

***Nkd1* Cas9-CKO Strategy**

Designer: Yun Li

Reviewer: Shuang Zhang

Design Date: 2021-7-16

Project Overview

Project Name

Nkd1

Project type

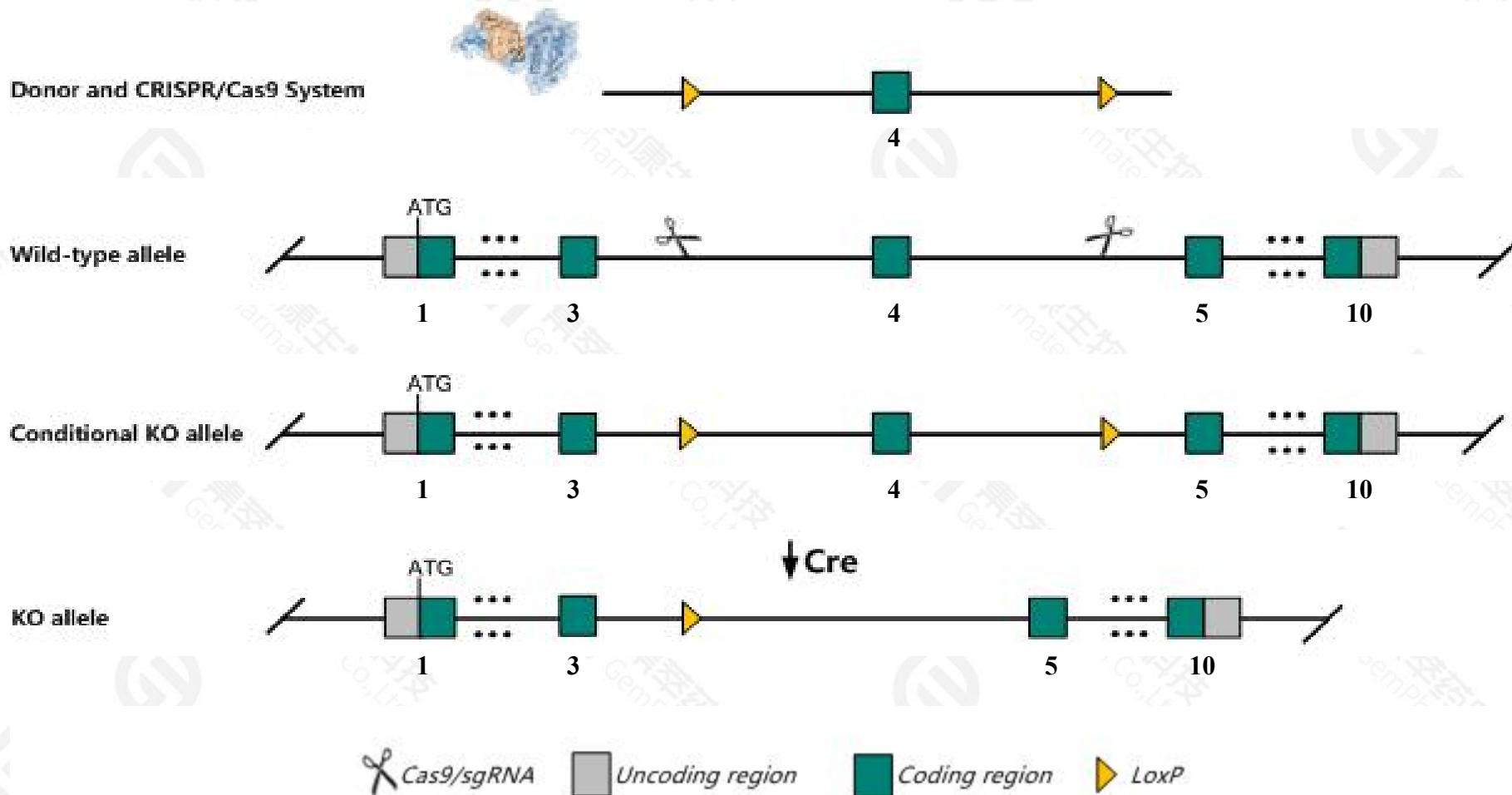
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Nkd1* gene. The schematic diagram is as follows:



Technical routes

- The *Nkd1* gene has 3 transcripts. According to the structure of *Nkd1* gene, exon4 of *Nkd1-201*(ENSMUST00000034086.13) transcript is recommended as the knockout region. The region contains 67bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Nkd1* 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, homozygous null mice display reduced male fertility with oligozoospermia, small testis, and small seminiferous tubules.
- The *Nkd1* gene is located on the Chr8. 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)

Nkd1 naked cuticle 1 [Mus musculus (house mouse)]

Gene ID: 93960, updated on 17-Nov-2020

Summary



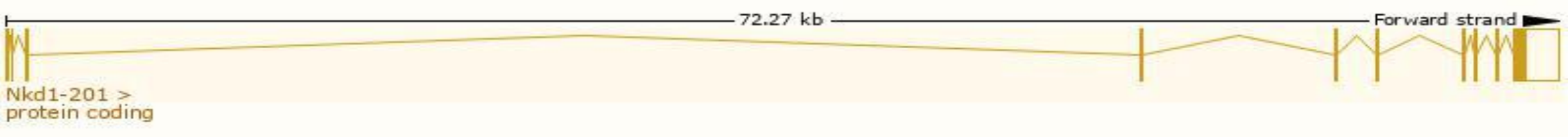
Official Symbol	Nkd1 provided by MGI
Official Full Name	naked cuticle 1 provided by MGI
Primary source	MGI:MGI:2135954
See related	Ensembl:ENSMUSG00000031661
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2810434J10Rik, 9030215G15Rik, Nkd
Expression	Broad expression in lung adult (RPKM 30.7), bladder adult (RPKM 15.6) and 21 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

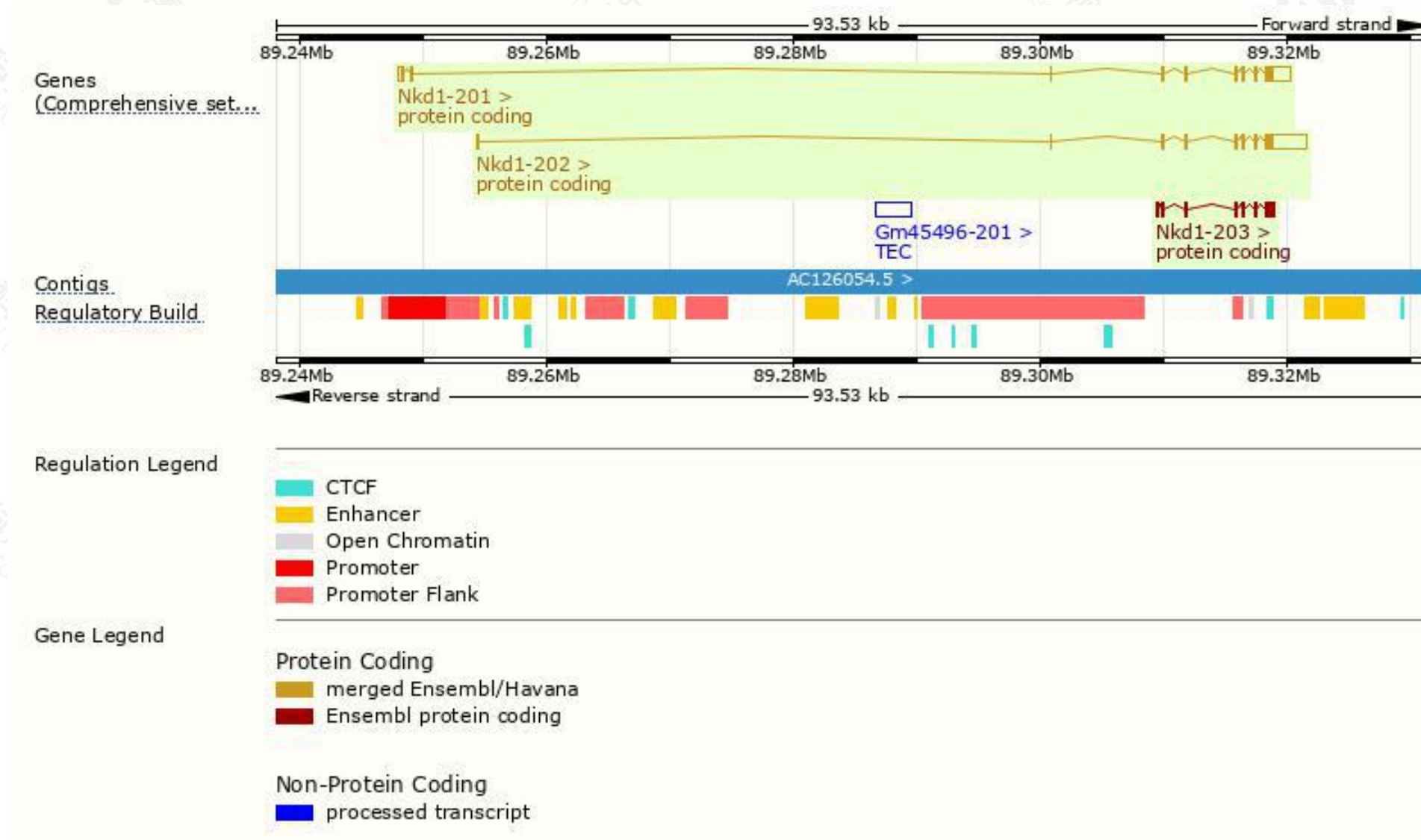
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nkd1-202	ENSMUST00000169179.3	4081	367aa	Protein coding	CCDS52631		TSL:2 , GENCODE basic ,
Nkd1-201	ENSMUST00000034086.13	3035	471aa	Protein coding	CCDS22511		TSL:1 , GENCODE basic , APPRIS P1 ,
Nkd1-203	ENSMUST00000211113.2	1474	367aa	Protein coding	CCDS52631		TSL:5 , GENCODE basic ,

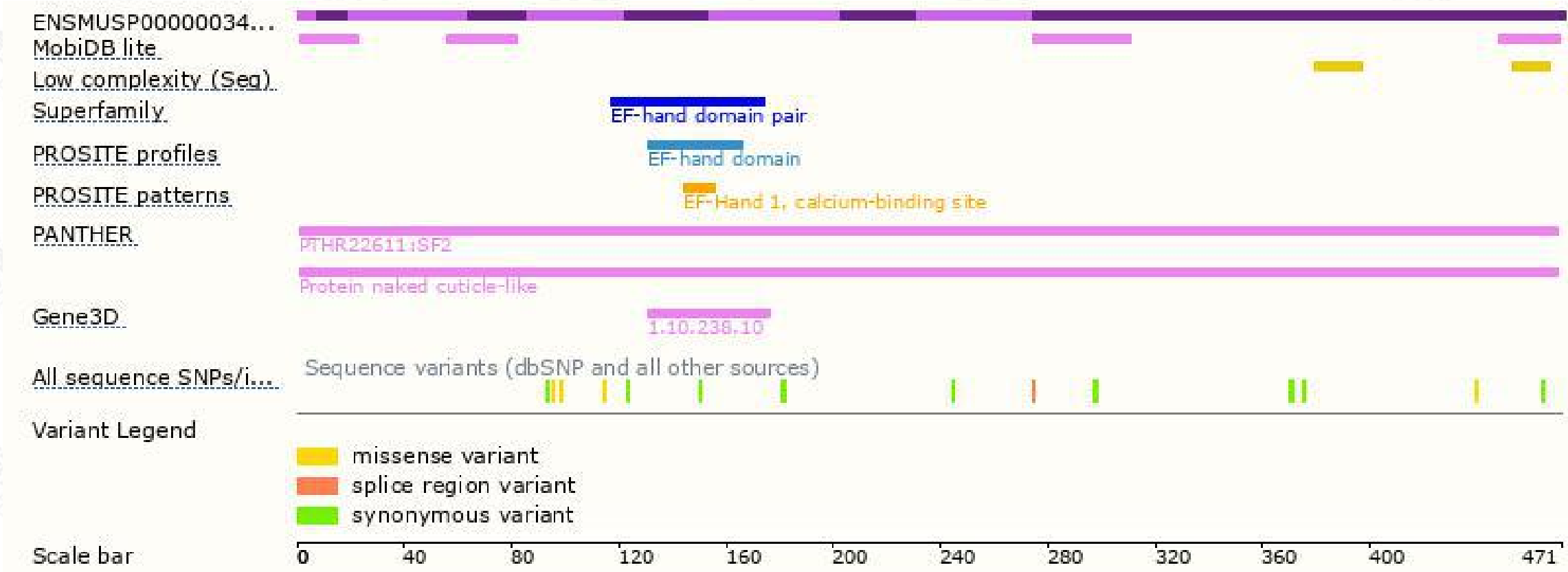
The strategy is based on the design of *Nkd1-201* transcript,the transcription is shown below:



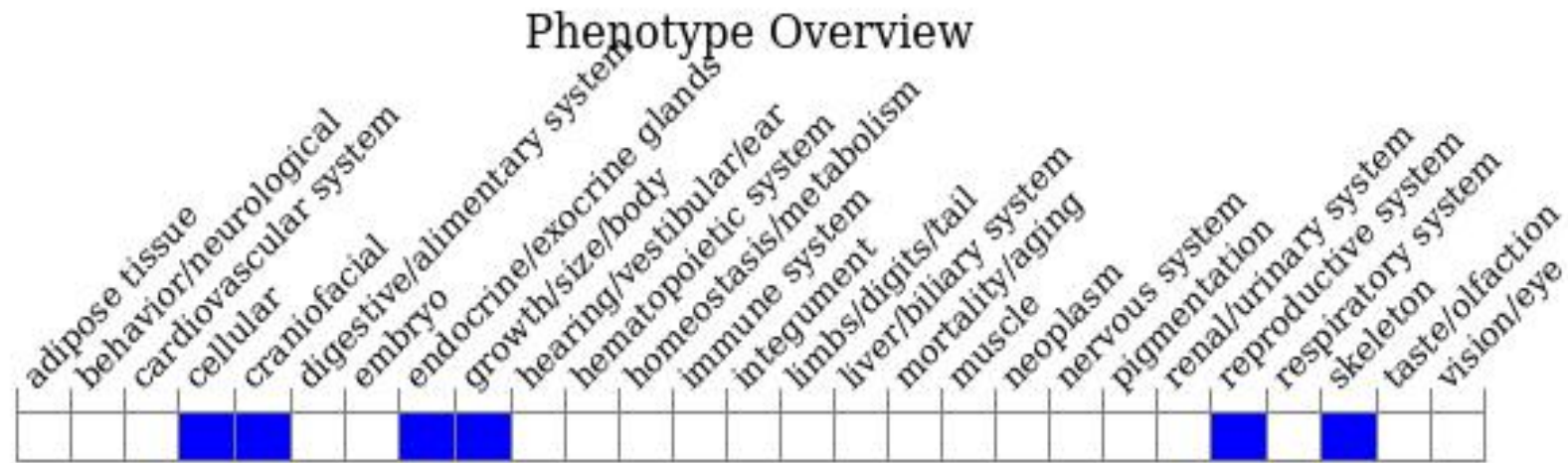
Genomic location distribution



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, homozygous null mice display reduced male fertility with oligozoospermia, small testis, and small seminiferous tubules.

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

Tel: 025-5864 1534

