

Spag16 Cas9-CKO Strategy

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Reviewer:

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Project Overview

Project Name

Spag16

Project type

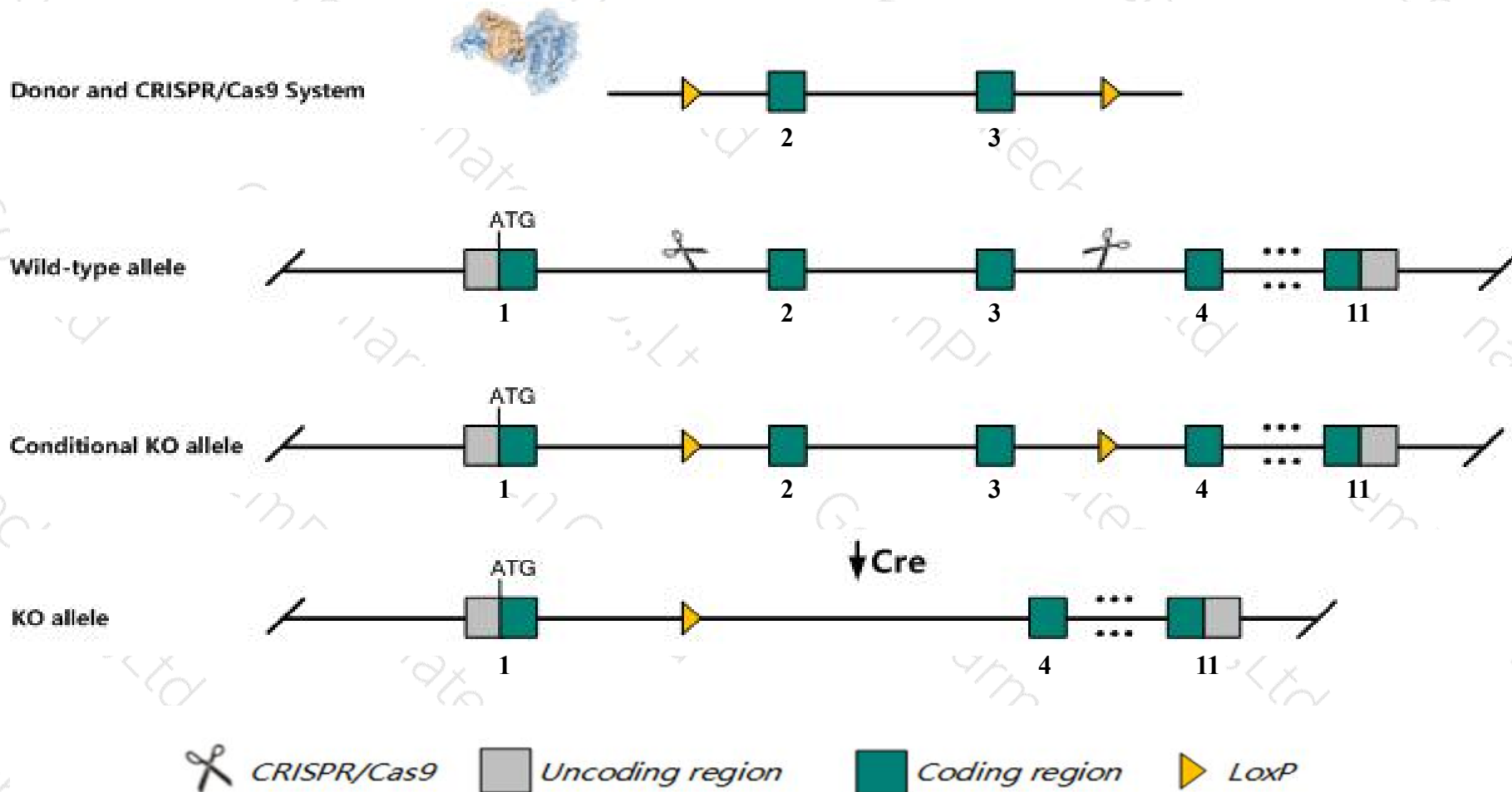
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Spag16* gene. The schematic diagram is as follows:



- The *Spag16* gene has 7 transcripts. According to the structure of *Spag16* gene, exon2-exon3 of *Spag16-202* (ENSMUST00000113940.3) transcript is recommended as the knockout region. The region contains 143bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Spag16* 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.

- According to the existing MGI data, chimeric males carrying one copy of the mutated allele have impaired spermatogenesis, a significant loss of germ cells at the round spermatid stage, and disorganized sperm axoneme structure. No offspring carrying the mutated allele are produced from matings using male chimeras.
- The transcript NM_025728.4 on the NCBI will not be affected.
- The *Spag16* gene is located on the Chr1. 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)

Spag16 sperm associated antigen 16 [*Mus musculus* (house mouse)]

Gene ID: 66722, updated on 10-Sep-2019

Summary



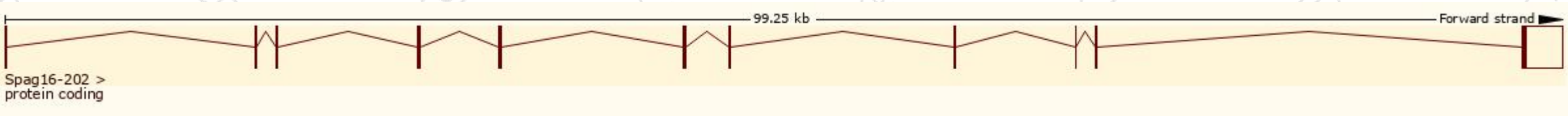
Official Symbol	Spag16 provided by MGI
Official Full Name	sperm associated antigen 16 provided by MGI
Primary source	MGI:MGI:1913972
See related	Ensembl:ENSMUSG000000053153
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Pf20; Wdr29; AV261009; 4921511D23Rik; 4930524F24Rik; 4930585K05Rik
Expression	Biased expression in testis adult (RPKM 9.4), lung adult (RPKM 1.1) and 1 other tissue See more
Orthologs	human all

Transcript information (Ensembl)

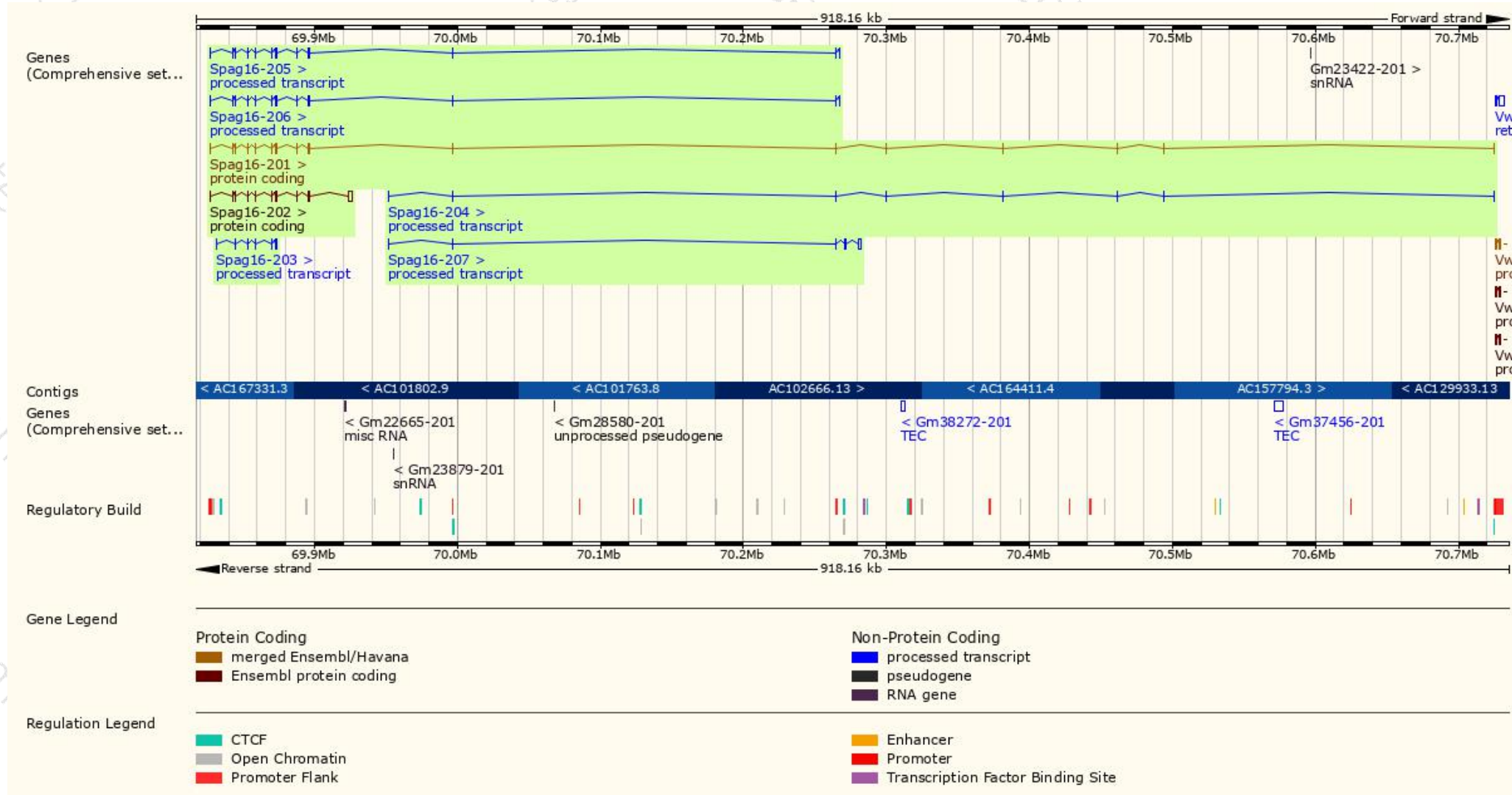
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Spag16-202	ENSMUST00000113940.3	3559	405aa	Protein coding	CCDS69901	Q8K450	TSL:1 GENCODE basic
Spag16-201	ENSMUST00000065425.11	2126	639aa	Protein coding	CCDS15027	Q8K450	TSL:1 GENCODE basic APPRIS P1
Spag16-207	ENSMUST00000191569.1	1714	No protein	Processed transcript	-	-	TSL:1
Spag16-205	ENSMUST00000187159.6	1433	No protein	Processed transcript	-	-	TSL:1
Spag16-206	ENSMUST00000190833.6	1338	No protein	Processed transcript	-	-	TSL:1
Spag16-204	ENSMUST00000161310.7	1297	No protein	Processed transcript	-	-	TSL:1
Spag16-203	ENSMUST00000161131.2	983	No protein	Processed transcript	-	-	TSL:1

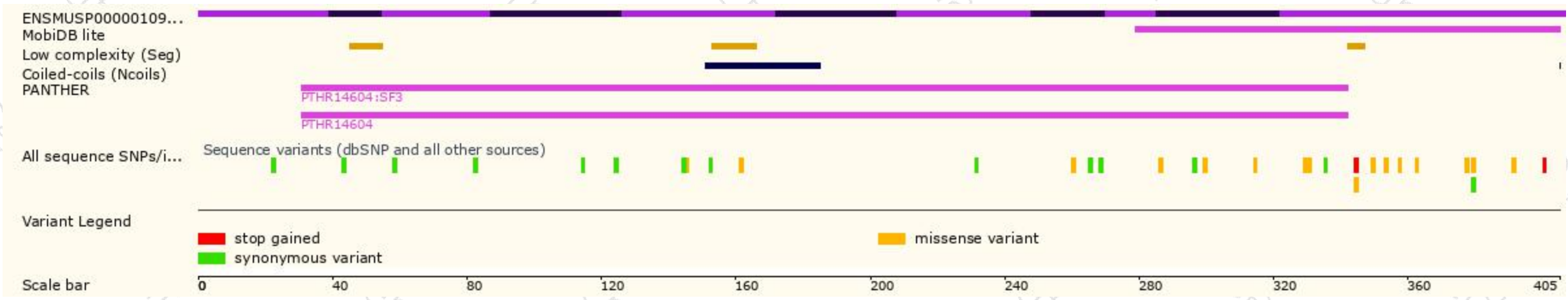
The strategy is based on the design of *Spag16-202* transcript, the transcription is shown below:



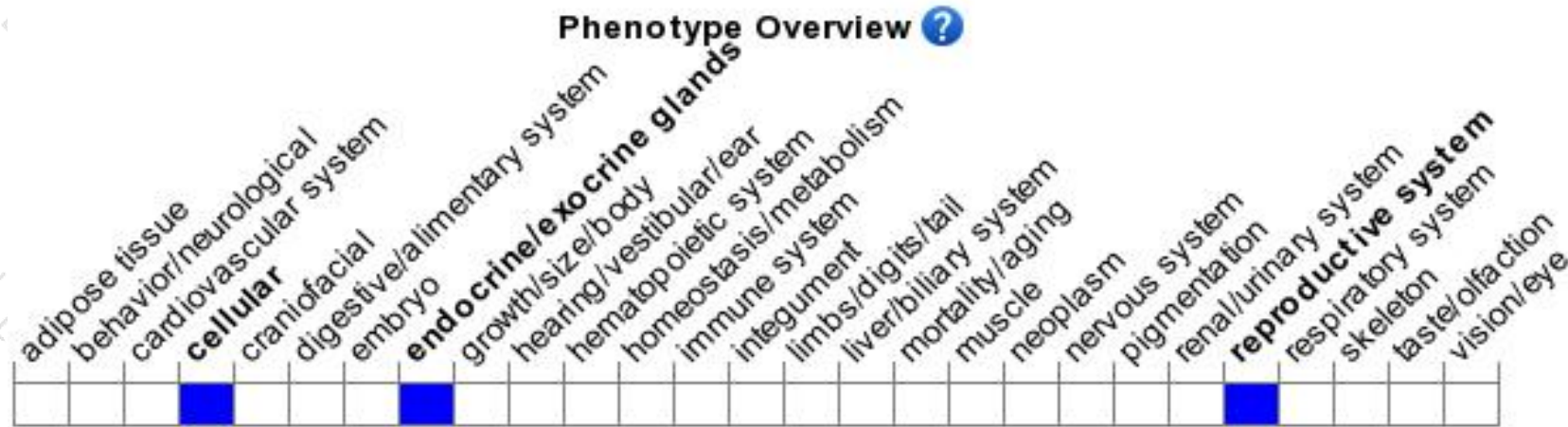
Genomic location distribution



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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