

# Spag4 Cas9-KO Strategy

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# Overview

## Target Gene Name

- Spag4

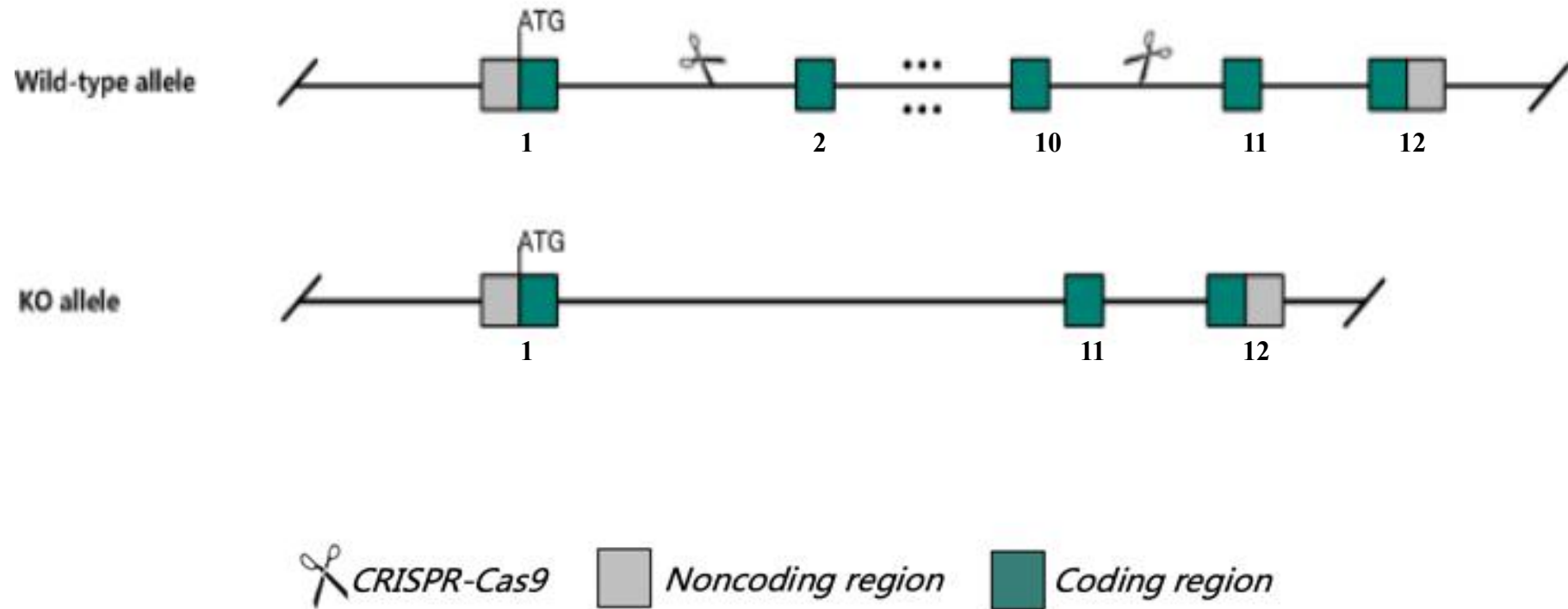
## Project Type

- Cas9-KO

## Genetic Background

- C57BL/6JGpt

# Strain Strategy



# Technical Information

- The *Spag4* gene has 8 transcripts. According to the structure of *Spag4* gene, exon2-exon10 of *Spag4*-201 (ENSMUST00000038860.12) transcript is recommended as the knockout region. The region contains 773bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Spag4* gene. The brief process is as follows: gRNAs were transcribed in vitro. Cas9 and gRNAs 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.

# Gene Information

## Spag4 sperm associated antigen 4 [Mus musculus (house mouse)]

Gene ID: 245865, updated on 13-Mar-2020

### Summary

<b>Official Symbol</b>	Spag4 <small>provided by <a href="#">MGI</a></small>
<b>Official Full Name</b>	sperm associated antigen 4 <small>provided by <a href="#">MGI</a></small>
<b>Primary source</b>	<a href="#">MGI:MGI:2444120</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000038180</a>
<b>Gene type</b>	protein coding
<b>RefSeq status</b>	VALIDATED
<b>Organism</b>	<a href="#">Mus musculus</a>
<b>Lineage</b>	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
<b>Also known as</b>	1700041K21Rik, MNCb-0953, Sun4, mKIAA4118
<b>Expression</b>	Biased expression in testis adult (RPKM 206.1) and CNS E14 (RPKM 2.7) <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>

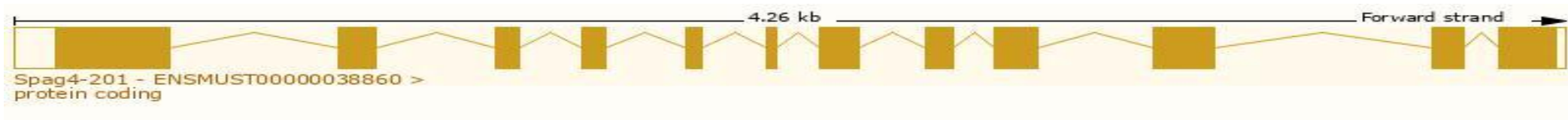
Source: <https://www.ncbi.nlm.nih.gov/>

# Transcript Information

The gene has 8 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Spag4-201	<a href="#">ENSMUST00000038860.11</a>	1473	<a href="#">443aa</a>	Protein coding	<a href="#">CCDS50777</a>	<a href="#">Q9J1F2</a>	TSL:1 GENCODE basic APPRIS P1
Spag4-206	<a href="#">ENSMUST00000137966.1</a>	747	<a href="#">210aa</a>	Protein coding	-	<a href="#">B7ZCP3</a>	CDS 3' incomplete TSL:3
Spag4-207	<a href="#">ENSMUST00000138178.7</a>	438	<a href="#">78aa</a>	Nonsense mediated decay	-	<a href="#">F6S430</a>	CDS 5' incomplete TSL:3
Spag4-203	<a href="#">ENSMUST00000131144.7</a>	370	<a href="#">40aa</a>	Nonsense mediated decay	-	<a href="#">F6XNA5</a>	CDS 5' incomplete TSL:3
Spag4-208	<a href="#">ENSMUST00000149139.1</a>	667	No protein	Processed transcript	-	-	TSL:5
Spag4-202	<a href="#">ENSMUST00000125996.7</a>	3892	No protein	Retained intron	-	-	TSL:2
Spag4-205	<a href="#">ENSMUST00000136765.7</a>	2203	No protein	Retained intron	-	-	TSL:1
Spag4-204	<a href="#">ENSMUST00000133511.7</a>	2022	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Spag4*-201 transcript, the transcription is shown below:

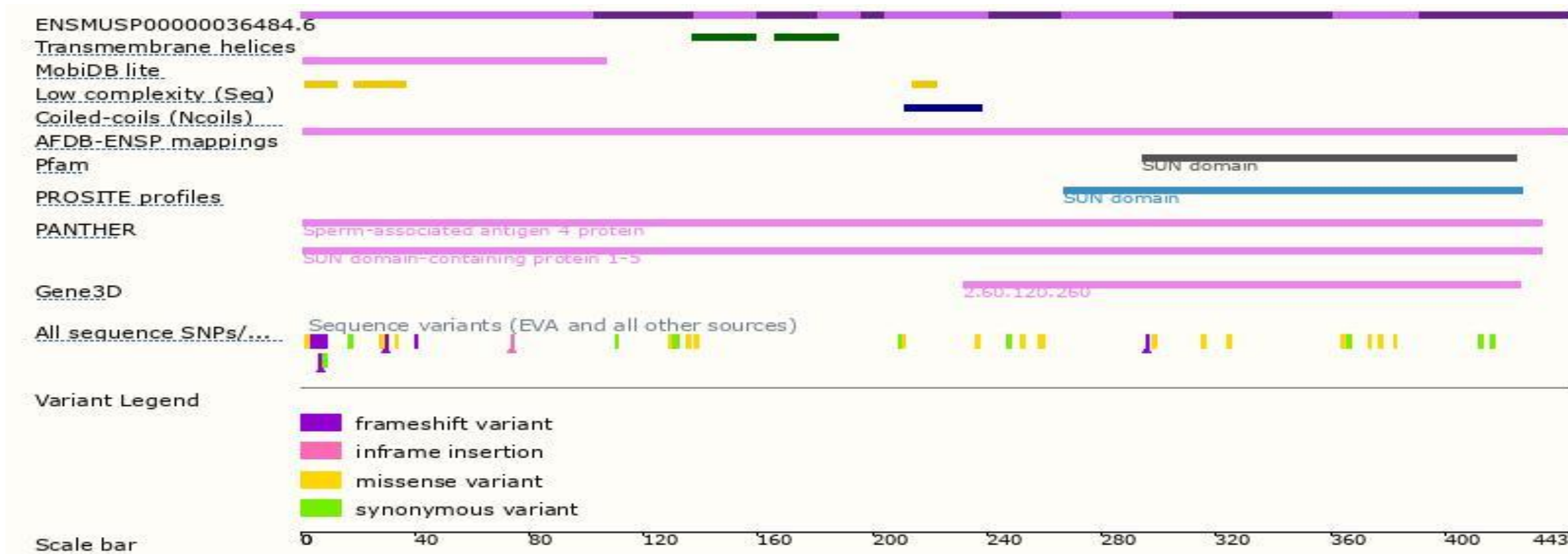


Source: <https://www.ensembl.org>

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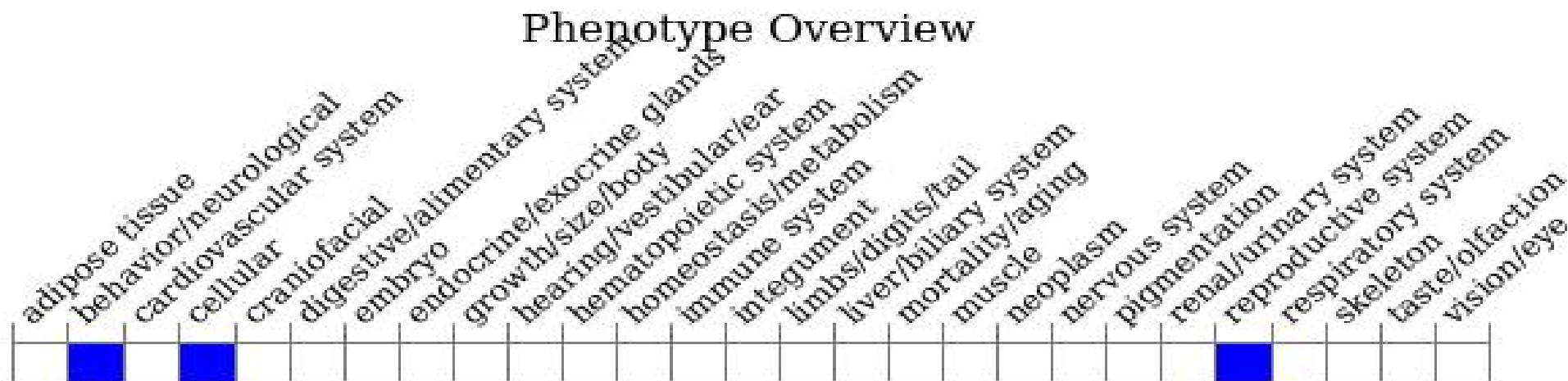


# Protein Information





# Mouse Phenotype Information (MGI)



- Mice homozygous for a knock-out allele show disrupted spermiogenesis, severe defects in sperm head formation, abnormal manchette morphology, globozoospermia, and male infertility.

# Important Information

- *Spag4* is located on Chr2. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.
- Intron 1-2(462 bp) and 10-11(598 bp), the effect of LOXP insertion on gene is unknown.