Zp2 Cas9-CKO Strategy

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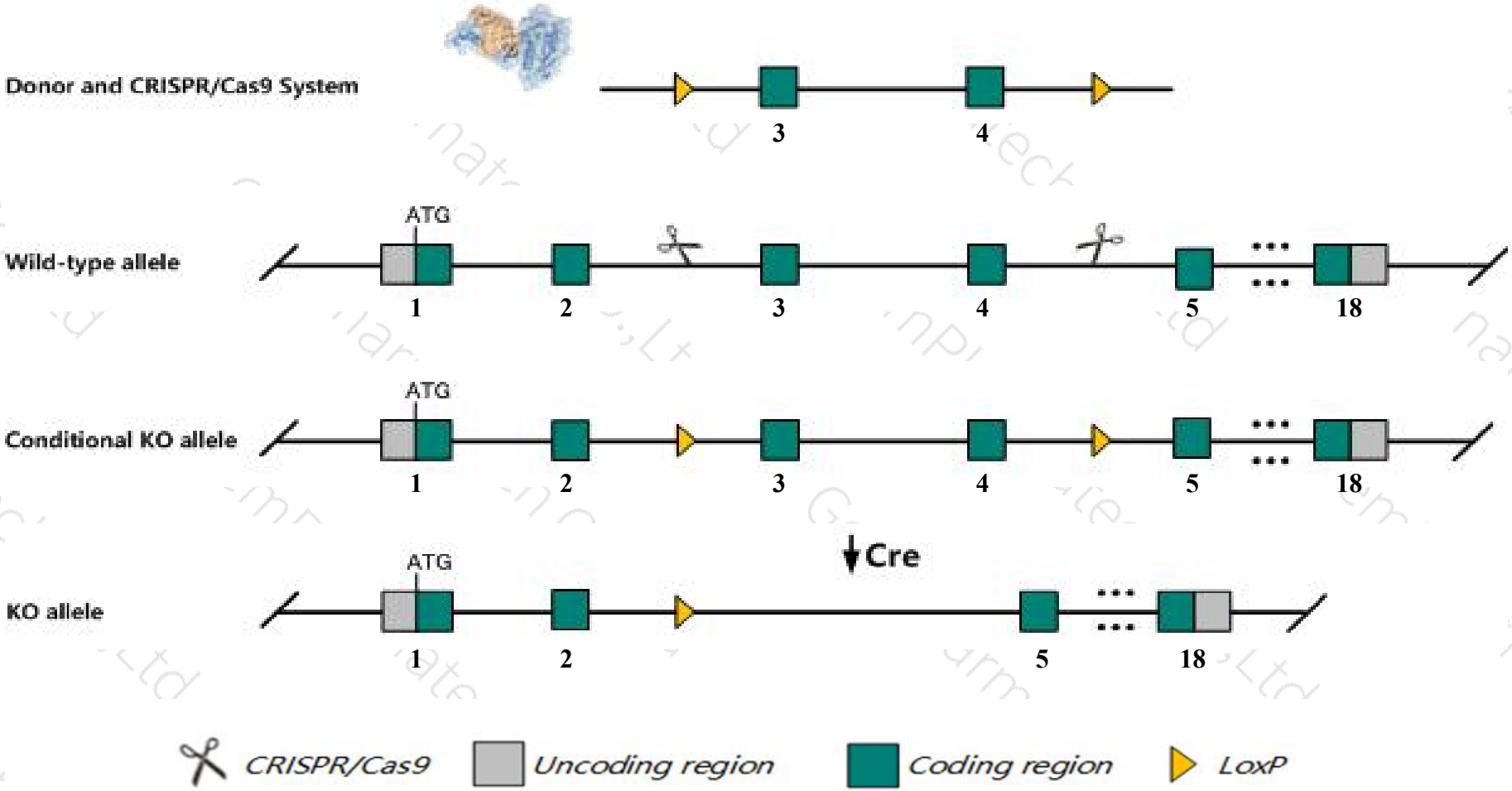
Project Overview

Project Name	***Zp2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Zp2* gene. The schematic diagram is as follows:



Technical routes

- The *Zp2* gene has 5 transcripts. According to the structure of *Zp2* gene, exon3-exon4 of *Zp2-201* (ENSMUST00000033207.5) transcript is recommended as the knockout region.The region contains 179bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Zp2* 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.

Notice

➢ According to the existing MGI data,Female homozygous mutants exhibit a thin zona pellucida matrix in early ovarian follicles that becomes disassociated in pre-ovulatory follicles. Few oocytes are produced, and any that are fertilized fail to survive to the two-cell stage.

➢ The *Zp2* gene is located on the Chr7. 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）

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Zp2 zona pellucida glycoprotein 2 [Mus musculus (house mouse)]

Gene ID: 22787, updated on 31-Jan-2019

Summary

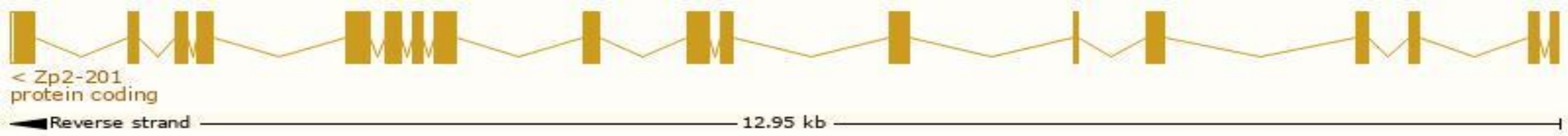
Official Symbol	Zp2 provided by MGI
Official Full Name	zona pellucida glycoprotein 2 provided byMGI
Primary source	MGI:MGI:99214
See related	Ensembl:ENSMUSG00000030911
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Zp-2
Summary	This gene encodes a member of the zona pellucida family of glycoproteins that play an important role in the survival of growing oocytes, successful fertilization and the passage of early embryos through the oviduct. The encoded preproprotein undergoes proteolytic processing to generate the mature polypeptide that is incorporated into the extracellular matrix surrounding mouse oocytes. Mice lacking the encoded protein develop defective zonae pellucidae that disrupt folliculogenesis, fertility and development. [provided by RefSeq, Sep 2016]
Expression	Restricted expression toward ovary adult (RPKM 15.6)See more
Orthologs	human all

Transcript information（Ensembl）

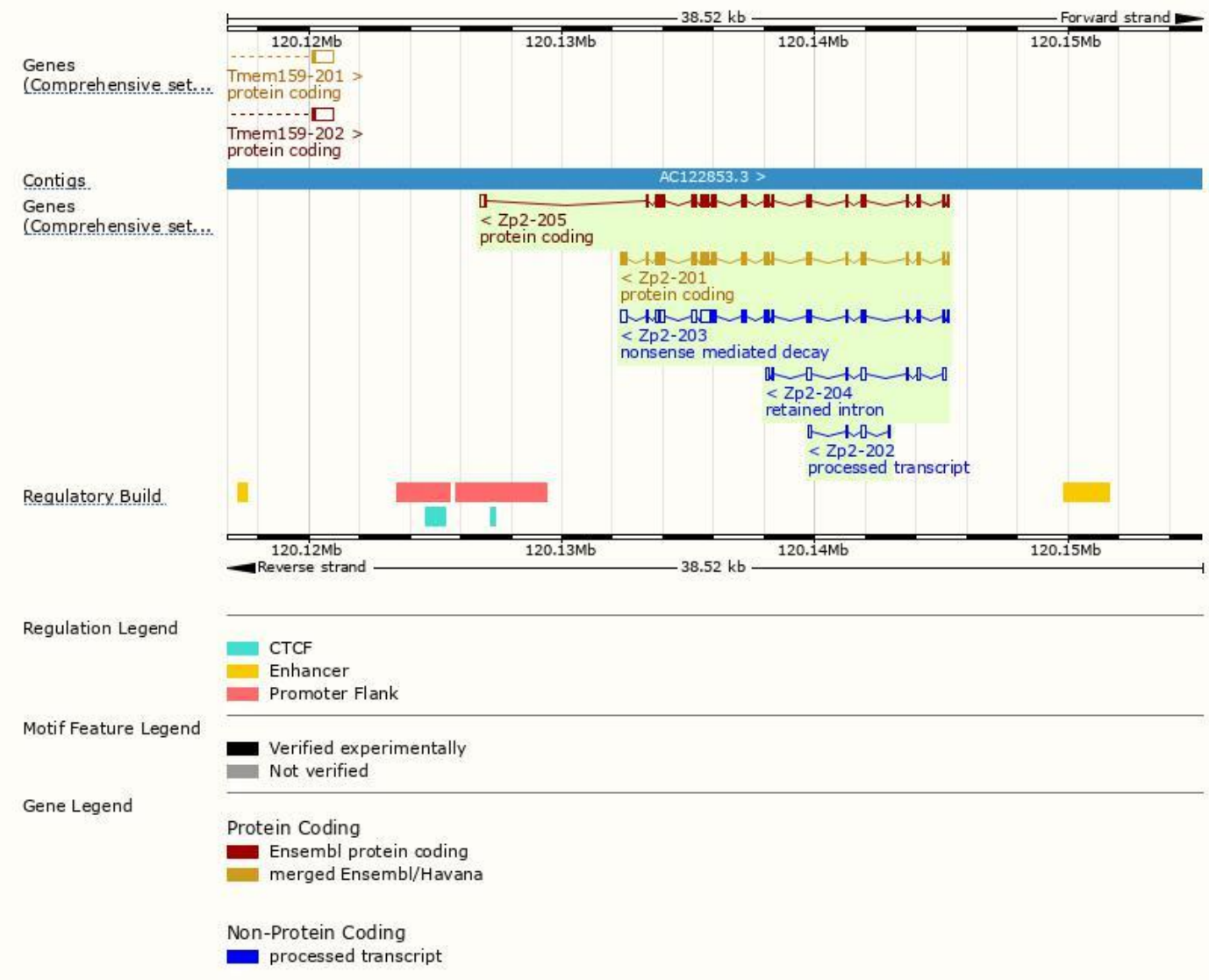
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Zp2-201	ENSMUST00000033207.5	2221	713aa	Protein coding	CCDS21792	P20239	TSL:1 GENCODE basic APPRIS P2
Zp2-205	ENSMUST00000208874.1	2233	676aa	Protein coding	-	Q3UX44	TSL:1 GENCODE basic APPRIS ALT2
Zp2-203	ENSMUST00000207726.1	2393	428aa	Nonsense mediated decay	-	A0A140LIR5	TSL:1
Zp2-202	ENSMUST00000207333.1	349	No protein	Processed transcript	-	-	TSL:5
Zp2-204	ENSMUST00000208122.1	914	No protein	Retained intron	-	-	TSL:5

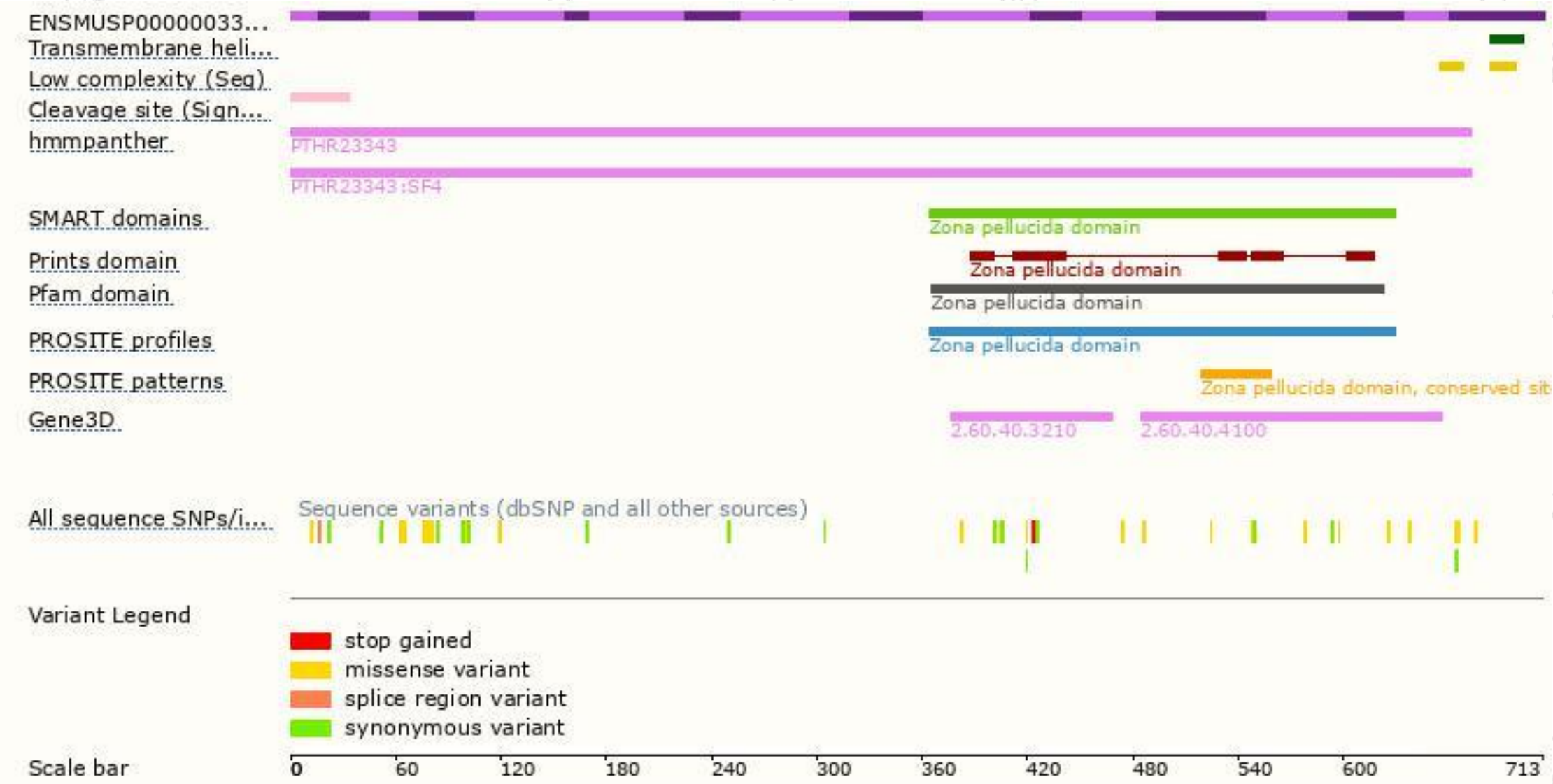
The strategy is based on the design of *Zp2-201* transcript,The transcription is shown below



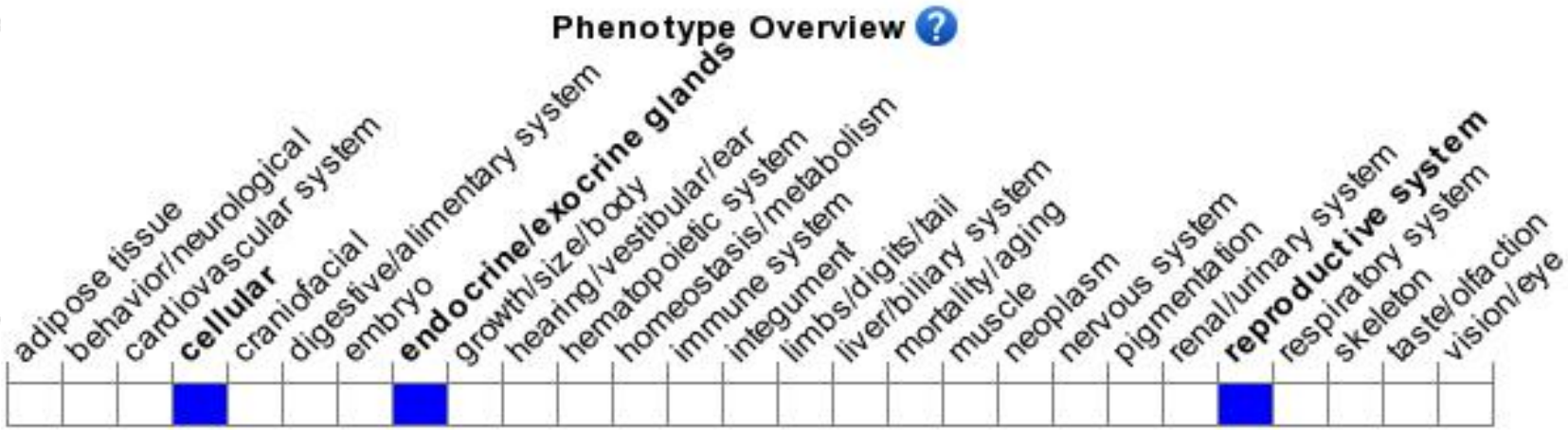
Genomic location distribution



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,Female homozygous mutants exhibit a thin zona pellucida matrix in early ovarian follicles that becomes disassociated in pre-ovulatory follicles. Few oocytes are produced, and any that are fertilized fail to survive to the two-cell stage.

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

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