

Spag4 Cas9-CKO Strategy

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Overview

Target Gene Name

- Spag4

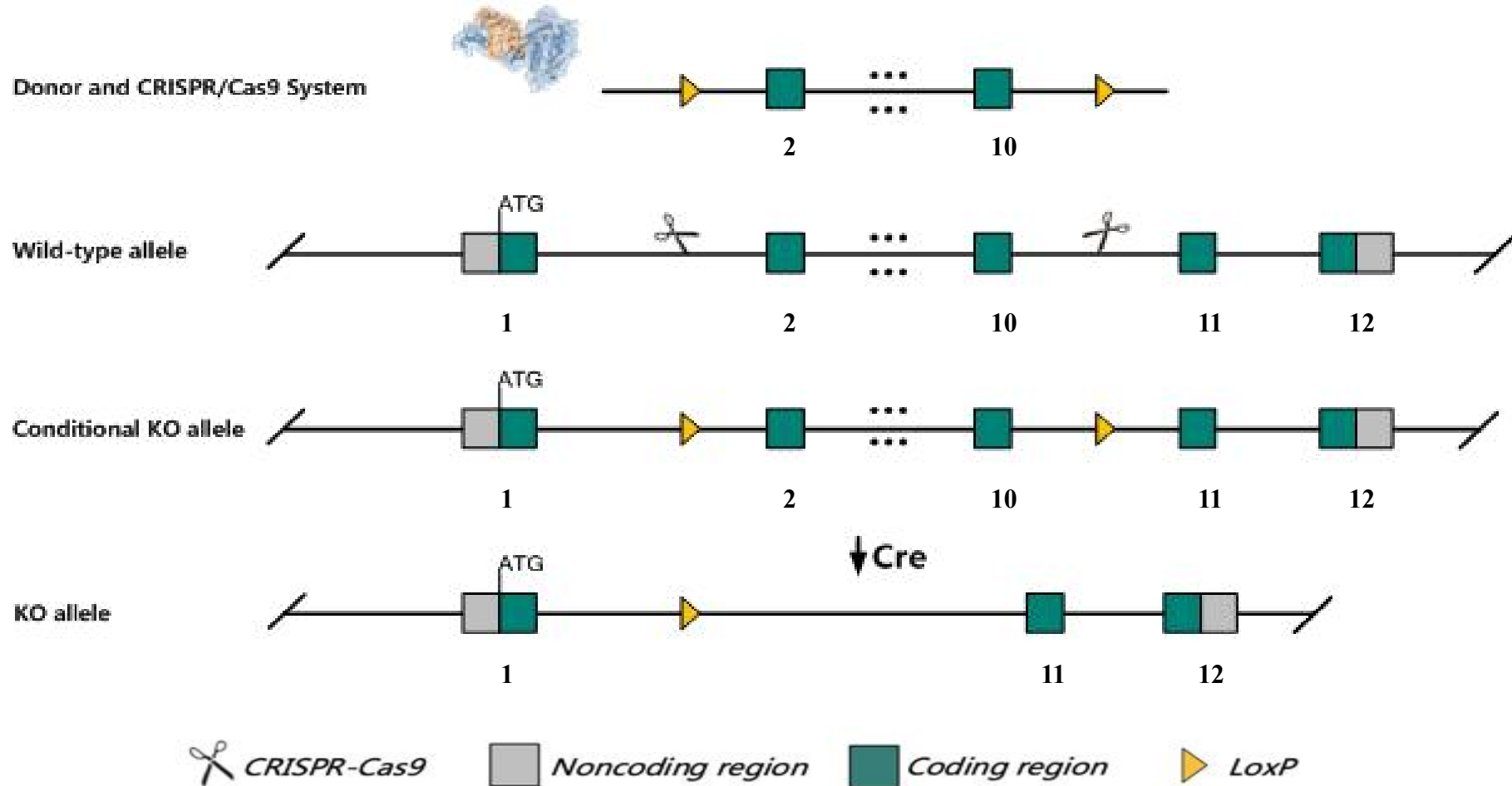
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Spag4* gene.

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: 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 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.
- 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.

Gene Information

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

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

Summary

Official Symbol	Spag4 <small>provided by MGI</small>
Official Full Name	sperm associated antigen 4 <small>provided by MGI</small>
Primary source	MGI:MGI:2444120
See related	Ensembl:ENSMUSG00000038180
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	1700041K21Rik, MNCb-0953, Sun4, mKIAA4118
Expression	Biased expression in testis adult (RPKM 206.1) and CNS E14 (RPKM 2.7) See more
Orthologs	human all

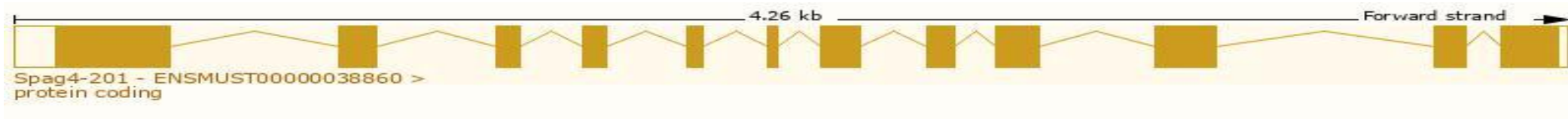
Source: <https://www.ncbi.nlm.nih.gov/>

Transcript Information

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

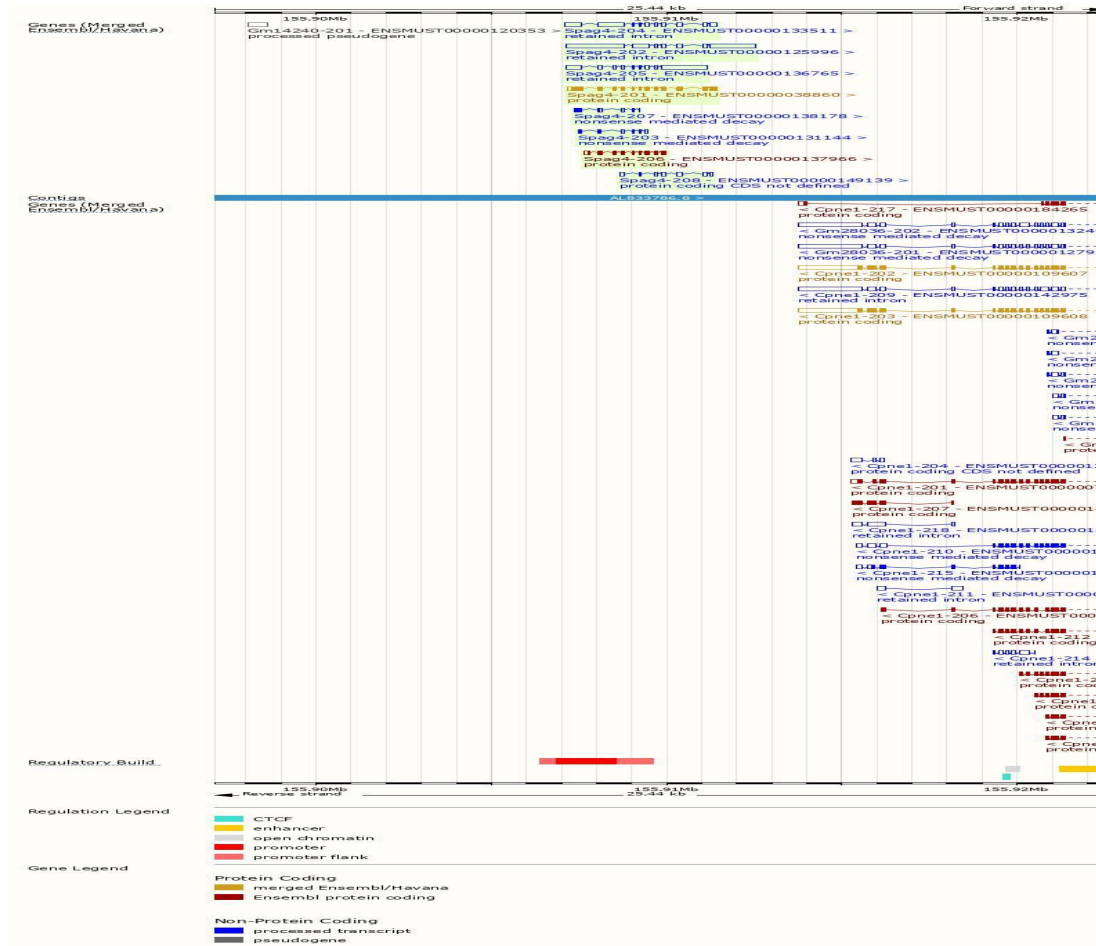
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Spag4-201	ENSMUST00000038860.11	1473	443aa	Protein coding	CCDS50777	Q9JIF2	TSL:1 GENCODE basic APPRIS P1
Spag4-206	ENSMUST00000137966.1	747	210aa	Protein coding	-	B7ZCP3	CDS 3' incomplete TSL:3
Spag4-207	ENSMUST00000138178.7	438	78aa	Nonsense mediated decay	-	F6S430	CDS 5' incomplete TSL:3
Spag4-203	ENSMUST00000131144.7	370	40aa	Nonsense mediated decay	-	F6XNA5	CDS 5' incomplete TSL:3
Spag4-208	ENSMUST00000149139.1	667	No protein	Processed transcript	-	-	TSL:5
Spag4-202	ENSMUST00000125996.7	3892	No protein	Retained intron	-	-	TSL:2
Spag4-205	ENSMUST00000136765.7	2203	No protein	Retained intron	-	-	TSL:1
Spag4-204	ENSMUST00000133511.7	2022	No protein	Retained intron	-	-	TSL:1

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

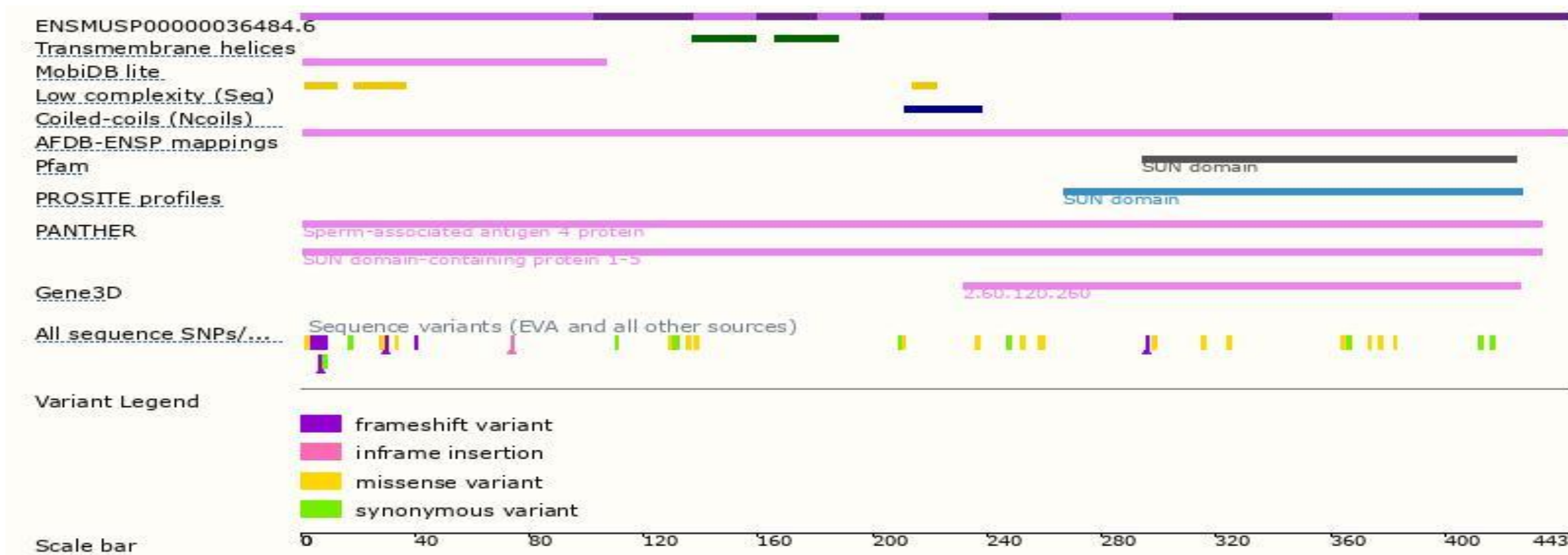


Source: <https://www.ensembl.org>

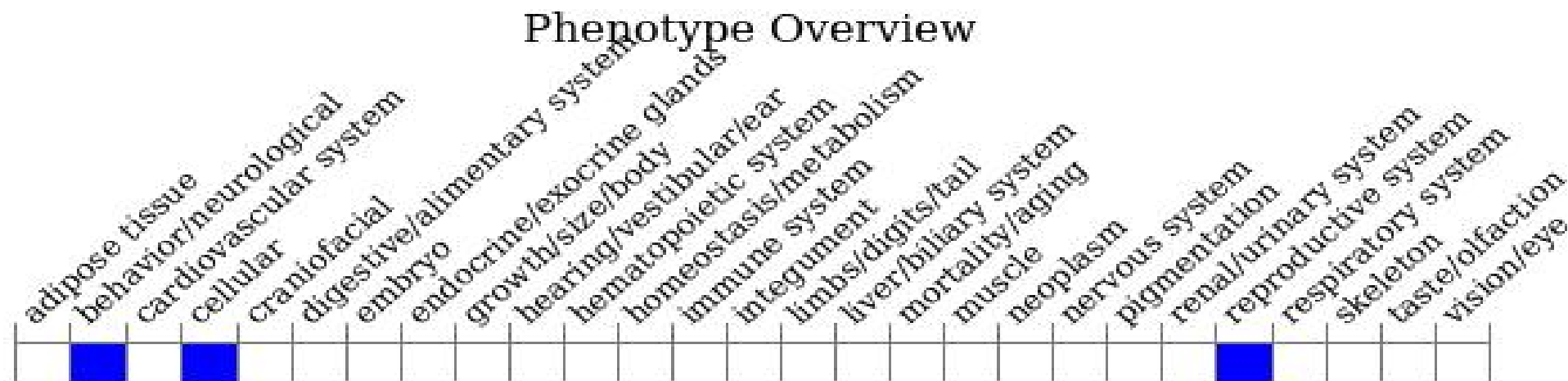
Genomic Information



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 risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.