

# *C2cd4c* Cas9-CKO Strategy

**Designer:** Daohua Xu

**Reviewer:** Huimin Su

**Design Date:** 2020-5-20

# Project Overview

**Project Name**

***C2cd4c***

**Project type**

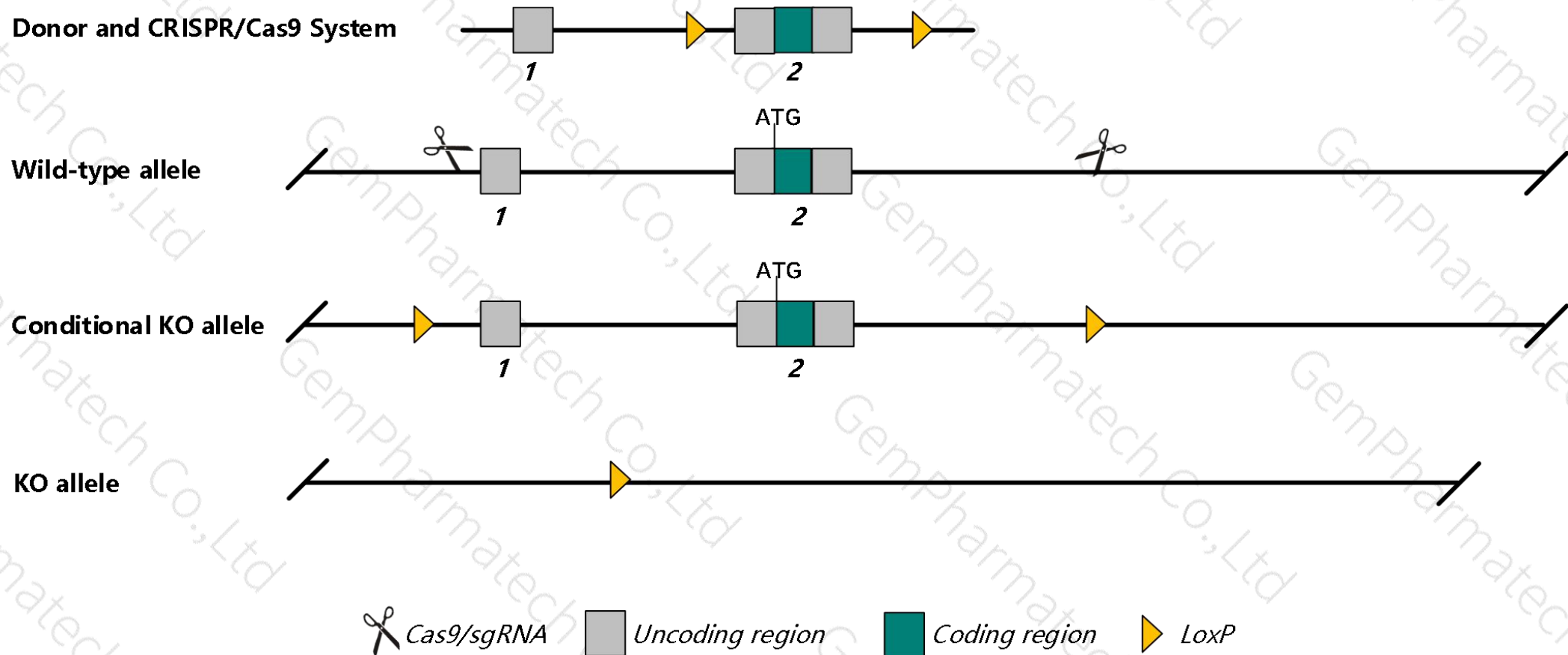
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *C2cd4c* gene. The schematic diagram is as follows:



# Technical routes

- The *C2cd4c* gene has 2 transcripts. According to the structure of *C2cd4c* gene, exon1-exon2 of *C2cd4c-201* (ENSMUST00000059699.8) transcript is recommended as the knockout region. The region contains all of the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *C2cd4c* 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, mice homozygous for a knock-out allele exhibit decreased body weight but normal glucose homeostasis and pancreas development.
- The *C2cd4c* gene is located on the Chr10. 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)

## C2cd4c C2 calcium-dependent domain containing 4C [Mus musculus (house mouse)]

Gene ID: 237397, updated on 13-Mar-2020

### Summary



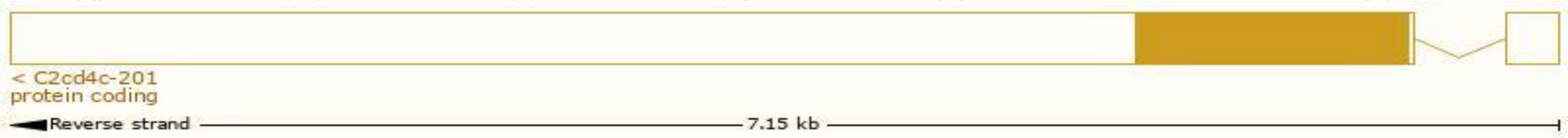
<b>Official Symbol</b>	C2cd4c provided by <a href="#">MGI</a>
<b>Official Full Name</b>	C2 calcium-dependent domain containing 4C provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:2685084</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000045912</a>
<b>Gene type</b>	protein 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>	4932409I22Rik, Fam148c, Gm238
<b>Expression</b>	Biased expression in CNS E18 (RPKM 2.9), cortex adult (RPKM 1.9) and 12 other tissues <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>

# Transcript information (Ensembl)

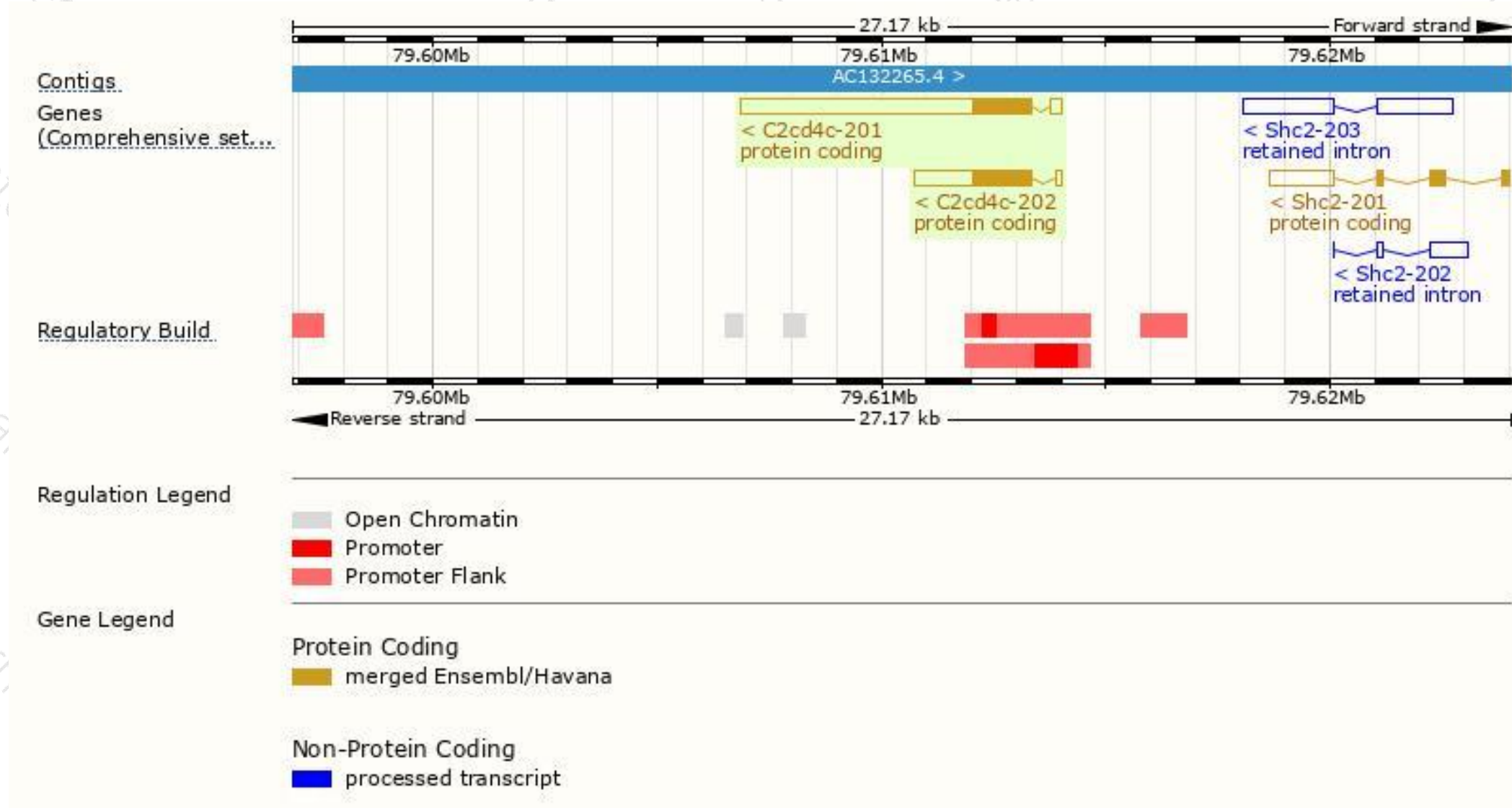
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
C2cd4c-201	<a href="#">ENSMUST00000059699.8</a>	6729	<a href="#">419aa</a>	Protein coding	<a href="#">CCDS23979</a>	<a href="#">Q5HZI2</a>	TSL:1 GENCODE basic APPRIS P1
C2cd4c-202	<a href="#">ENSMUST00000178228.2</a>	2761	<a href="#">419aa</a>	Protein coding	<a href="#">CCDS23979</a>	<a href="#">Q5HZI2</a>	TSL:1 GENCODE basic APPRIS P1

The strategy is based on the design of *C2cd4c-201* transcript,the transcription is shown below:

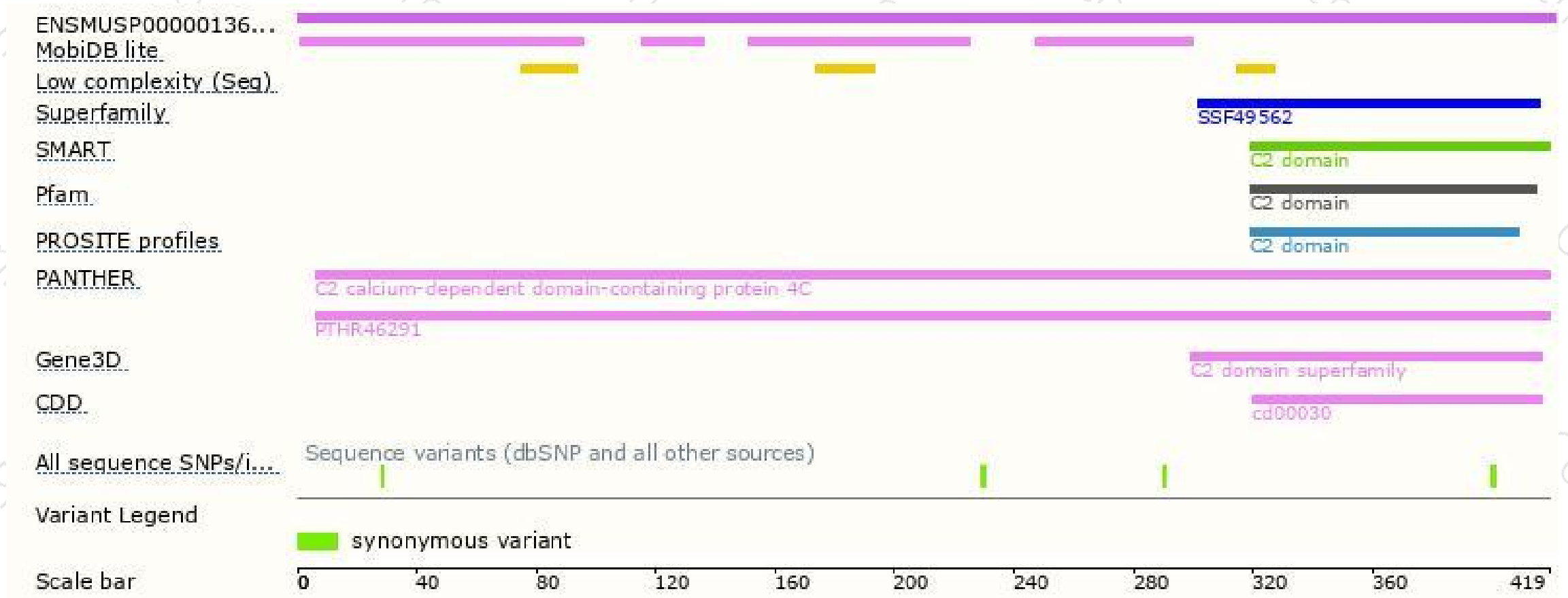


# Genomic location distribution





# 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 decreased body weight but normal glucose homeostasis and pancreas development.

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

Tel: 400-9660890

