

# Sec24d Cas9-CKO Strategy

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## **Project Overview**



**Project Name** 

Sec24d

**Project type** 

Cas9-CKO

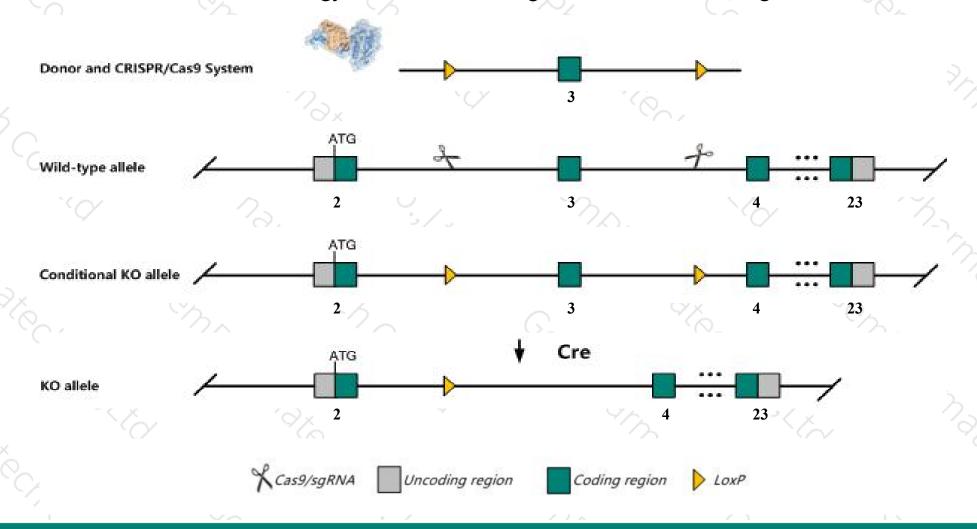
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Sec24d gene. The schematic diagram is as follows:



### Technical routes



- The Sec24d gene has 8 transcripts. According to the structure of Sec24d gene, exon3 of Sec24d-201(ENSMUST00000047923.11) transcript is recommended as the knockout region. The region contains 124bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Sec24d* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector 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.

### **Notice**



- > According to the existing MGI data,mice homozygous for a knock-out allele exhibit early embryonic lethality. A hypomorphic gene trap allele results in lethality during organogenesis.
- ➤ The transcript of *Sec24d*-202&203&204&205&207&208 may not be affected.
- > The Sec24d gene is located on the Chr3. 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)



#### Sec24d Sec24 related gene family, member D (S. cerevisiae) [Mus musculus (house mouse)]

Gene ID: 69608, updated on 13-Mar-2020

#### Summary

☆ ?

Official Symbol Sec24d provided by MGI

Official Full Name Sec24 related gene family, member D (S. cerevisiae) provided by MGI

Primary source MGI:MGI:1916858

See related Ensembl:ENSMUSG00000039234

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 2310020L09Rik, Gm1349

Expression Broad expression in adrenal adult (RPKM 64.8), duodenum adult (RPKM 50.4) and 24 other tissuesSee more

Orthologs <u>human</u> all

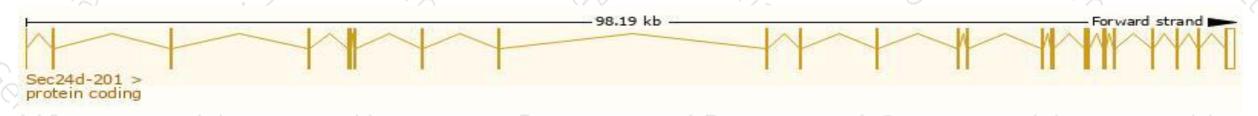
## Transcript information (Ensembl)



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

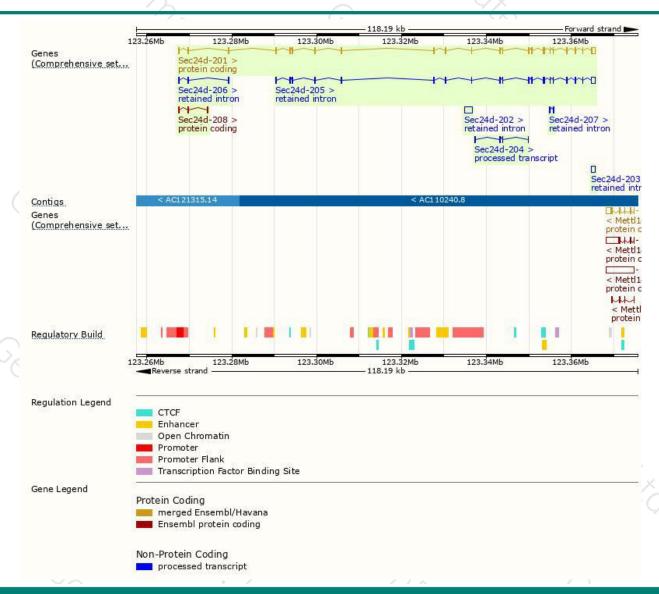
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Sec24d-201	ENSMUST00000047923.11	3903	1032aa	Protein coding	CCDS38621	Q6NXL1	TSL:1 GENCODE basic APPRIS P1
Sec24d-208	ENSMUST00000200333.1	307	<u>47aa</u>	Protein coding	1 <del>-</del>	A0A0G2JGJ5	TSL:1 GENCODE basic
Sec24d-204	ENSMUST00000196631.1	376	No protein	Processed transcript	1/4	-	TSL:3
Sec24d-205	ENSMUST00000197291.4	3649	No protein	Retained intron	2	-	TSL:5
Sec24d-202	ENSMUST00000196167.1	1947	No protein	Retained intron	15	ē	TSL:NA
Sec24d-203	ENSMUST00000196482.1	923	No protein	Retained intron	. e <del>.</del>		TSL:NA
Sec24d-207	ENSMUST00000200309.1	421	No protein	Retained intron	<u> </u>	ū.	TSL:2
Sec24d-206	ENSMUST00000198210.1	409	No protein	Retained intron	( <u>4</u>	2	TSL:2

The strategy is based on the design of Sec24d-201 transcript, the transcription is shown below:



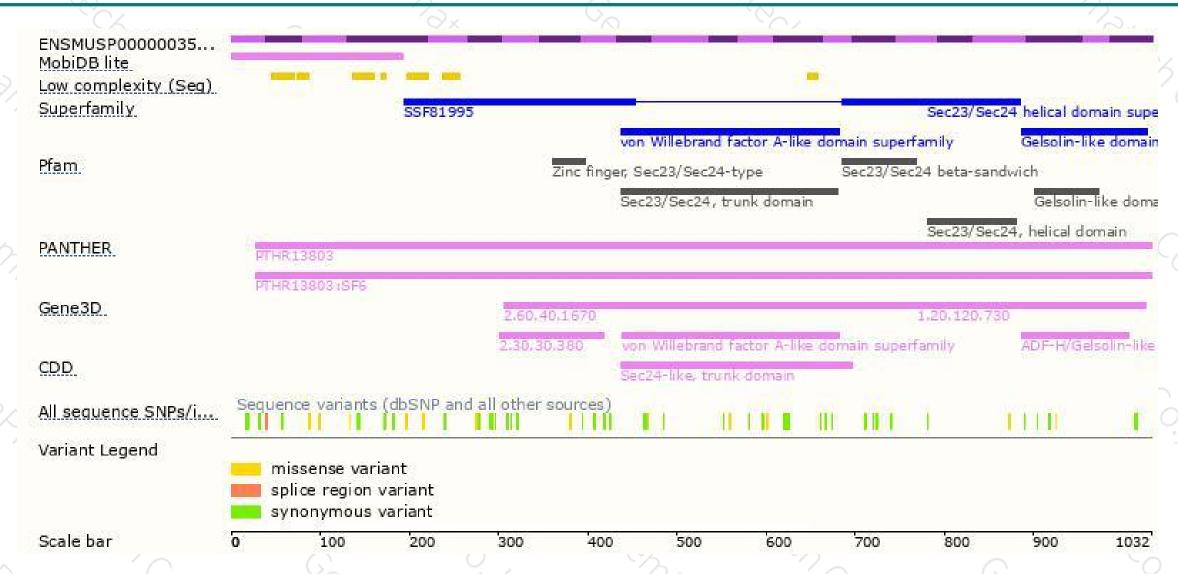
### Genomic location distribution





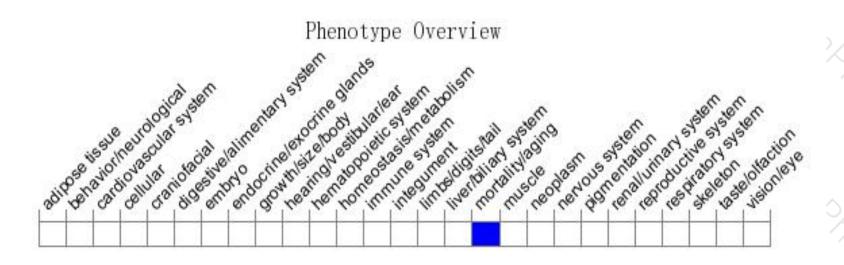
### Protein domain





## 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 knock-out allele exhibit early embryonic lethality. A hypomorphic gene trap allele results in lethality during organogenesis.



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

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