

# Spata5 Cas9-CKO Strategy

Designer: Xueting Zhang

Reviewer: Yanhua Shen

Date:2020-03-10

# **Project Overview**



**Project Name** 

Spata5

**Project type** 

Cas9-CKO

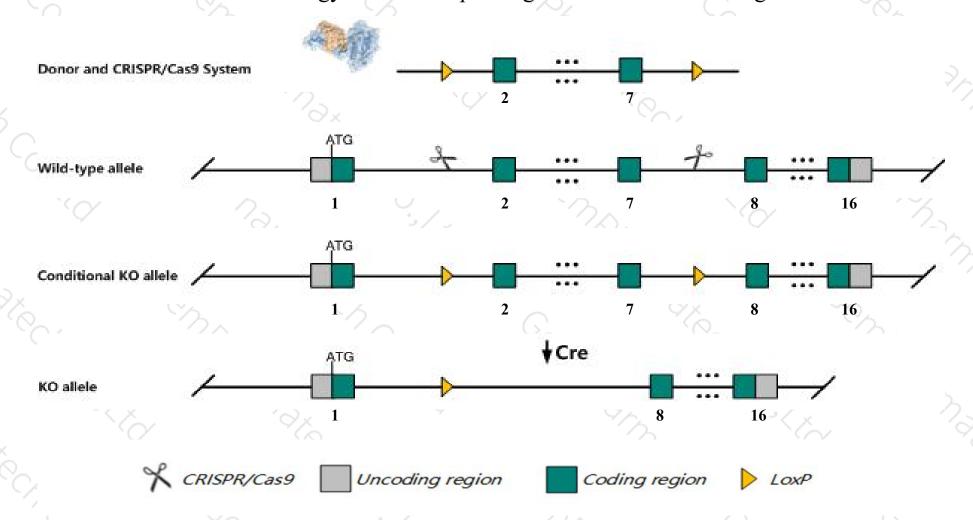
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Spata5* gene. The schematic diagram is as follows:



### Technical routes



- ➤ The *Spata5* gene has 10 transcripts. According to the structure of *Spata5* gene, exon2-exon7 of *Spata5-202* (ENSMUST00000108112.9) transcript is recommended as the knockout region. The region contains 1174bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Spata5* 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**



- The floxed region is near to the N-terminal of Nudt6 gene, this strategy may influence the regulatory function of the N-terminal of *Nudt6* gene.
- > Transcript *Spata5-204&205&207&210* may not be affected.
- > The *Spata5* gene is located on the Chr3. 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)



#### Spata5 spermatogenesis associated 5 [ Mus musculus (house mouse) ]

Gene ID: 57815, updated on 12-Aug-2019

#### Summary

2 2

Official Symbol Spata5 provided by MGI

Official Full Name spermatogenesis associated 5 provided by MGI

Primary source MGI:MGI:1927170

See related Ensembl: ENSMUSG00000027722

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 Spaf; C78064; 2510048F20Rik

Expression Ubiquitous expression in CNS E11.5 (RPKM 3.8), placenta adult (RPKM 3.4) and 25 other tissues See more

Orthologs <u>human</u> all

#### Genomic context



Location: 3; 3 B

See Spata5 in Genome Data Viewer

Exon count: 18

Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	3	NC_000069.6 (3741990337579096)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	3	NC_000069.5 (3731920237478017)

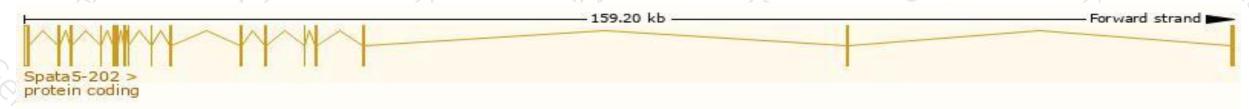
# Transcript information (Ensembl)



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

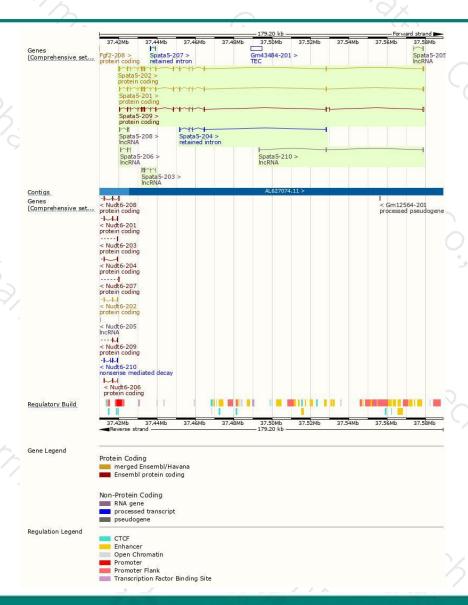
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Spata5-202	ENSMUST00000108112.9	3295	893aa	Protein coding	CCDS50895	Q3UMC0	TSL:1 GENCODE basic APPRIS ALT2
Spata5-209	ENSMUST00000198968.1	2916	<u>842aa</u>	Protein coding	CCDS79900	A0A0G2JFY0	TSL:1 GENCODE basic APPRIS ALT2
Spata5-201	ENSMUST00000029277.12	2890	892aa	Protein coding	CCDS17321	A0A0A0MQ80	TSL:1 GENCODE basic APPRIS P3
Spata5-204	ENSMUST00000130674.1	592	No protein	Retained intron	1520	21	TSL:3
Spata5-207	ENSMUST00000142199.1	371	No protein	Retained intron	120	-	TSL:2
Spata5-203	ENSMUST00000124347.1	599	No protein	IncRNA		*	TSL:3
Spata5-210	ENSMUST00000200093.1	482	No protein	IncRNA	-	-	TSL:3
Spata5-208	ENSMUST00000147687.7	404	No protein	IncRNA	72	24	TSL:3
Spata5-205	ENSMUST00000132958.2	379	No protein	IncRNA	120	5	TSL:3
Spata5-206	ENSMUST00000138225.1	370	No protein	IncRNA	1-1	-8	TSL:5

The strategy is based on the design of Spata5-202 transcript, The transcription is shown below



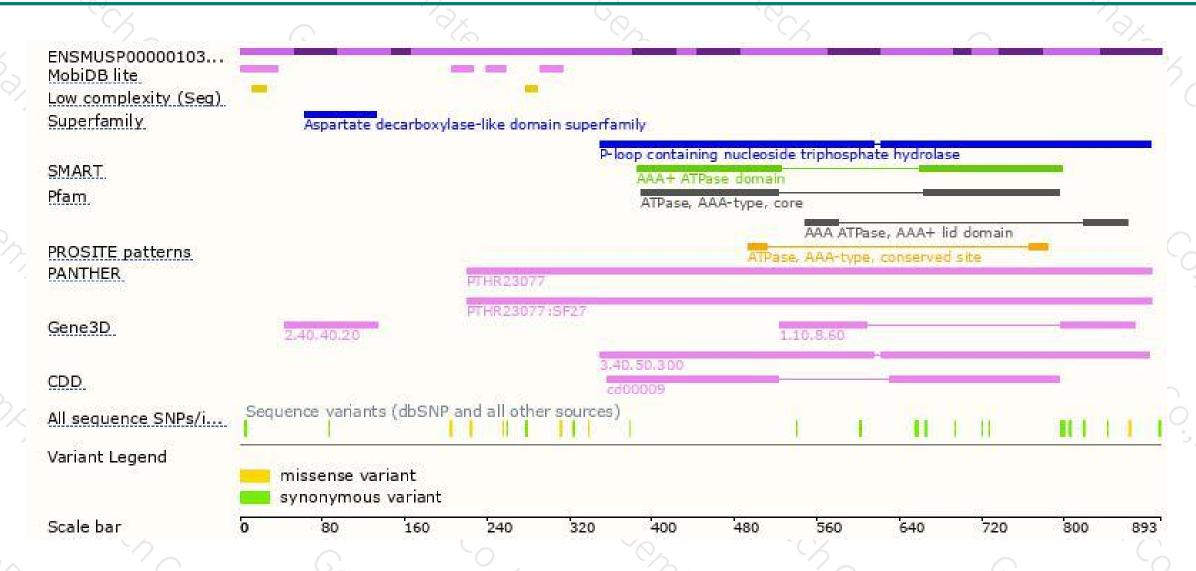
### Genomic location distribution





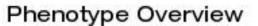
### Protein domain

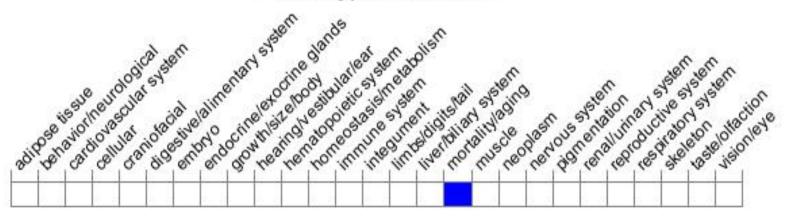




# Mouse phenotype description(MGI)







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



If you have any questions, you are welcome to inquire. Tel: 400-9660890





