

***Hectd2* Cas9-CKO Strategy**

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Project Overview

Project Name

Hectd2

Project type

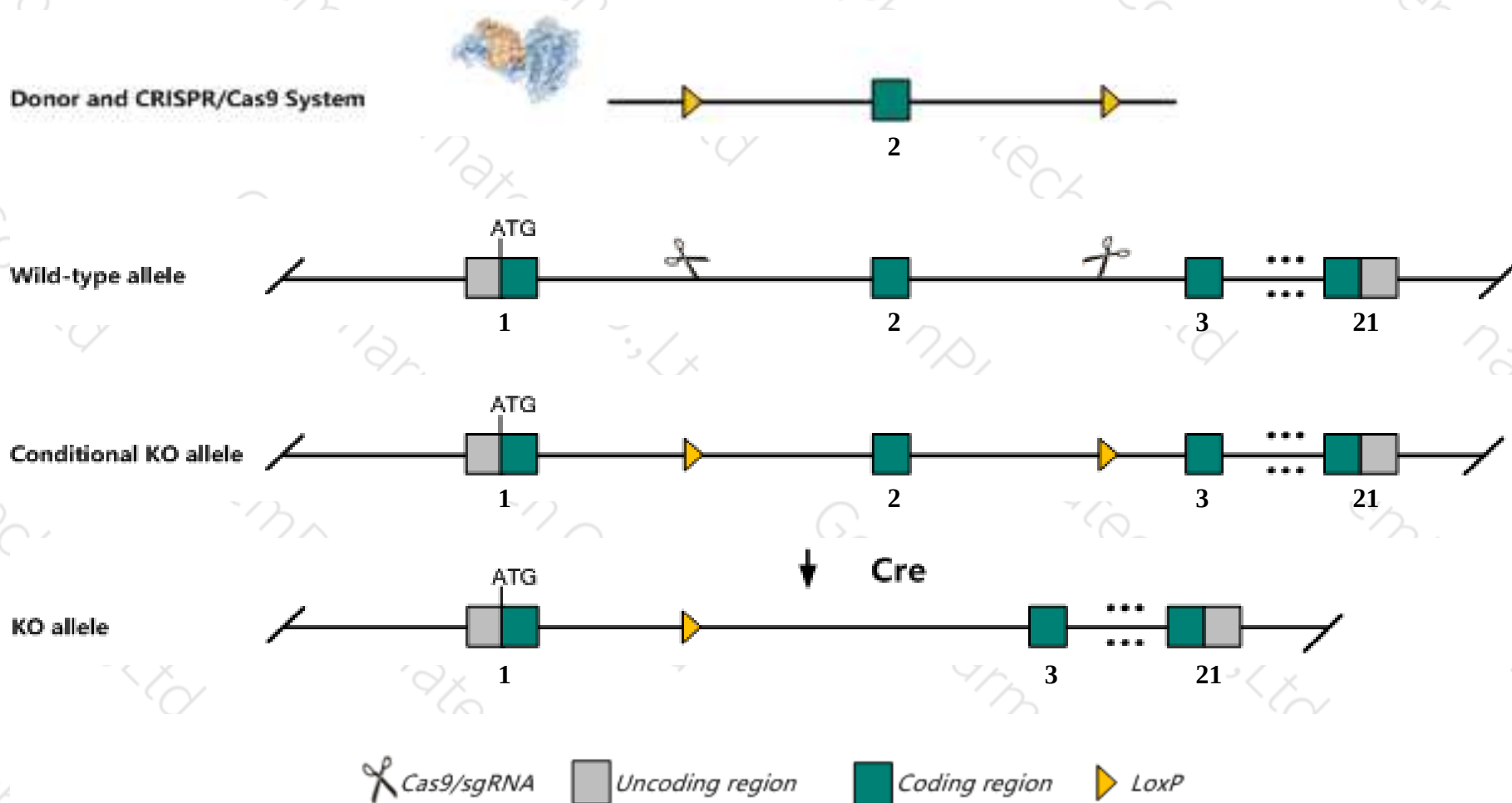
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Hectd2* gene. The schematic diagram is as follows:



- The *Hectd2* gene has 9 transcripts. According to the structure of *Hectd2* gene, exon2 of *Hectd2*-201 (ENSMUST00000047247.11) transcript is recommended as the knockout region. The region contains 130bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Hectd2* 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.

- The *Hectd2* gene is located on the Chr19. 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)

Hectd2 HECT domain E3 ubiquitin protein ligase 2 [Mus musculus (house mouse)]

Gene ID: 226098, updated on 3-Feb-2019

Summary



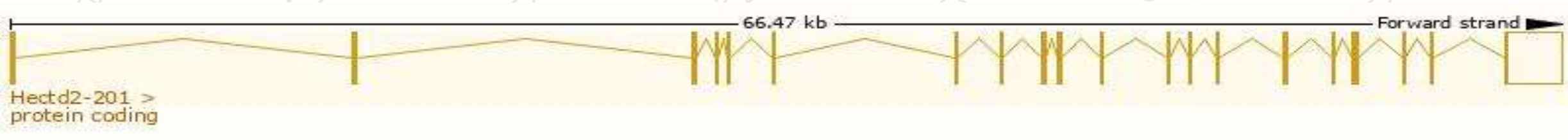
Official Symbol	Hectd2 provided by MGI
Official Full Name	HECT domain E3 ubiquitin protein ligase 2 provided by MGI
Primary source	MGI:MGI:2442663
See related	Ensembl:ENSMUSG00000041180
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	4921524L07, A630025O09Rik, AW212605
Expression	Broad expression in CNS E18 (RPKM 4.4), whole brain E14.5 (RPKM 3.6) and 16 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

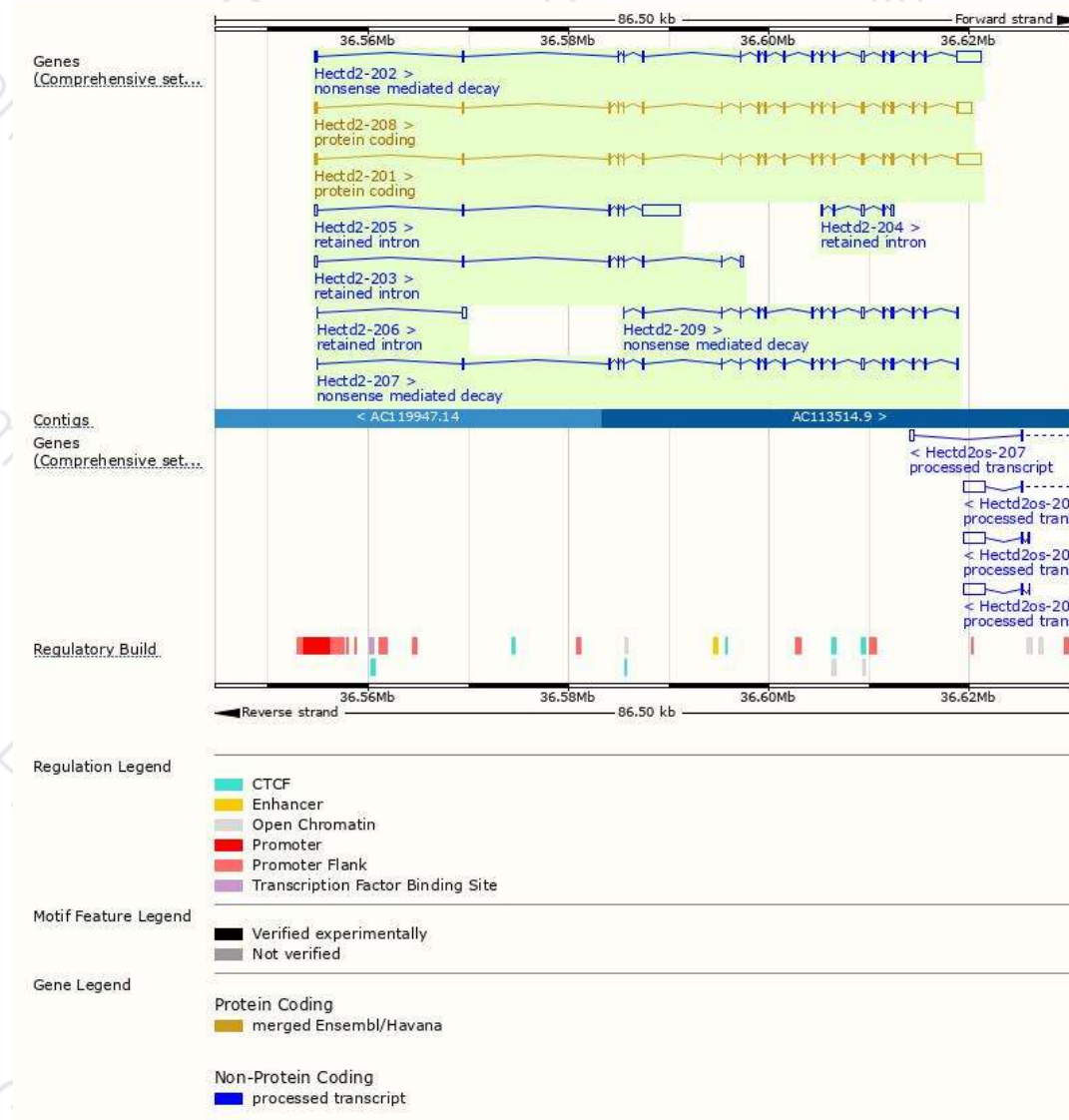
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Hectd2-201	ENSMUST00000047247.11	4731	774aa	Protein coding	CCDS29773	Q8CDU6	TSL:1 GENCODE basic APPRIS P3
Hectd2-208	ENSMUST00000169036.8	3842	775aa	Protein coding	CCDS50425	B2RPZ5	TSL:1 GENCODE basic APPRIS ALT 1
Hectd2-202	ENSMUST00000139215.7	4585	90aa	Nonsense mediated decay	-	D6REW0	TSL:1
Hectd2-207	ENSMUST00000155594.8	2214	360aa	Nonsense mediated decay	-	D6RI27	CDS 5' incomplete TSL:5
Hectd2-209	ENSMUST00000177381.7	1679	194aa	Nonsense mediated decay	-	H3BJF4	CDS 5' incomplete TSL:5
Hectd2-205	ENSMUST00000149629.8	4491	No protein	Retained intron	-	-	TSL:1
Hectd2-203	ENSMUST00000141503.1	1116	No protein	Retained intron	-	-	TSL:1
Hectd2-204	ENSMUST00000142404.1	712	No protein	Retained intron	-	-	TSL:3
Hectd2-206	ENSMUST00000155447.1	506	No protein	Retained intron	-	-	TSL:3

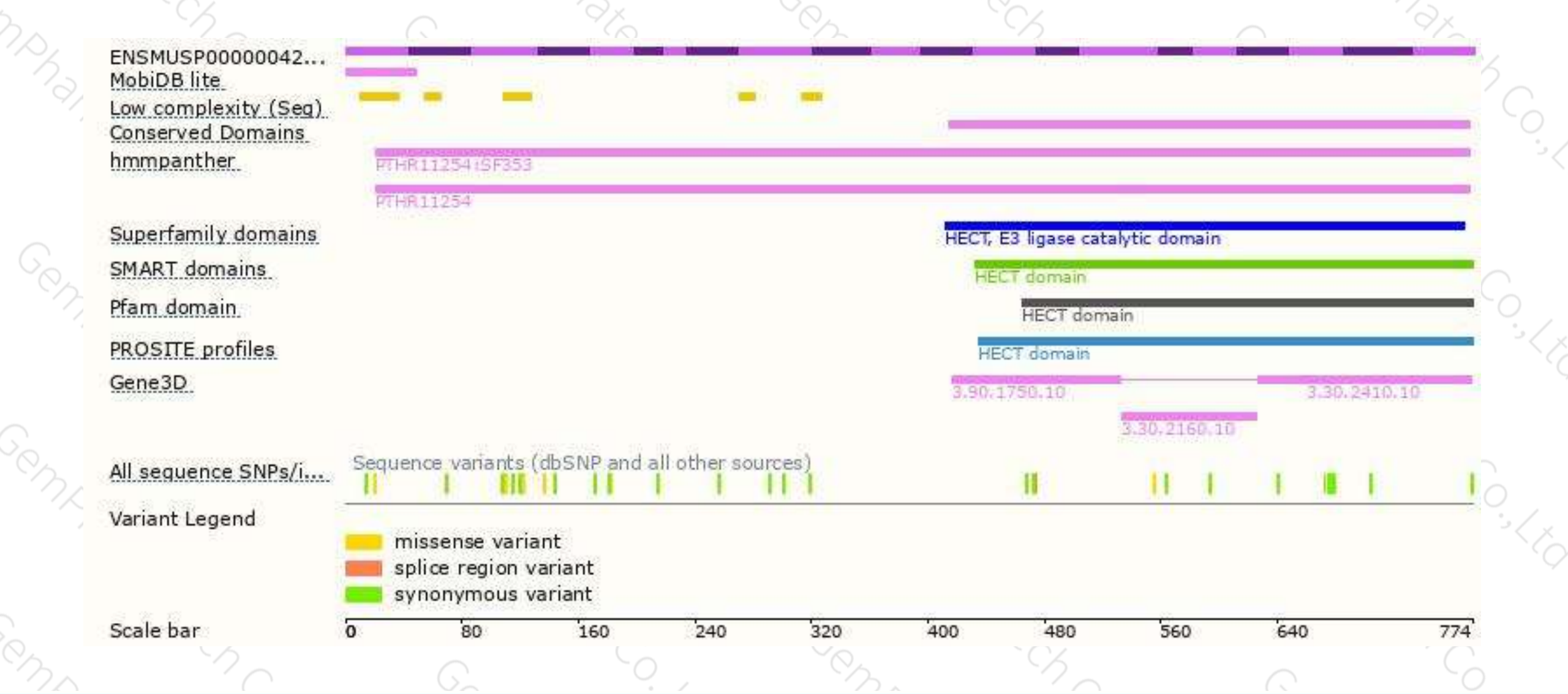
The strategy is based on the design of *Hectd2-201* transcript,The transcription is shown below



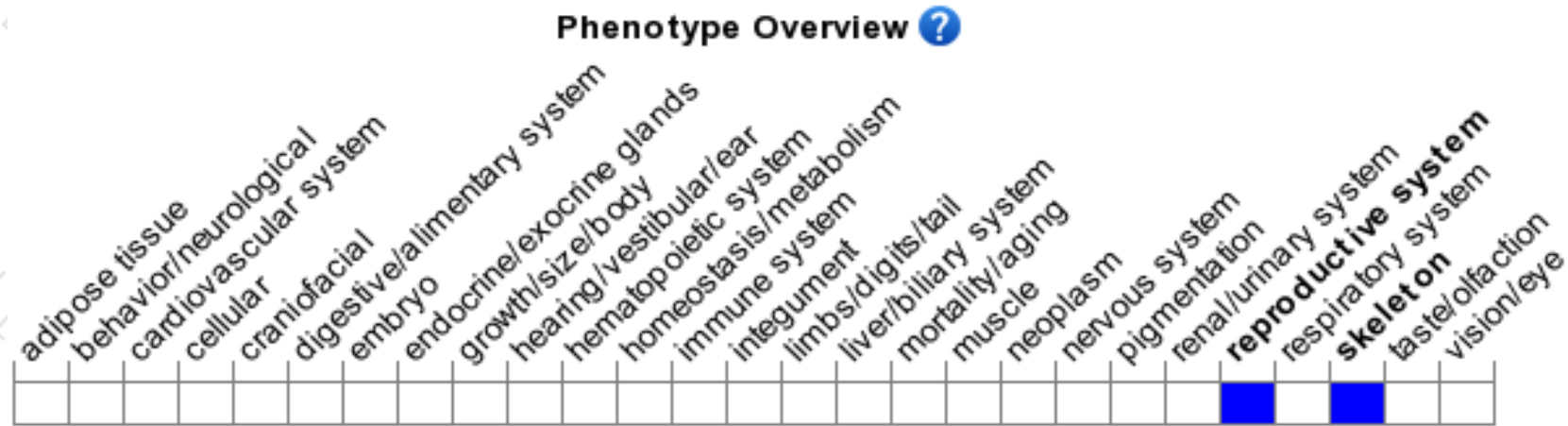
Genomic location distribution



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/>).

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

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