

***Dennd1b* Cas9-CKO Strategy**

Designer: Jia Yu

Reviewer: Xiaojing Li

Design Date: 2020-10-13

Project Overview

Project Name

Dennd1b

Project type

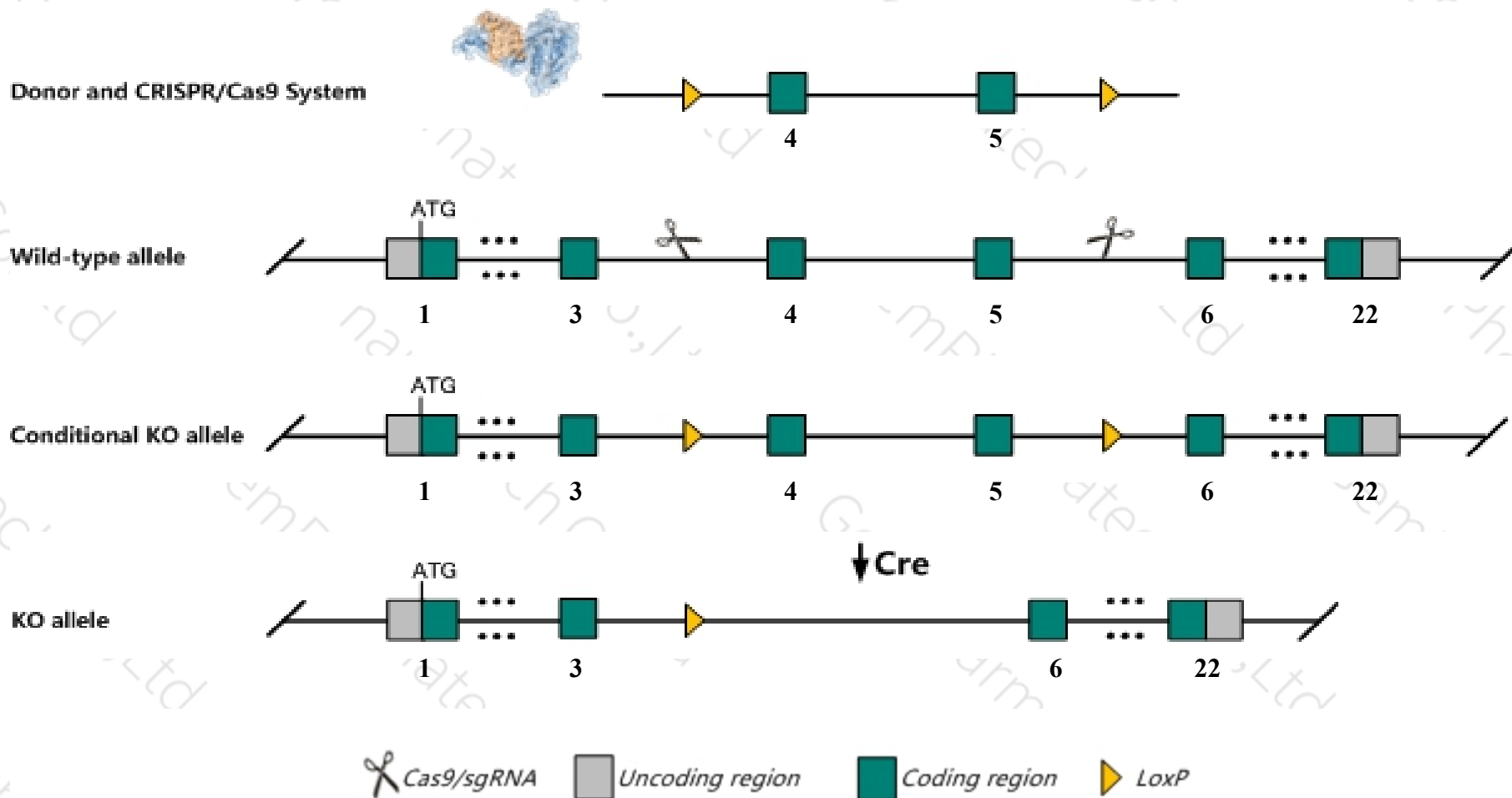
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Dennd1b* gene. The schematic diagram is as follows:



- The *Dennd1b* gene has 9 transcripts. According to the structure of *Dennd1b* gene, exon4-exon5 of *Dennd1b*-202(ENSMUST00000168527.7) transcript is recommended as the knockout region. The region contains 170bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Dennd1b* 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.

- According to the existing MGI data, homozygous KO results in enhanced allergic responses to aerosolized antigen challenges caused by delayed TCR down-modulation following receptor activation in T helper 2 cells.
- The *Dennd1b* gene is located on the Chr1. 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)

Dennd1b DENN/MADD domain containing 1B [Mus musculus (house mouse)]

Gene ID: 329260, updated on 13-Mar-2020

Summary



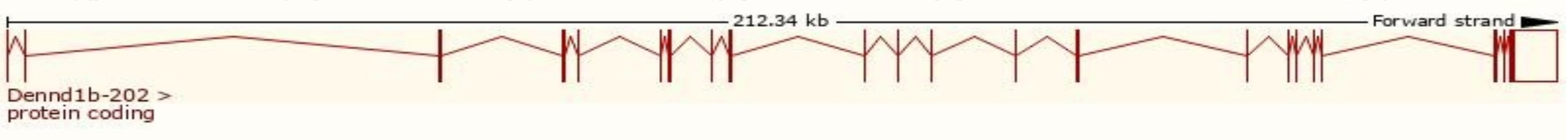
Official Symbol	Dennd1b provided by MGI
Official Full Name	DENN/MADD domain containing 1B provided by MGI
Primary source	MGI:MGI:2447812
See related	Ensembl:ENSMUSG00000056268
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	4632404N19Rik, 4930467M19Rik, 6820401H01Rik, F730008N07Rik
Expression	Ubiquitous expression in placenta adult (RPKM 2.2), thymus adult (RPKM 1.6) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

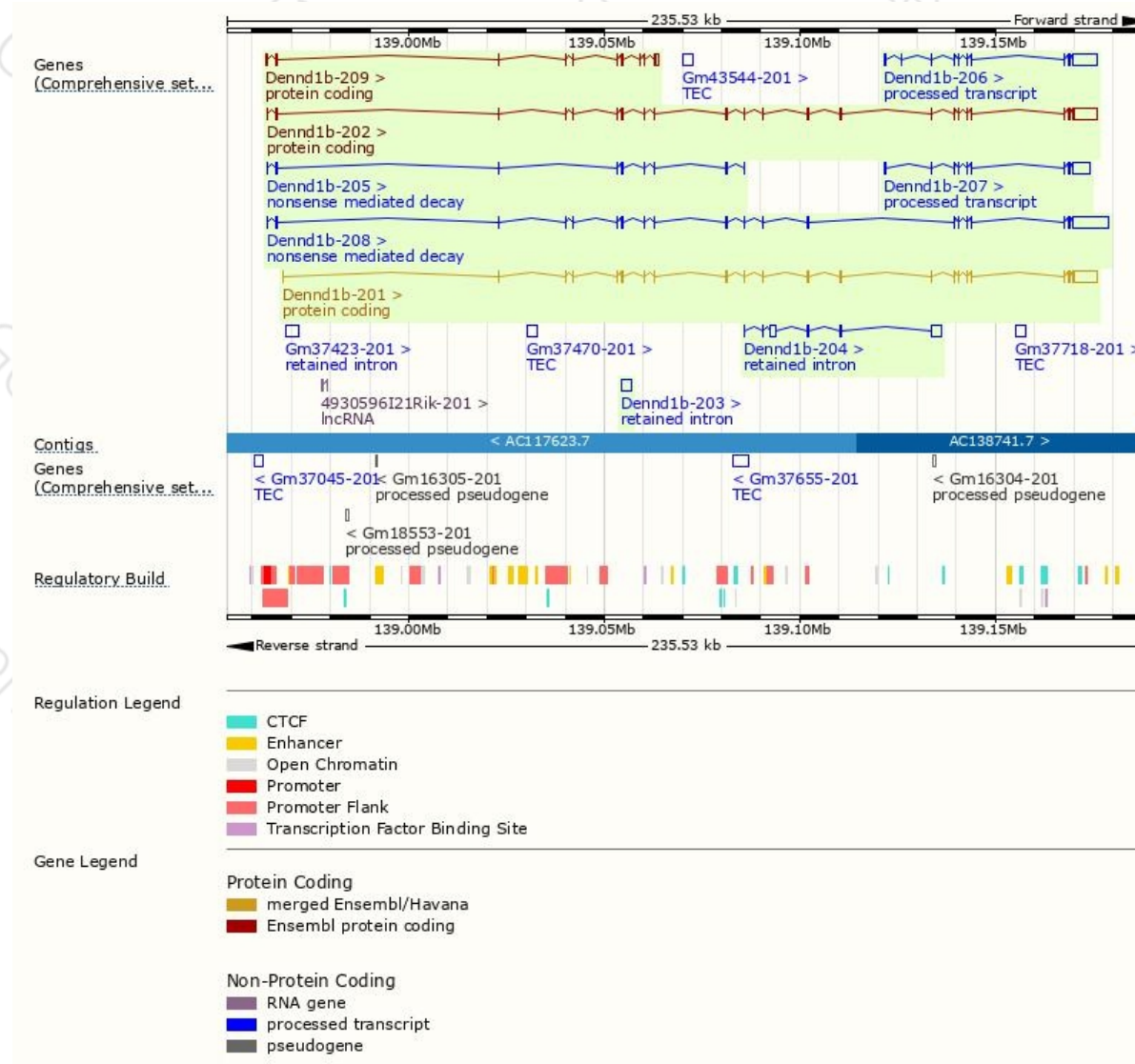
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Dennd1b-202	ENSMUST00000168527.7	8206	766aa	Protein coding	CCDS48384	Q3U1T9	TSL:5 GENCODE basic APPRIS P1
Dennd1b-201	ENSMUST00000094505.5	8073	691aa	Protein coding	CCDS35728	Q3U1T9	TSL:1 GENCODE basic
Dennd1b-209	ENSMUST00000200533.4	1853	232aa	Protein coding	-	Q3U1T9	TSL:1 GENCODE basic
Dennd1b-208	ENSMUST00000200429.4	10770	352aa	Nonsense mediated decay	-	A0A0G2JH11	TSL:5
Dennd1b-205	ENSMUST00000198759.4	730	47aa	Nonsense mediated decay	-	A0A0G2JGT6	TSL:5
Dennd1b-206	ENSMUST00000199634.1	7297	No protein	Processed transcript	-	-	TSL:1
Dennd1b-207	ENSMUST00000200295.4	5337	No protein	Processed transcript	-	-	TSL:1
Dennd1b-204	ENSMUST00000198418.1	4406	No protein	Retained intron	-	-	TSL:5
Dennd1b-203	ENSMUST00000196816.1	2796	No protein	Retained intron	-	-	TSL:NA

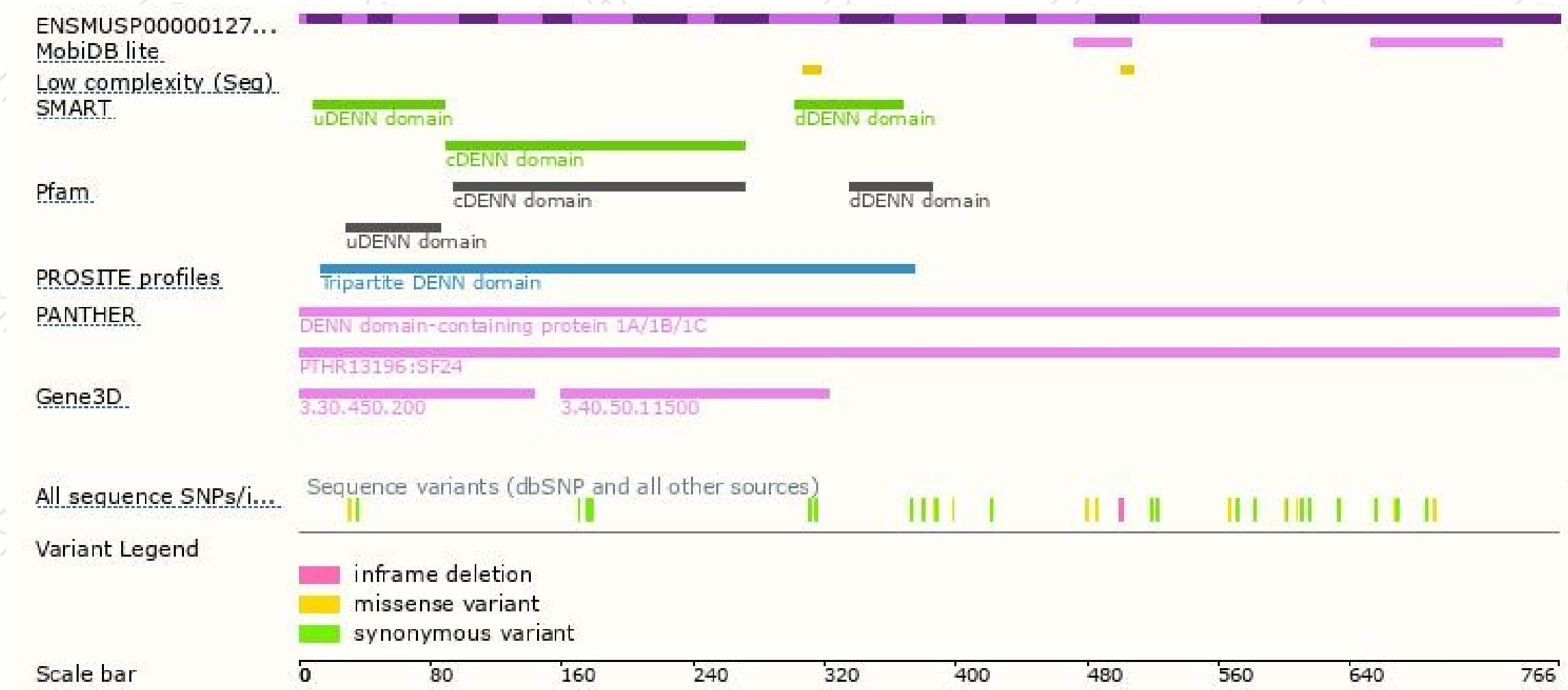
The strategy is based on the design of *Dennd1b-202* transcript,the transcription is shown below:



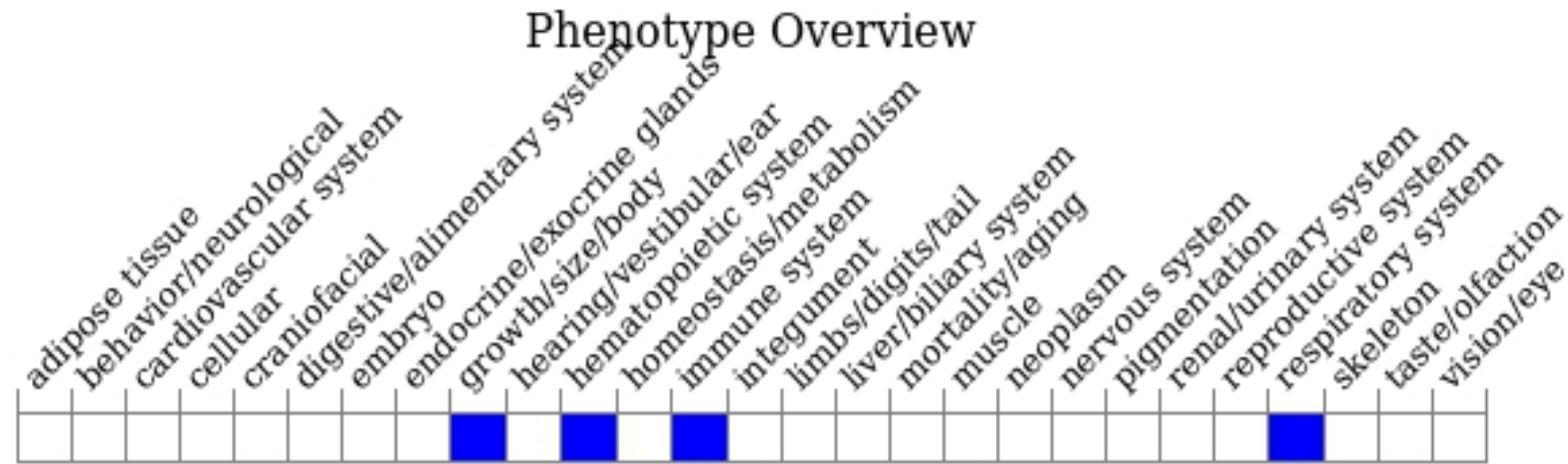
Genomic location distribution



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 KO results in enhanced allergic responses to aerosolized antigen challenges caused by delayed TCR down-modulation following receptor activation in T helper 2 cells.

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

Tel: 025-5864 1534

