

# Spata13 Cas9-CKO Strategy

Designer: Daohua Xu

Reviewer: Huimin Su

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# **Project Overview**



**Project Name** 

Spata13

**Project type** 

Cas9-CKO

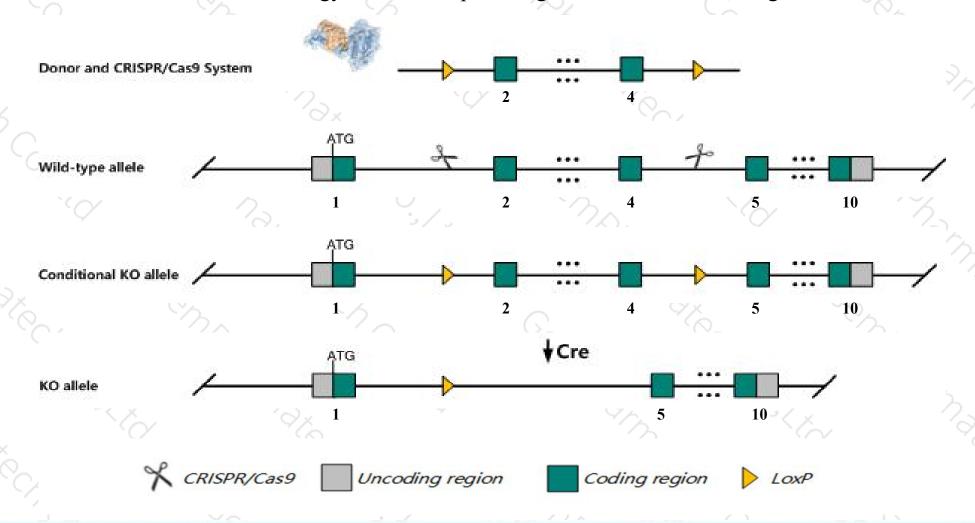
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Spata13 gene. The schematic diagram is as follows:



### Technical routes



- The Spata13 gene has 9 transcripts. According to the structure of Spata13 gene, exon2-exon4 of Spata13-209 (ENSMUST00000162945.1) transcript is recommended as the knockout region. The region contains 503bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Spata13* 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, mice homozygous for a knock-out allele are viable, fertile and overtly normal. behavioral tests indicate that mutant mice exhibit submissive social hierarchy behavior and females exhibit increased voluntary activity in the dark phase of the light-dark cycle.
- ➤ Transcript Spata13-203, Spata13-204 and Spata13-205 may not be affected.
- > The *Spata13* gene is located on the Chr14. 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)



#### Spata13 spermatogenesis associated 13 [Mus musculus (house mouse)]

Gene ID: 219140, updated on 13-Mar-2020

#### Summary

☆ ?

Official Symbol Spata 13 provided by MGI

Official Full Name spermatogenesis associated 13 provided by MGI

Primary source MGI:MGI:104838

See related Ensembl: ENSMUSG00000021990

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 C79050, ESTM11, X83327, mFLJ00298

Expression Ubiquitous expression in thymus adult (RPKM 11.9), adrenal adult (RPKM 5.6) and 28 other tissuesSee more

Orthologs <u>human</u> all

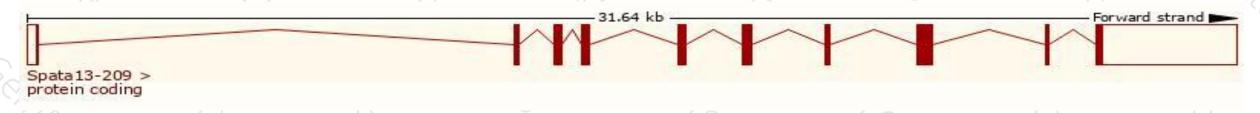
# Transcript information (Ensembl)



The gene has 9 transcripts, all transcripts are shown below:

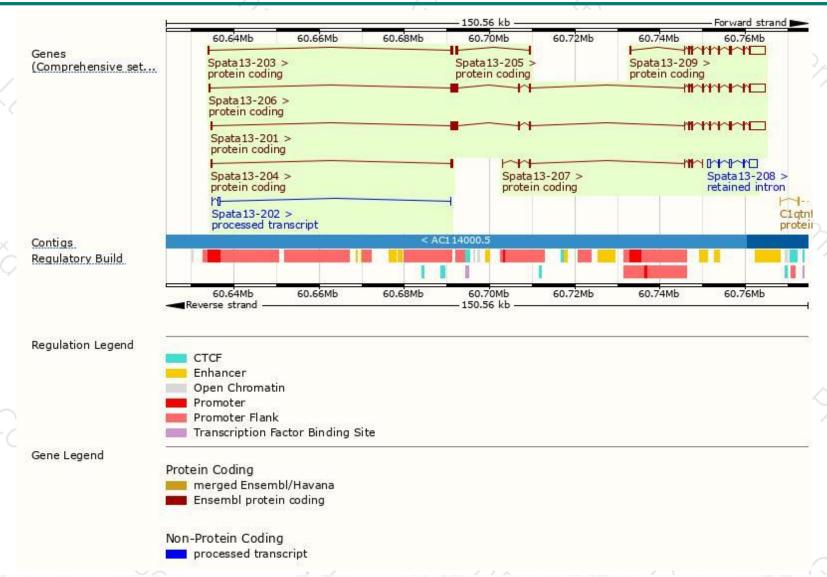
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Spata13-201	ENSMUST00000022566.13	7517	1244aa	Protein coding	CCDS49516	E9Q3I3	TSL:5 GENCODE basic
Spata13-206	ENSMUST00000160973.7	7467	<u>1244aa</u>	Protein coding	CCDS49516	E9Q3I3	TSL:1 GENCODE basic
Spata13-209	ENSMUST00000162945.1	5469	<u>574aa</u>	Protein coding	CCDS79328	E0CYU0	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Spata13-207	ENSMUST00000162131.8	1208	369aa	Protein coding	798	F6SRZ4	CDS 3' incomplete TSL:2
Spata13-205	ENSMUST00000160095.2	719	240aa	Protein coding		F6V9S1	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Spata13-203	ENSMUST00000159169.1	697	<u>124aa</u>	Protein coding	1993	E0CXN6	CDS 3' incomplete TSL:2
Spata13-204	ENSMUST00000159729.1	686	<u>138aa</u>	Protein coding	(2)	E0CZ51	CDS 3' incomplete TSL:2
Spata13-202	ENSMUST00000159135.1	429	No protein	Processed transcript	798	-	TSL:3
Spata13-208	ENSMUST00000162939.1	3161	No protein	Retained intron			TSL:5
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The strategy is based on the design of *Spata13-209* transcript, the transcription is shown below:



### Genomic location distribution





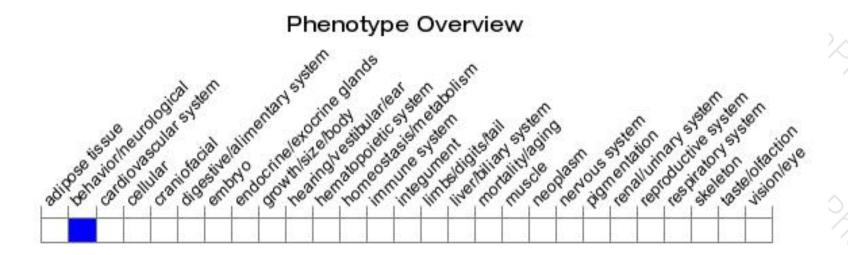
### Protein domain





# 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 knock-out allele are viable, fertile and overtly normal.

Behavioral tests indicate that mutant mice exhibit submissive social hierarchy behavior and females exhibit increased voluntary activity in the dark phase of the light-dark cycle.



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





