

Astl Cas9-CKO Strategy

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Reviewer:

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Project Overview

Project Name

Astl

Project type

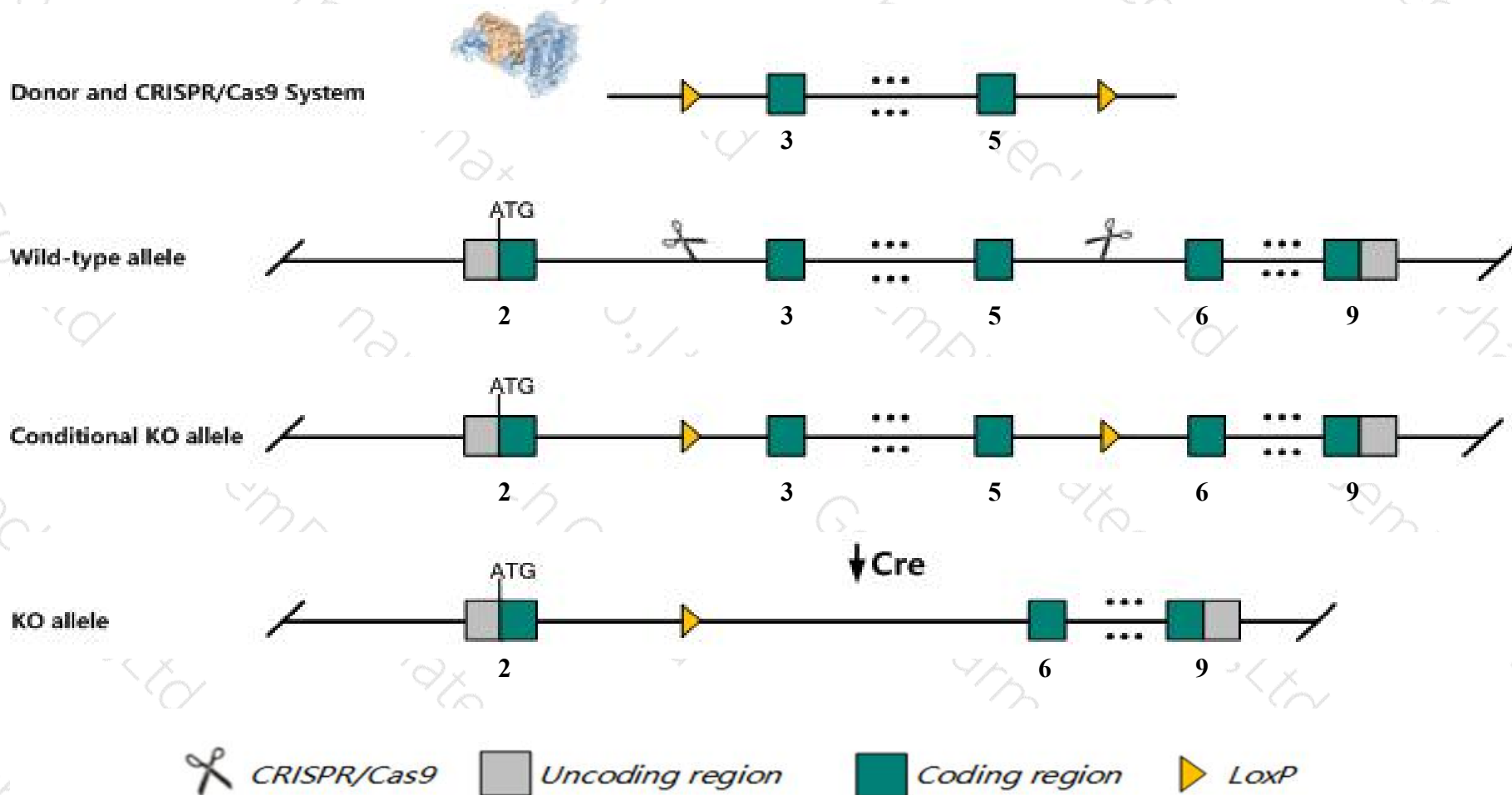
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



- The *Astl* gene has 4 transcripts. According to the structure of *Astl* gene, exon3-exon5 of *Astl-201* (ENSMUST00000059839.8) transcript is recommended as the knockout region. The region contains 274bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Astl* 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 knock-out alleles exhibit reduced female fertility.
- The *Astl* gene is located on the Chr2. 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)

Astl astacin-like metalloendopeptidase (M12 family) [*Mus musculus* (house mouse)]

Gene ID: 215095, updated on 13-Mar-2020

Summary

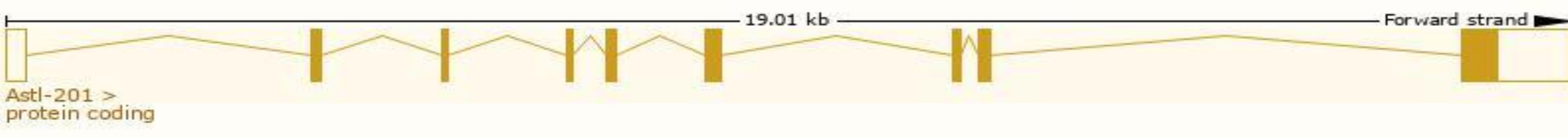
Official Symbol	Astl provided by MGI
Official Full Name	astacin-like metalloendopeptidase (M12 family) provided by MGI
Primary source	MGI:MGI:3046414
See related	Ensembl:ENSMUSG00000050468
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Sas1b; C87576; 7420700F21
Expression	Restricted expression toward ovary adult (RPKM 7.3) See more
Orthologs	human all

Transcript information (Ensembl)

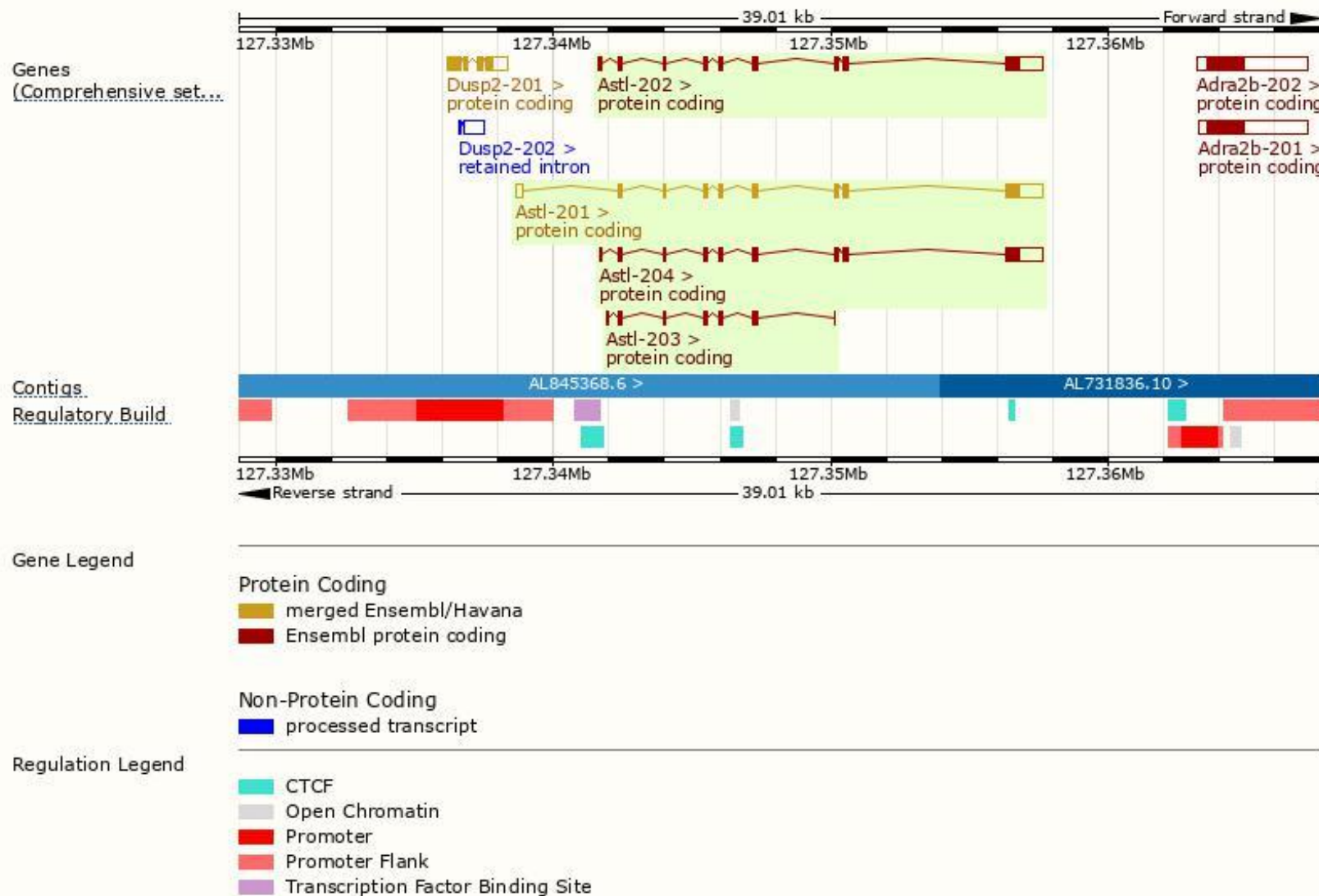
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Astl-201	ENSMUST00000059839.8	2377	414aa	Protein coding	CCDS16700	Q6HA09	TSL:1 GENCODE basic APPRIS P3
Astl-202	ENSMUST00000089673.9	2251	435aa	Protein coding	CCDS71138	Q6HA09	TSL:1 GENCODE basic APPRIS ALT2
Astl-204	ENSMUST00000179618.7	2236	414aa	Protein coding	CCDS16700	Q6HA09	TSL:1 GENCODE basic APPRIS P3
Astl-203	ENSMUST00000156747.1	648	193aa	Protein coding	-	A2ARG6	CDS 3' incomplete TSL:5

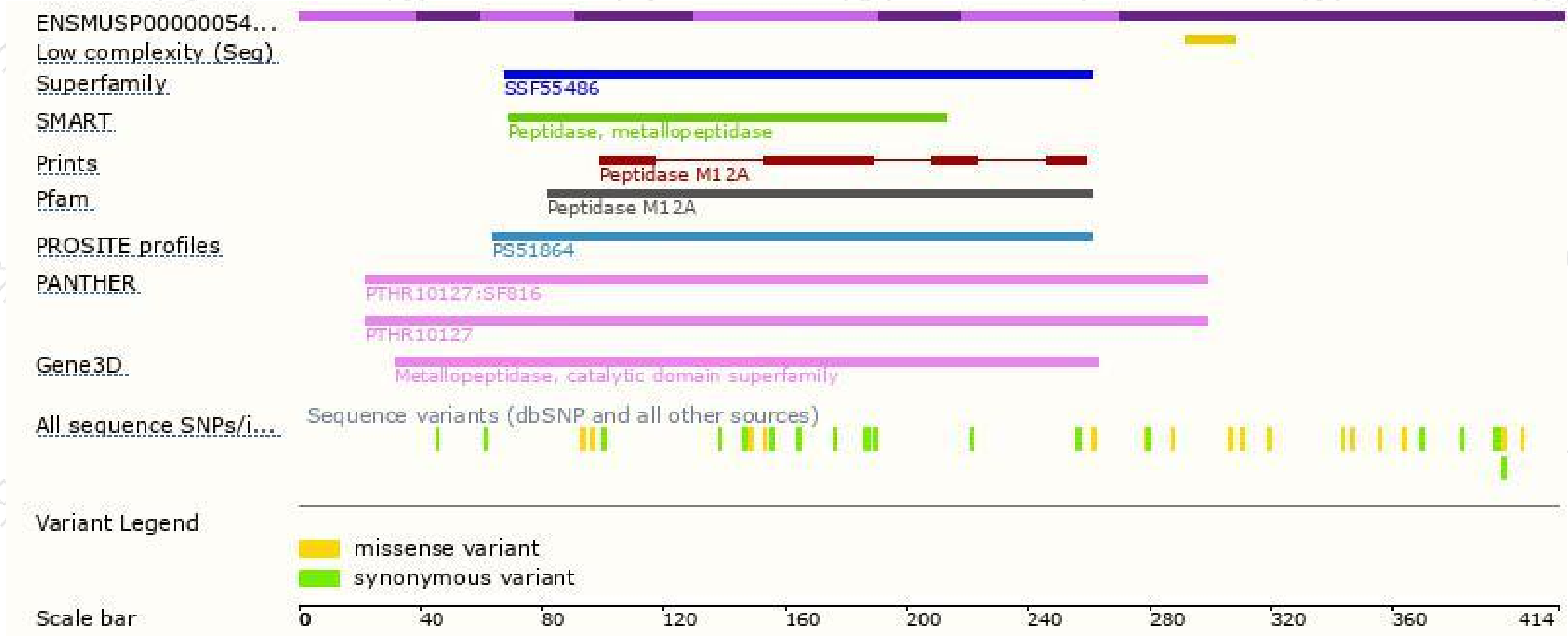
The strategy is based on the design of *Astl-201* transcript,The transcription is shown below



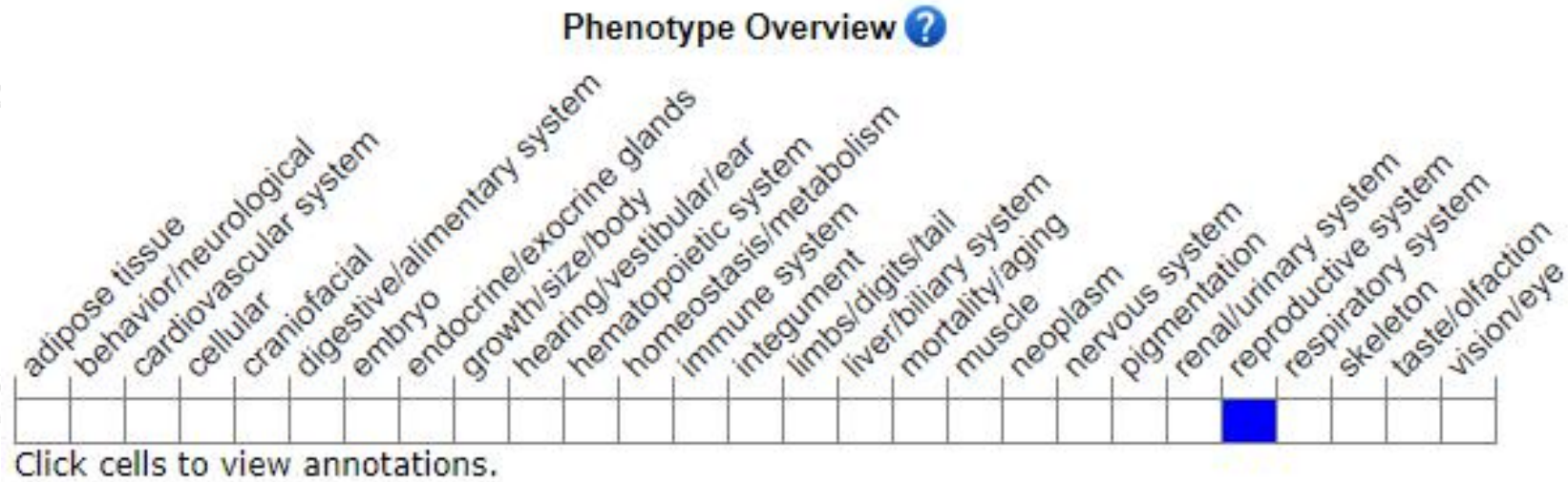
Genomic location distribution



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 knock-out alleles exhibit reduced female fertility.

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

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