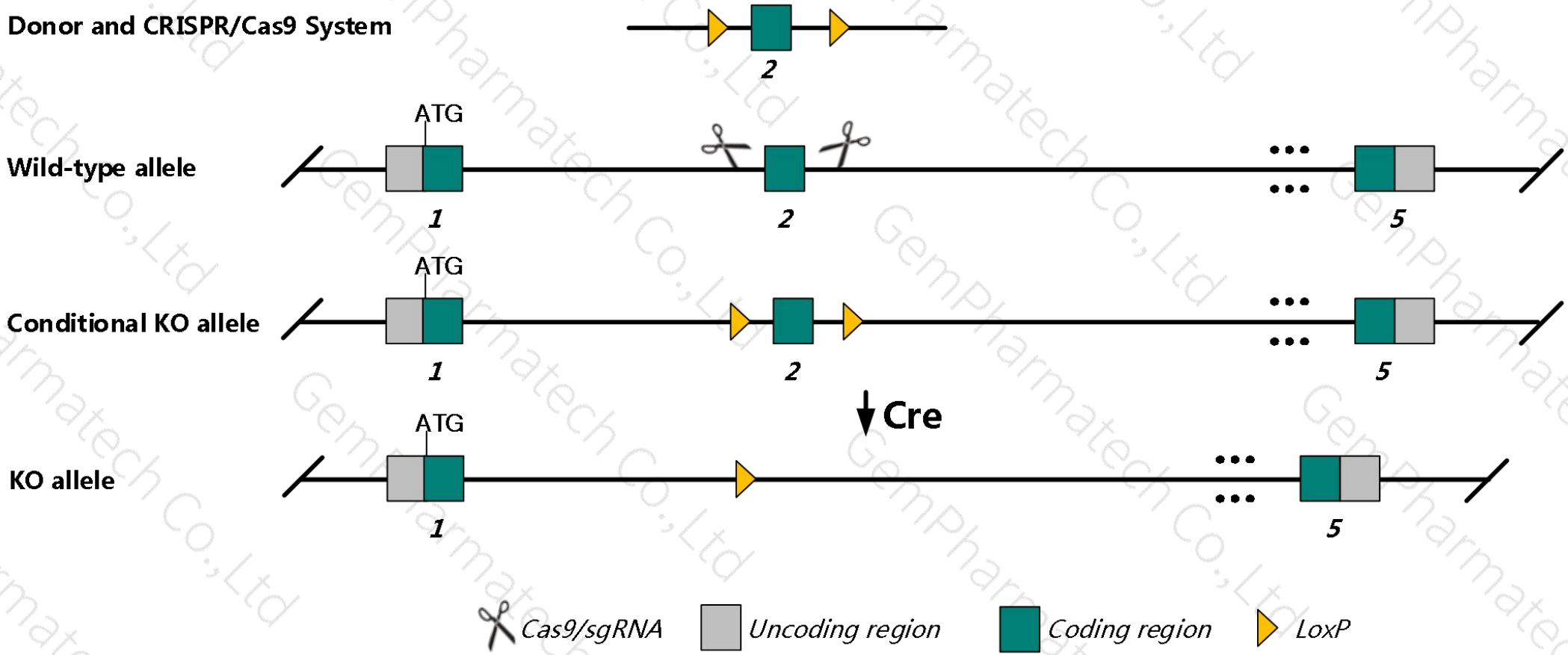
This model will use CRISPR/Cas9 technology to edit the *Spi1* gene. The schematic diagram is as follows:



Technical routes

- The *Spi1* gene has 4 transcripts.According to the structure of *Spi1* gene, exon2 of *Spi1*-201(ENSMUST00000002180.7) transcript is recommended as the knockout region.The region contains 97bp coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Spi1* 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.

Notice

- According to the existing MGI data，Nullizygous mice may exhibit fetal or perinatal lethality, absence of myeloid and B cells, and altered T cell and NK cell development. Mice carrying hypomorphic alleles display impaired B-cell and myeloid development, develop T cell derived lymphomas andacute myeloid leukemia, and die prematurely.
- The *Spi1* gene is located on the Chr2. 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.

Gene information （NCBI）

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Spi1 spleen focus forming virus (SFFV) proviral integration oncogene [*Mus musculus* (house mouse)]

Gene ID: 20375, updated on 14-Mar-2020

Summary

Official Symbol	Spi1 provided by MGI
Official Full Name	spleen focus forming virus (SFFV) proviral integration oncogene provided by MGI
Primary source	MGI:MGI:98282
See related	Ensembl:ENSMUSG00000002111
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	Dis1; PU.1; Dis-1; Sfpi1; Spi-1; Sfpi-1; Tcfpu1; Tfpu.1
Expression	Broad expression in spleen adult (RPKM 51.8), lung adult (RPKM 18.0) and 15 other tissues See more
Orthologs	human all

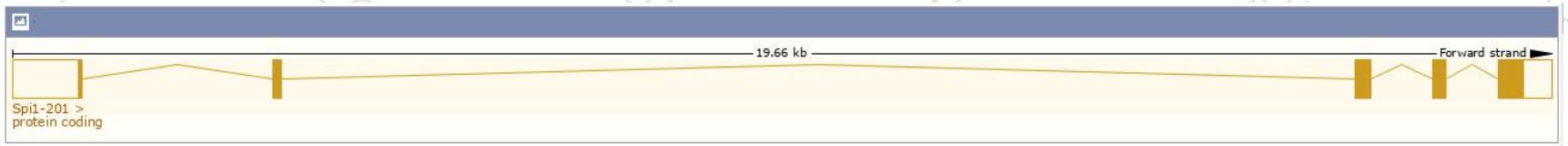
Transcript information（Ensembl）

The gene has 4 transcripts, and all transcripts are shown below:

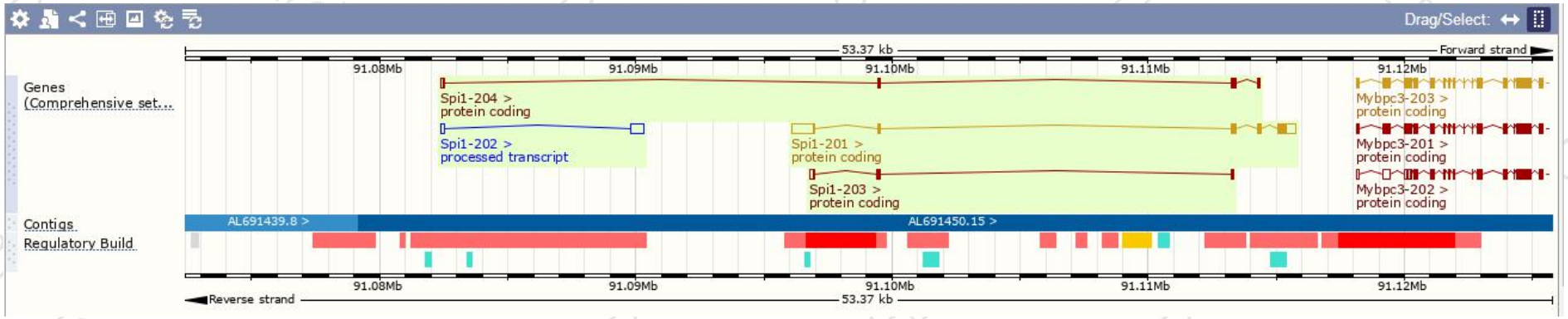
Show/hide columns (1 hidden) Filter

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Spi1-201	ENSMUST00000002180.7	2034	272aa	Protein coding	CCDS16425	P17433 Q3U5L4	TSL:1 GENCODE basic APPRIS P1
Spi1-204	ENSMUST00000169852.1	544	143aa	Protein coding	-	E9Q6W1	CDS 3' incomplete TSL:3
Spi1-203	ENSMUST00000132741.2	402	77aa	Protein coding	-	E9QAI9	CDS 3' incomplete TSL:3
Spi1-202	ENSMUST00000131400.1	613	No protein	Processed transcript	-	-	TSL:2

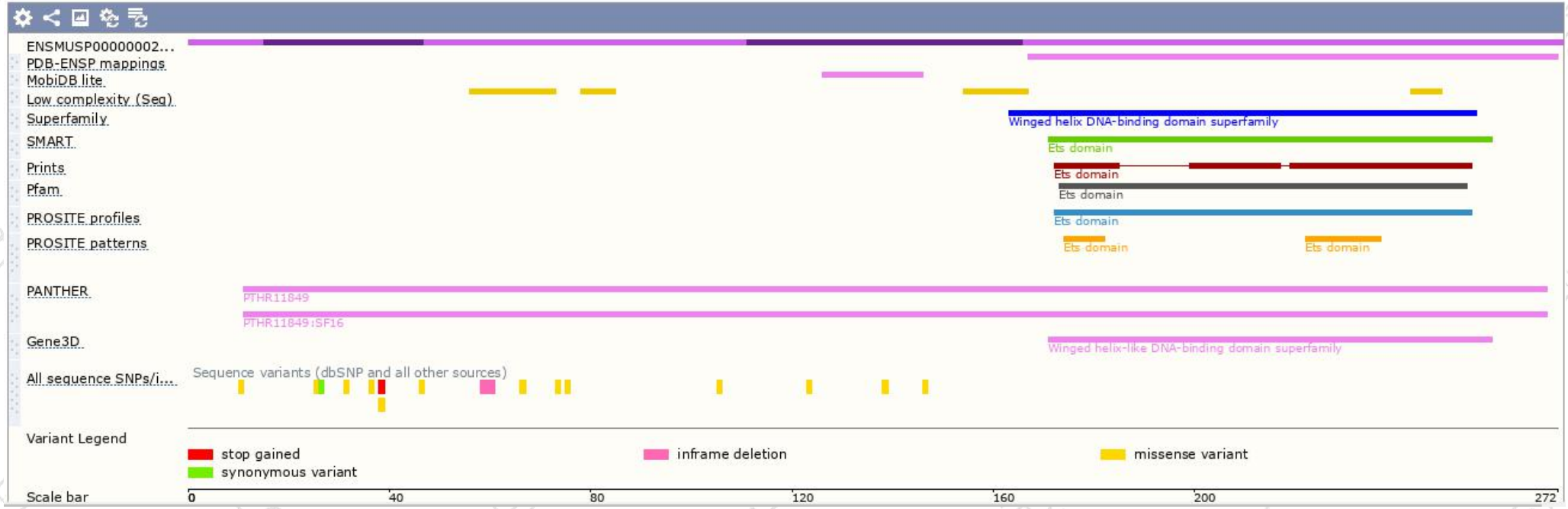
The strategy is based on the design of *Spi1*-201 transcript,The transcription is shown below



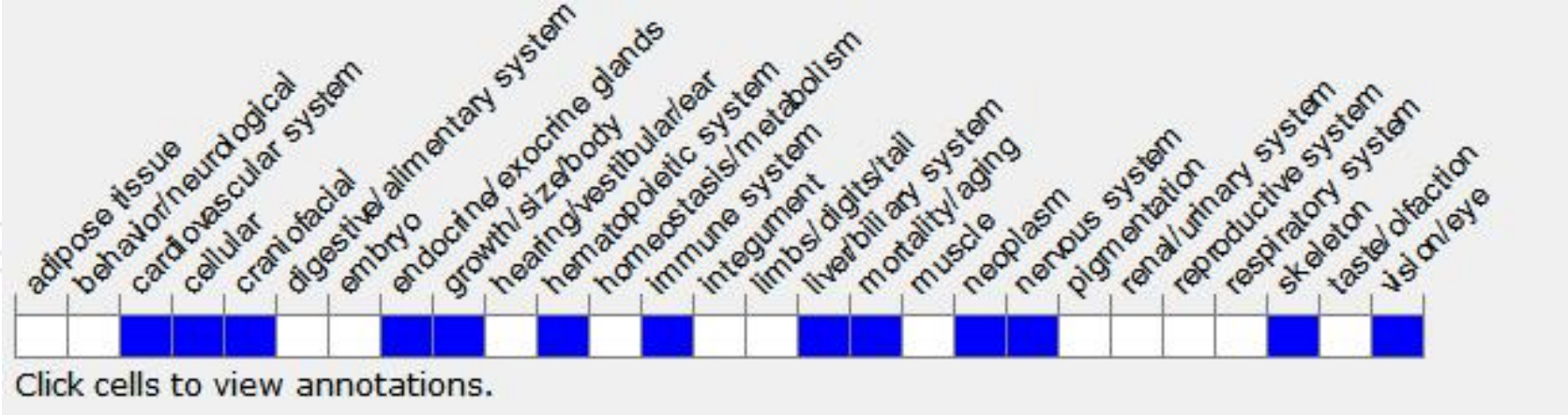
Genomic location distribution



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/) .

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.

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