

***Gorasp1* Cas9-CKO Strategy**

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Project Overview

Project Name

Gorasp1

Project type

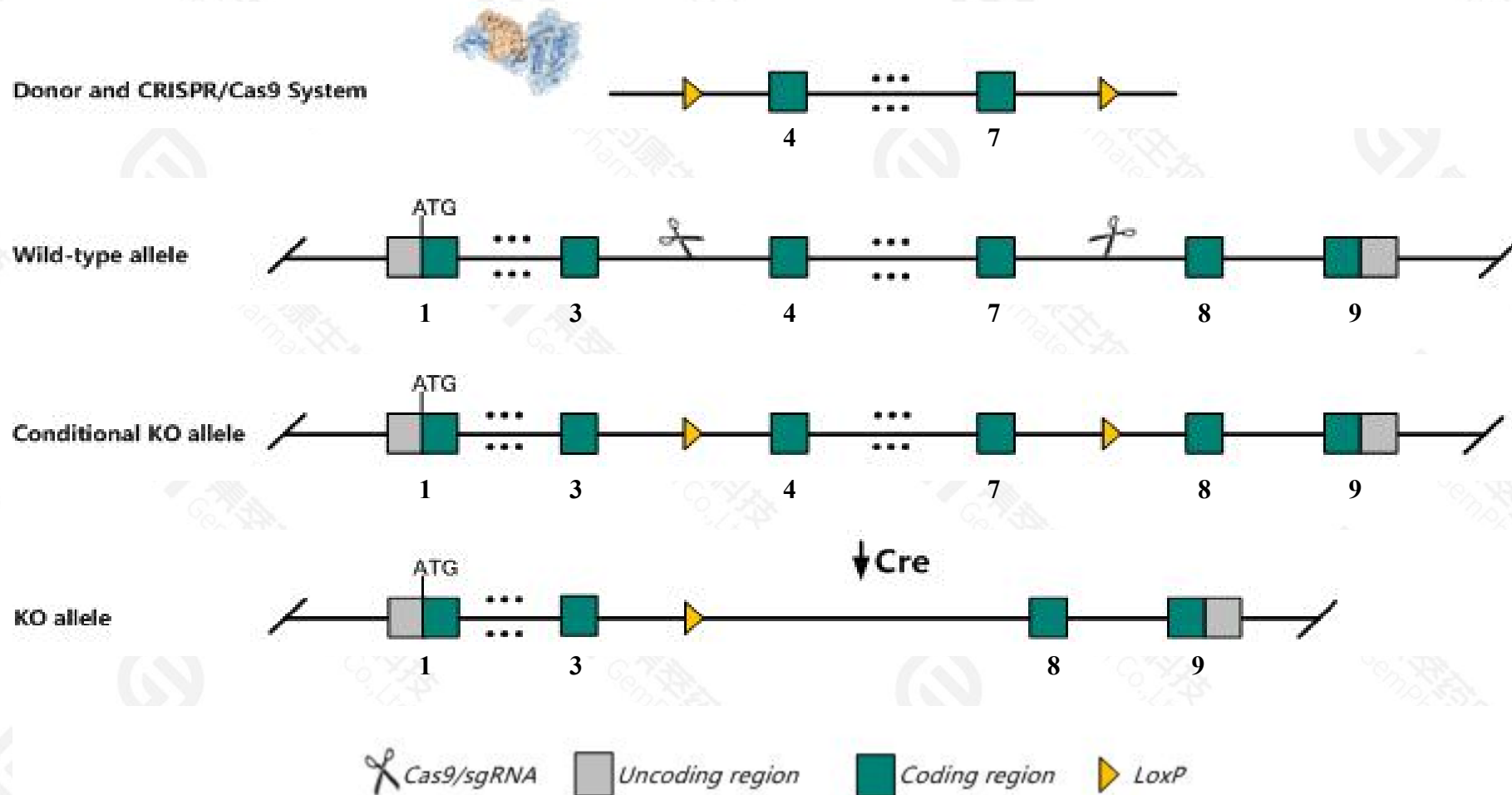
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Gorasp1* gene. The schematic diagram is as follows:



Technical routes

- The *Gorasp1* gene has 3 transcripts. According to the structure of *Gorasp1* gene, exon4-exon7 of *Gorasp1*-201(ENSMUST00000035099.9) transcript is recommended as the knockout region. The region contains 580bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Gorasp1* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor was constructed. Cas9, sgRNA 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 was 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, mice homozygous for a targeted disruption of this gene are viable, fertile and healthy with no detectable tissue defects. However, immortalized mutant embryonic fibroblasts show loss of cis Golgi integrity and glycosylation defects.
- The Intron7 is only 433bp, loxp insertion may affect mRNA splicing.
- The partial intron of *Cx3cr1* gene will be deleted together in this strategy.
- The N-terminal of *Gorasp1* gene will remain several amino acids, it may remain the partial function of *Gorasp1* gene.
- The *Gorasp1* gene is located on the Chr9. 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)

Gorasp1 golgi reassembly stacking protein 1 [Mus musculus (house mouse)]

Gene ID: 74498, updated on 19-Jan-2021

Summary



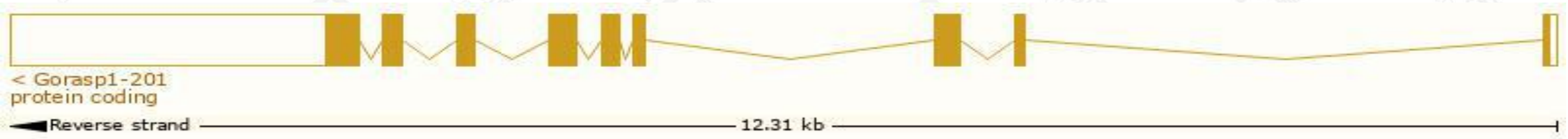
Official Symbol	Gorasp1 provided by MGI
Official Full Name	golgi reassembly stacking protein 1 provided by MGI
Primary source	MGI:MGI:1921748
See related	Ensembl:ENSMUSG00000032513
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	5430411C10Rik, GOL, GOLPH5, GRA, GRASP65, P6, P65
Expression	Ubiquitous expression in adrenal adult (RPKM 25.3), genital fat pad adult (RPKM 21.1) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

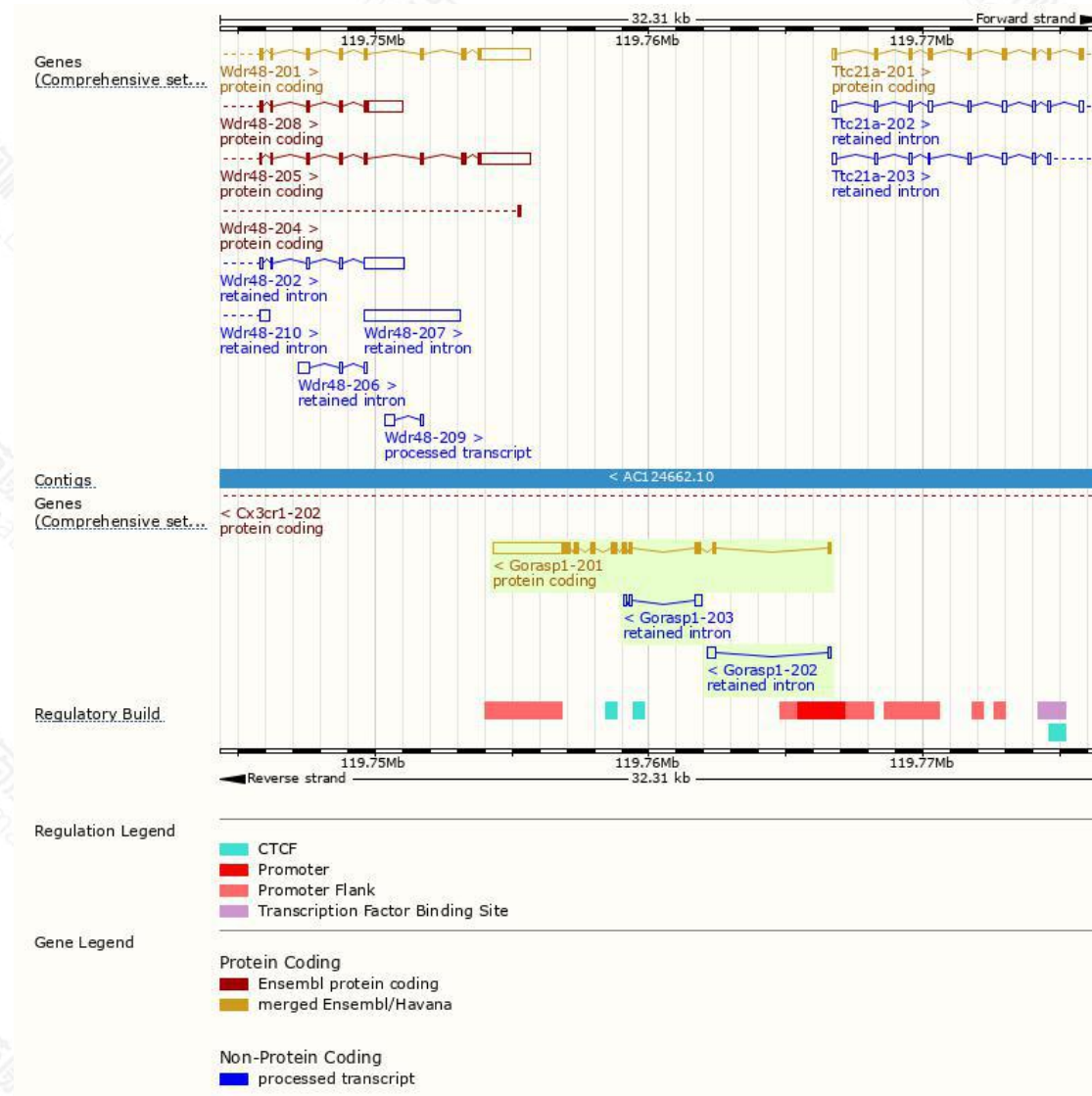
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Gorasp1-201	ENSMUST00000035099.9	3906	446aa	Protein coding	CCDS23619		TSL:1 , GENCODE basic , APPRIS P1 ,
Gorasp1-203	ENSMUST00000214118.2	434	No protein	Retained intron	-		TSL:2 ,
Gorasp1-202	ENSMUST00000213409.2	386	No protein	Retained intron	-		TSL:2 ,

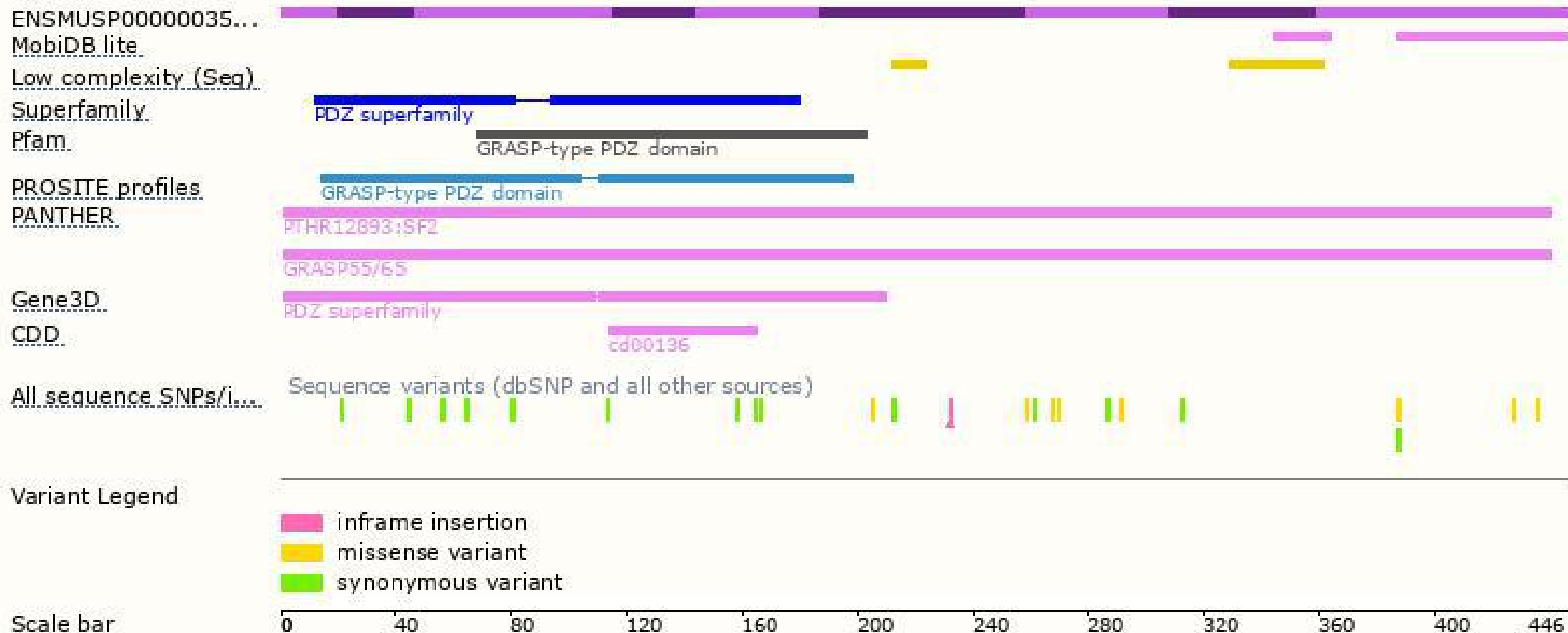
The strategy is based on the design of *Gorasp1-201* transcript,the transcription is shown below:



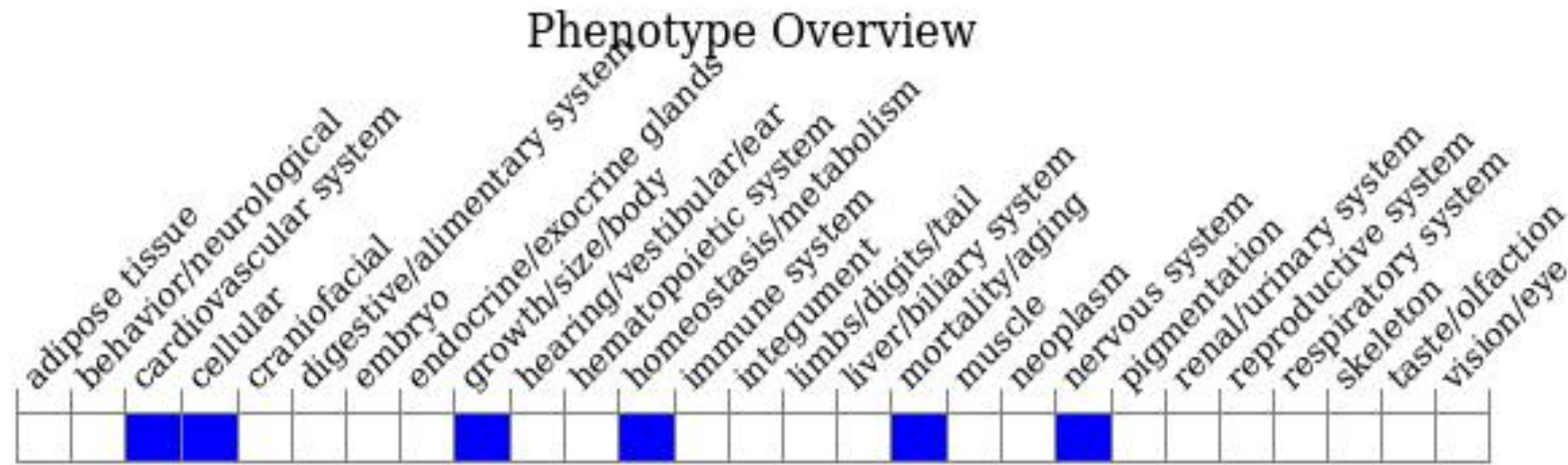
Genomic location distribution



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 targeted disruption of this gene are viable, fertile and healthy with no detectable tissue defects. However, immortalized mutant embryonic fibroblasts show loss of cis Golgi integrity and glycosylation defects.

If you have any questions, you are welcome to inquire.

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