Spink2 Cas9-KO Strategy

Designer: Xiaojing Li

Reviewer: JiaYu

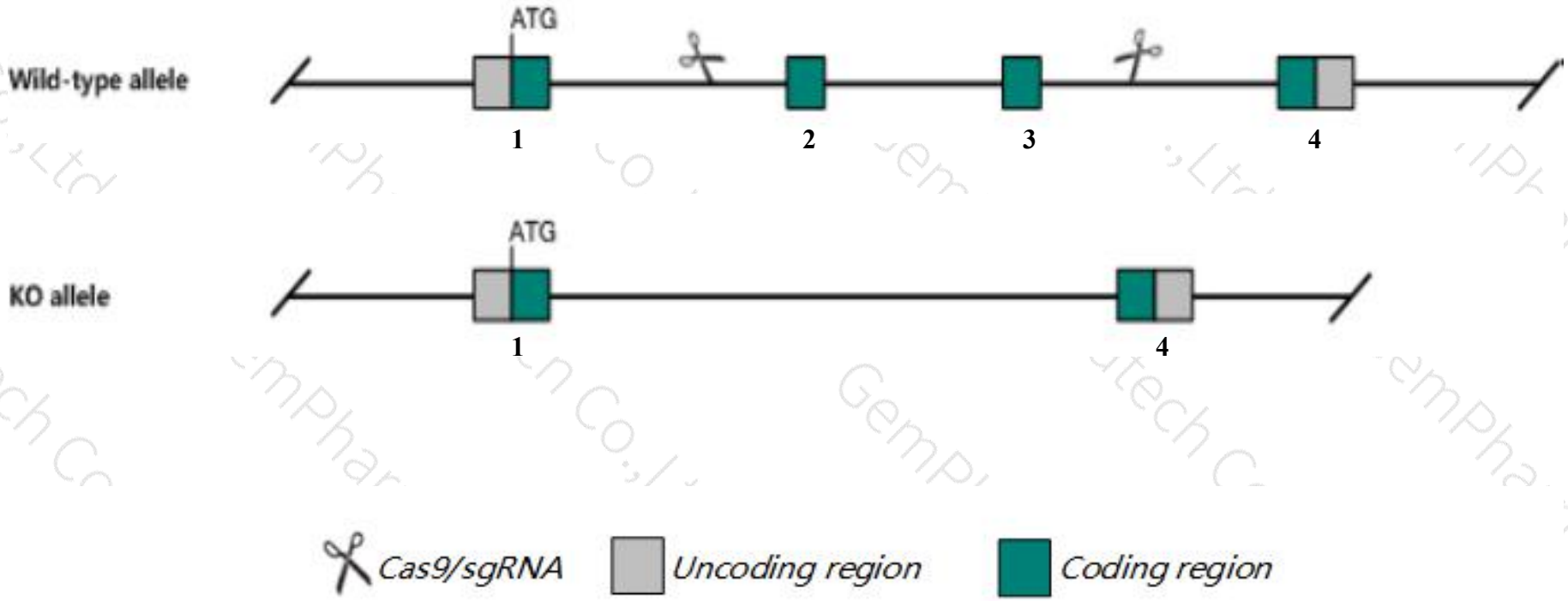
Design Date: 2020-8-20

Project Overview

Project Name	***Spink2***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Spink2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Spink2* gene has 3 transcripts. According to the structure of *Spink2* gene, exon2-exon3 of *Spink2-201*(ENSMUST00000065216.10) transcript is recommended as the knockout region.The region contains 172bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Spink2* 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 gene trap allele exhibit reduced male fertility associated with oligozoospermia, teratozoospermia, male germ cell apoptosis, and small testis.

➢ The *Spink2* gene is located on the Chr5. 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）

集萃药康 GemPharmatech

Spink2 serine peptidase inhibitor, Kazal type 2 [Mus musculus (house mouse)]

Gene ID: 69982, updated on 13-Mar-2020

Summary

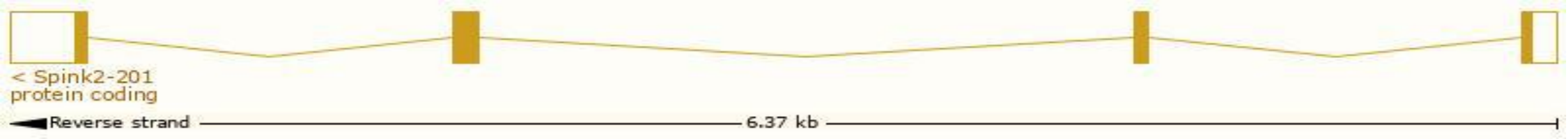
Official Symbol	Spink2 provided by MGI
Official Full Name	serine peptidase inhibitor, Kazal type 2 provided byMGI
Primary source	MGI:MGI:1917232
See related	Ensembl:ENSMUSG00000053030
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1700007F22Rik, AV038945, HUSI-II
Expression	Restricted expression toward testis adult (RPKM 405.6)See more
Orthologs	human all

Transcript information（Ensembl）

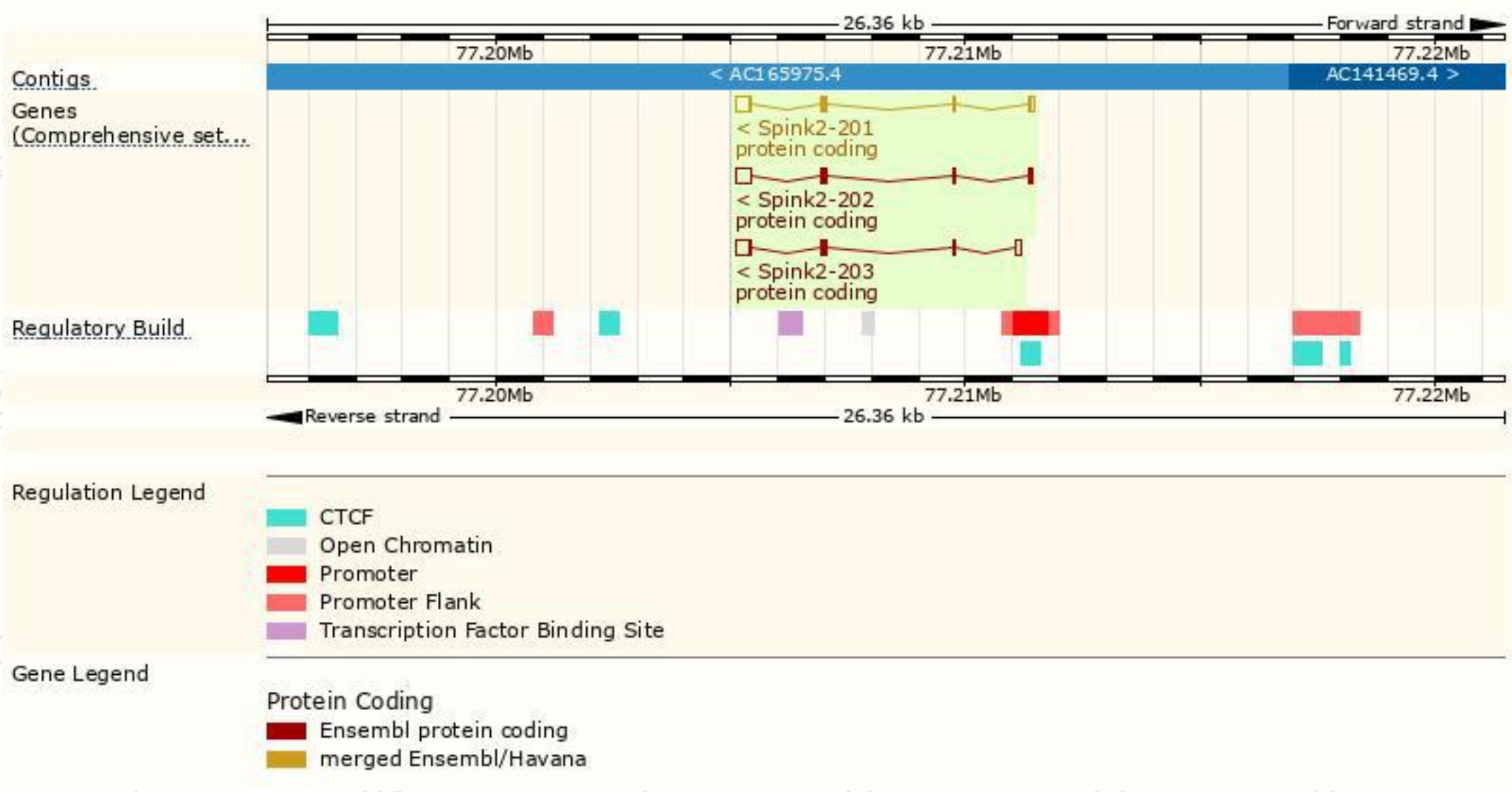
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Spink2-203	ENSMUST00000121825.1	643	59aa	Protein coding	CCDS80306	D3Z3X9	TSL:3 GENCODE basic APPRIS ALT2
Spink2-201	ENSMUST00000065216.10	631	86aa	Protein coding	CCDS39121	Q8BMY7	TSL:1 GENCODE basic APPRIS P3
Spink2-202	ENSMUST00000120429.7	594	56aa	Protein coding	CCDS80307	D3YTY0	TSL:1 GENCODE basic

The strategy is based on the design of *Spink2-201* transcript,the transcription is shown below:

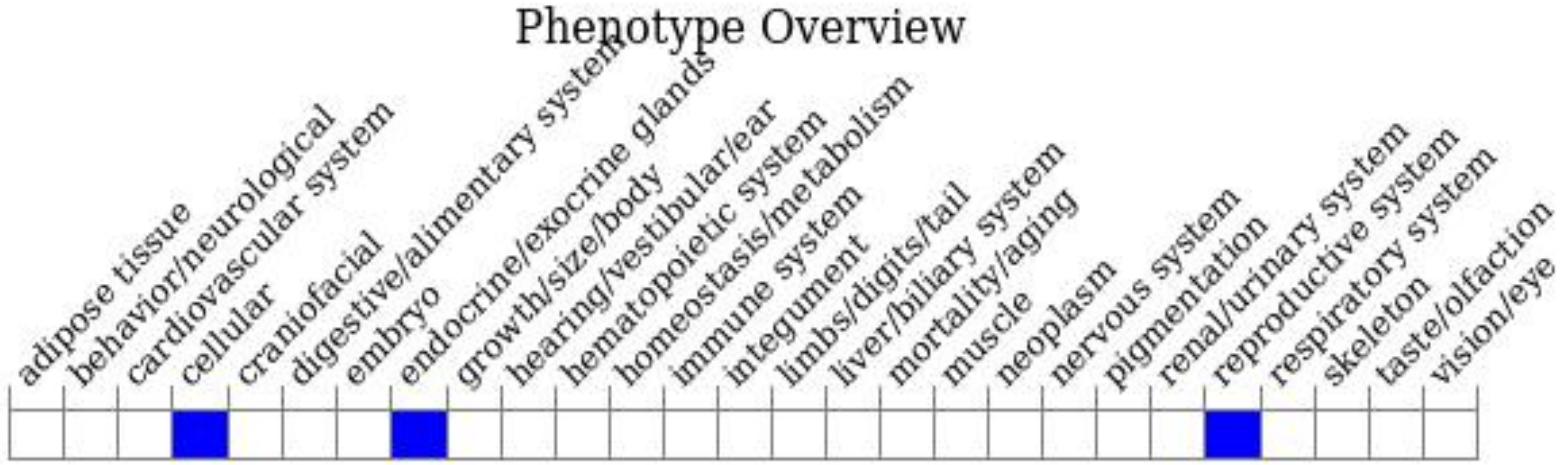


Genomic location distribution



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,mice homozygous for a gene trap allele exhibit reduced male fertility associated with oligozoospermia, teratozoospermia, male germ cell apoptosis, and small testis.

集萃药康
GemPharmatech

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

Tel: 025-5864 1534

集萃药康生物科技
GemPharmatech Co.,Ltd