Met Cas9-CKO Strategy

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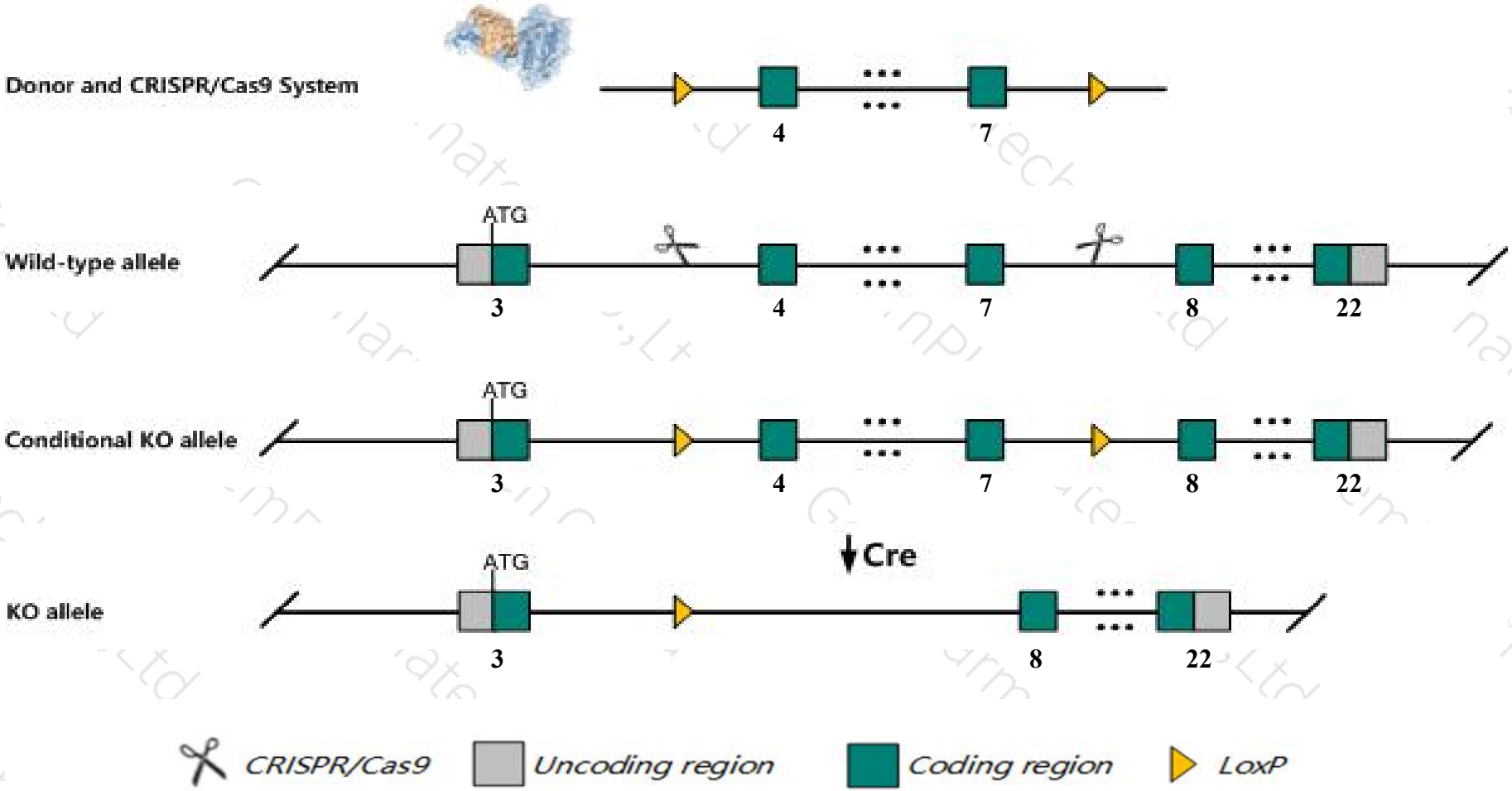
Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Met* gene has 7 transcripts. According to the structure of *Met* gene, exon4-exon7 of *Met-203* (ENSMUST00000115443.7) transcript is recommended as the knockout region.The region contains 662bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Met* 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,Homozygous null mutants exhibit impaired embryonic development resulting in death. Abnormalities observed in various mutant lines include muscle agenesis due to impaired migration of myogenic precursors, defects of motor axon migration, and placental andliver defects.

➢ The influence of *Met*-206&207 is unknown in this strategy.

➢ The N-terminal of *Met* gene will remain 399aa,it may remain the partial function of *Met* gene.

➢ The *Met* gene is located on the Chr6. 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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Met met proto-oncogene [Mus musculus (house mouse)]

Gene ID: 17295, updated on 19-Mar-2019

Summary

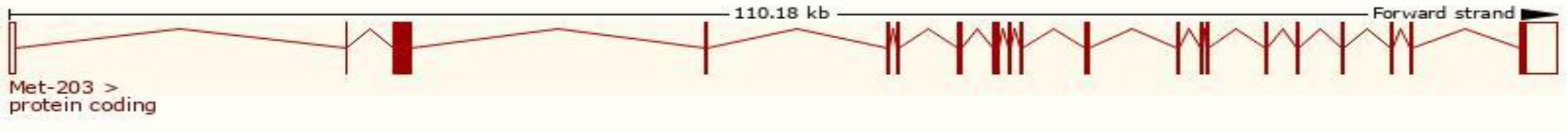
Official Symbol	Met provided by MGI
Official Full Name	met proto-oncogene provided by MGI
Primary source	MGI:MGI:96969
See related	Ensembl:ENSMUSG00000009376
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI838057, HGF, HGFR, Par4, c-Met
Expression	Broad expression in kidney adult (RPKM 10.5), bladder adult (RPKM 6.1) and 21 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

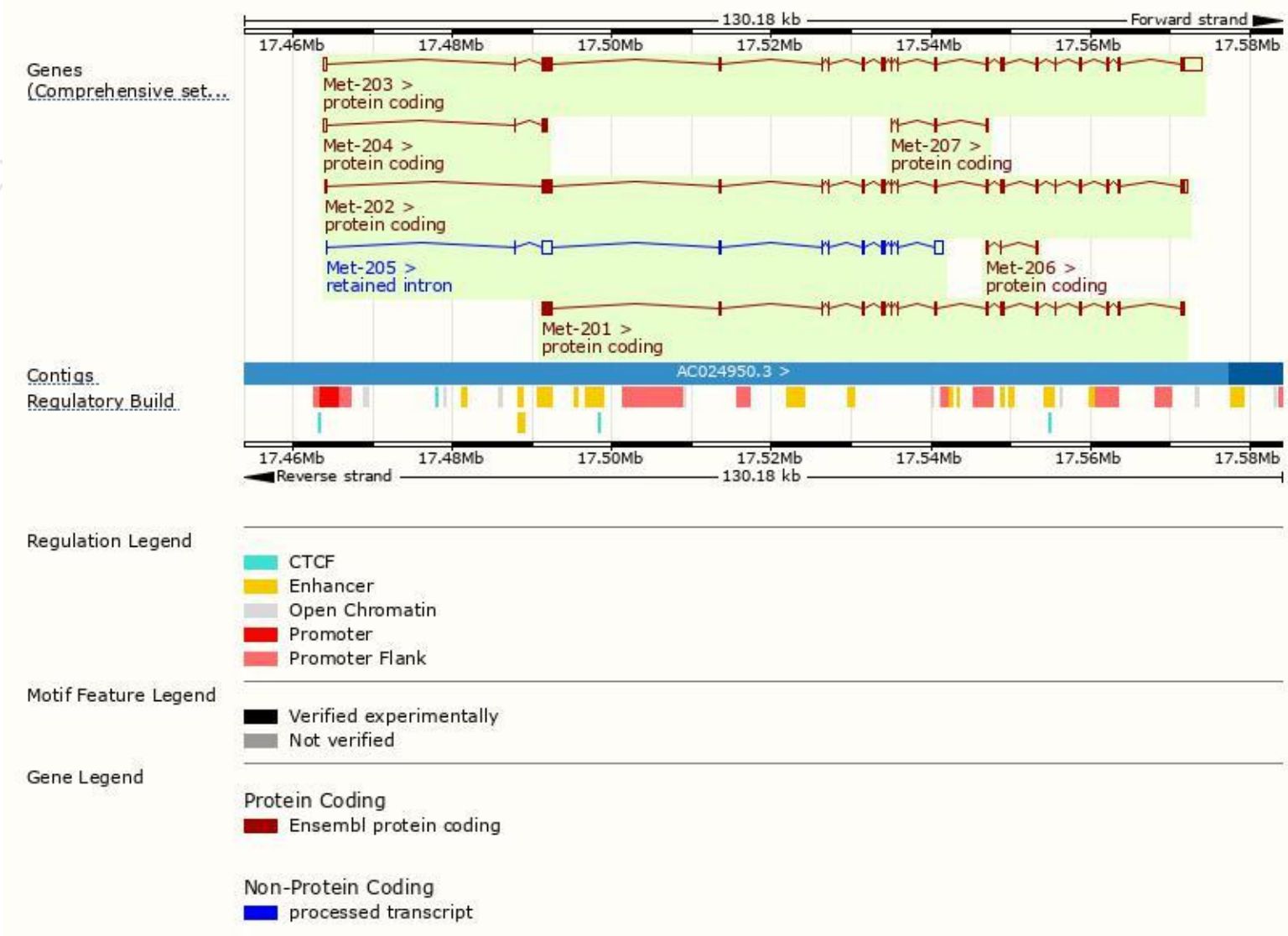
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Met-203	ENSMUST00000115443.7	6809	1379aa	Protein coding	CCDS19925	F8VQL0	TSL:5 GENCODE basic APPRIS P1
Met-202	ENSMUST00000115442.7	4663	1379aa	Protein coding	CCDS19925	F8VQL0	TSL:5 GENCODE basic APPRIS P1
Met-201	ENSMUST00000080469.11	4140	1379aa	Protein coding	CCDS19925	F8VQL0	TSL:1 GENCODE basic APPRIS P1
Met-204	ENSMUST00000140070.7	884	169aa	Protein coding	-	D3YVY2	CDS 3' incomplete TSL:3
Met-207	ENSMUST00000152802.1	683	214aa	Protein coding	-	F6X333	CDS 5' incomplete TSL:5
Met-206	ENSMUST00000148903.1	470	157aa	Protein coding	-	F6RCC5	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Met-205	ENSMUST00000145473.1	3541	No protein	Retained intron	-	-	TSL:1

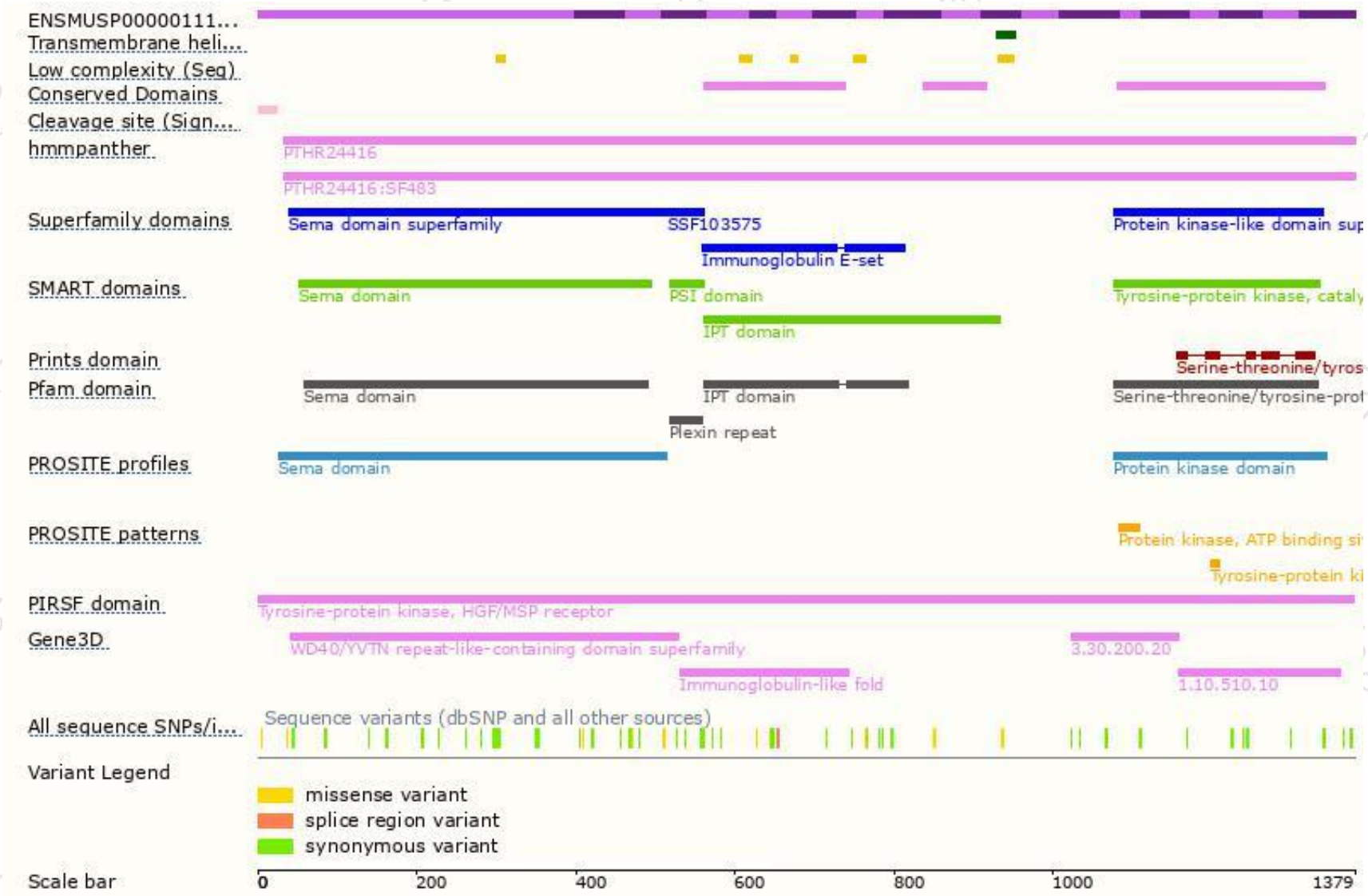
The strategy is based on the design of *Met-203* transcript,The transcription is shown below



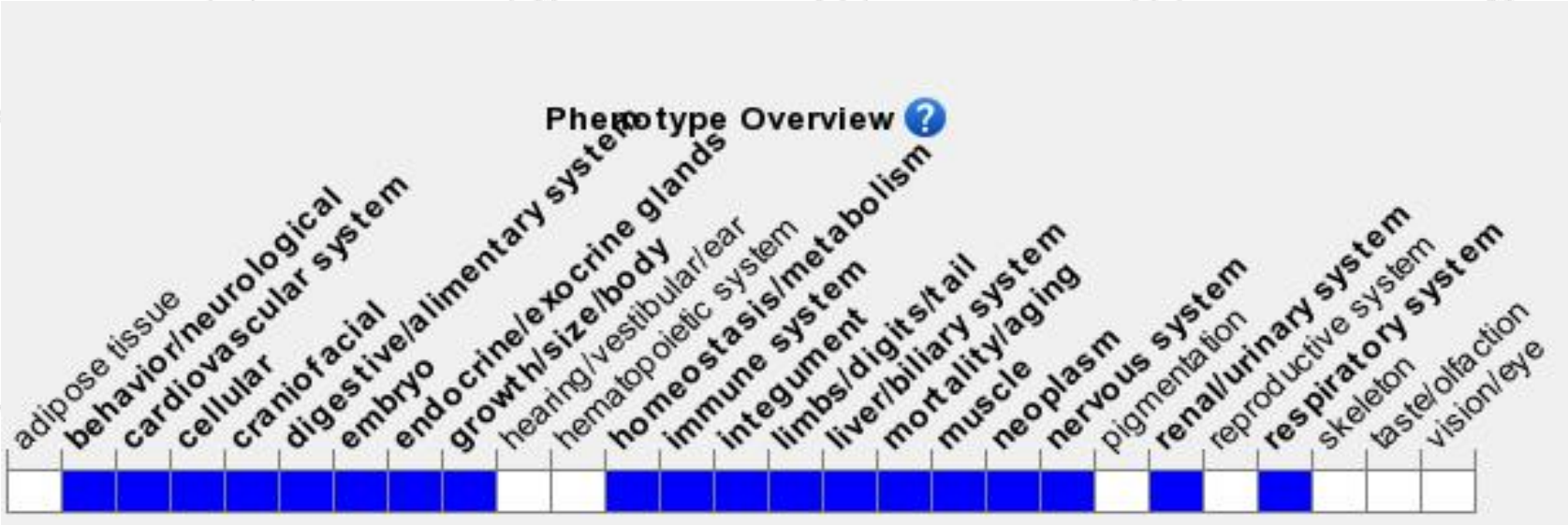
Genomic location distribution



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/).

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

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