Kcnn2 Cas9-CKO Strategy

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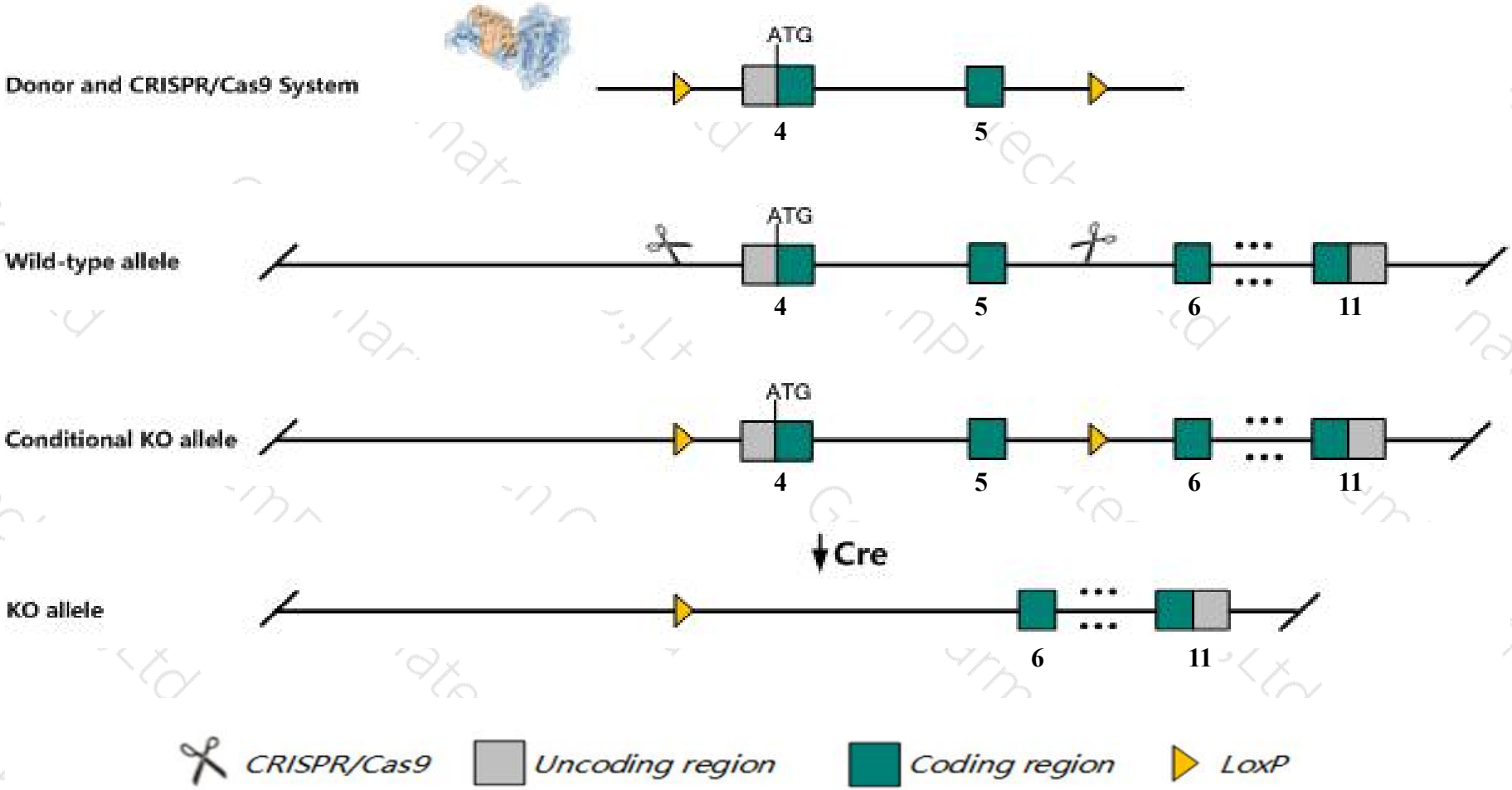
Project Overview

Project Name	***Kcnn2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Kcnn2* gene. The schematic diagram is as follows:



Technical routes

- The *Kcnn2* gene has 10 transcripts. According to the structure of *Kcnn2* gene, exon4-exon5 of *Kcnn2-201* (ENSMUST00000066890.13) transcript is recommended as the knockout region.The region contains start codon ATG. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Kcnn2* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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,Mice homozygous for a point mutation exhibit tremor and gait abnormalities. Homozygous null mice lack the apamin sensitive component of the medium afterhyperpolarization current but have normal hippocampal morphology.
- The floxed region in this strategy is exon4-5(include the start codon ATG),and there may have a risk of restarting protein translation.
- *AC122852.1* gene will be disrupted together in this strategy.
- The *Kcnn2* gene is located on the Chr18. 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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Kcnn2 potassium intermediate/small conductance calcium-activated channel, subfamily N, member 2 [Mus musculus (house mouse)]

Gene ID: 140492, updated on 2-Mar-2019

Summary

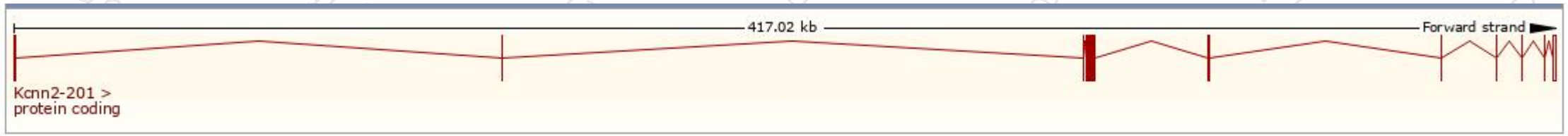
Official Symbol	Kcnn2 provided by MGI
Official Full Name	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 2 provided by MGI
Primary source	MGI:MGI:2153182
See related	Ensembl:ENSMUSG00000054477
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	KCa2.2, SK2, SKCA2, bc, fri
Expression	Biased expression in adrenal adult (RPKM 77.6), cerebellum adult (RPKM 7.7) and 3 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

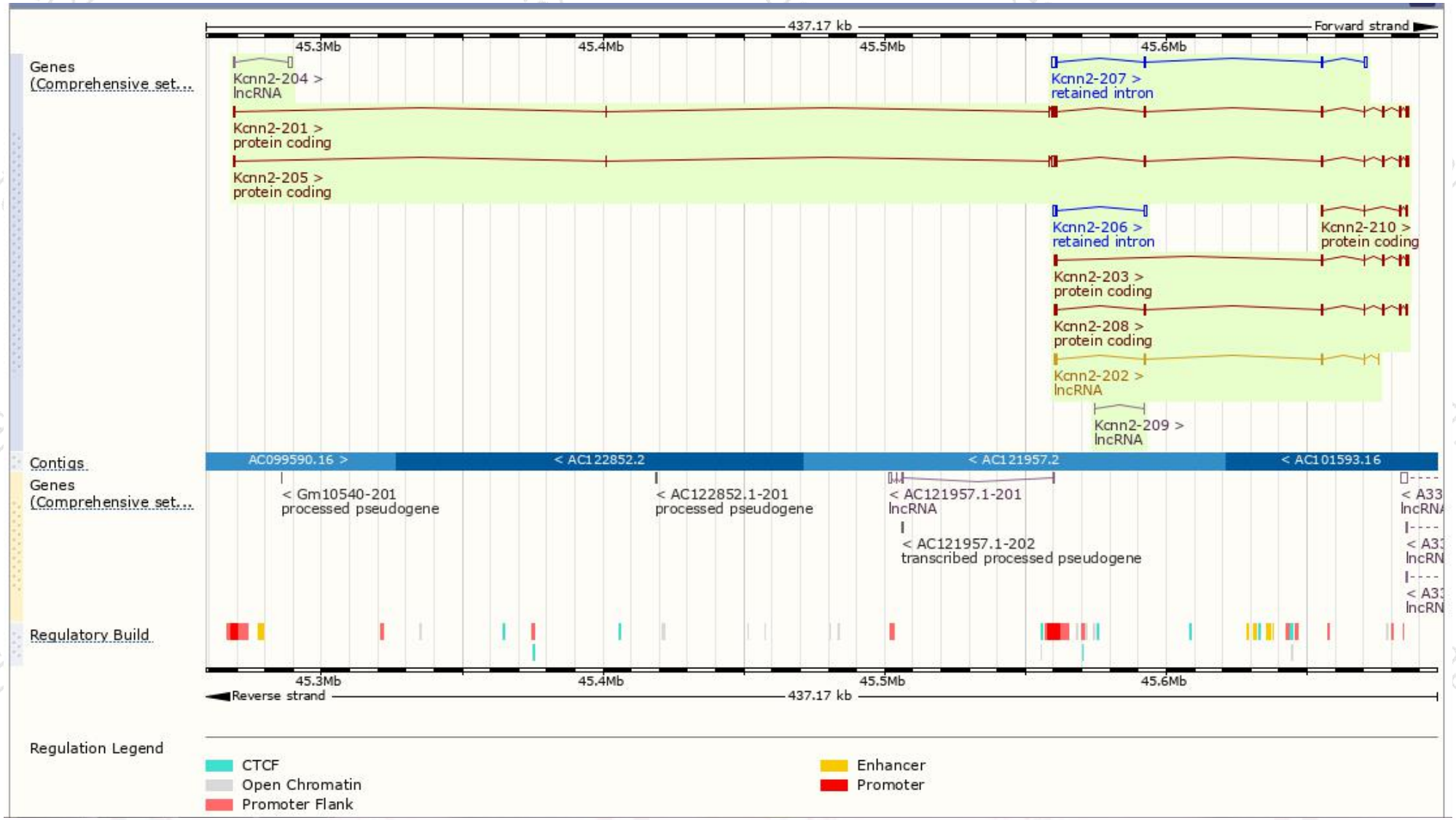
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Kcnn2-205	ENSMUST00000183850.7	3546	574aa	Protein coding	CCDS84380	A0A1B0GT12	TSL:1 GENCODE basic APPRIS P2
Kcnn2-208	ENSMUST00000211323.2	1725	574aa	Protein coding	CCDS84380	A0A1B0GT12	TSL:1 GENCODE basic APPRIS P2
Kcnn2-201	ENSMUST00000066890.13	3682	839aa	Protein coding	-	P58390	TSL:5 GENCODE basic APPRIS ALT2
Kcnn2-203	ENSMUST00000169783.1	1625	434aa	Protein coding	-	B4YDY0	TSL:1 GENCODE basic
Kcnn2-210	ENSMUST00000236405.1	389	78aa	Protein coding	-	-	CDS 5' incomplete
Kcnn2-204	ENSMUST00000183623.1	2376	No protein	Processed transcript	-	-	TSL:1
Kcnn2-202	ENSMUST00000167895.1	1347	No protein	Processed transcript	-	-	TSL:1
Kcnn2-209	ENSMUST00000235217.1	476	No protein	Processed transcript	-	-	
Kcnn2-207	ENSMUST00000184101.7	2845	No protein	Retained intron	-	-	TSL:1
Kcnn2-206	ENSMUST00000183897.7	1970	No protein	Retained intron	-	-	TSL:1

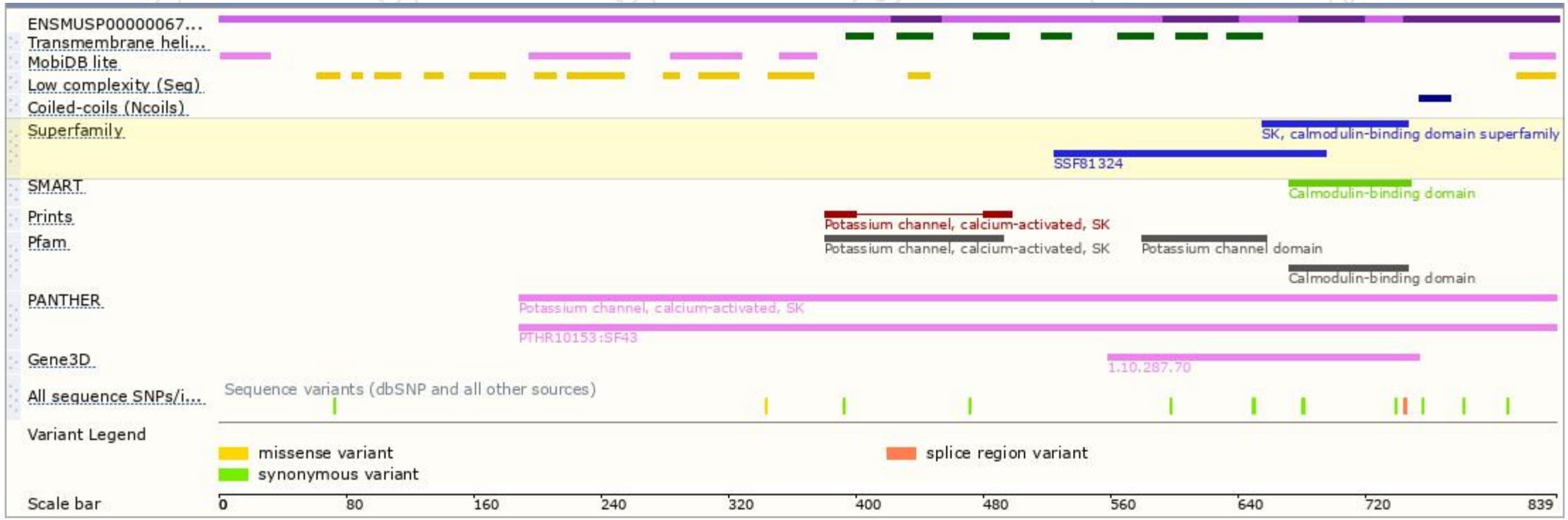
The strategy is based on the design of *Kcnn2-201* transcript,The transcription is shown below



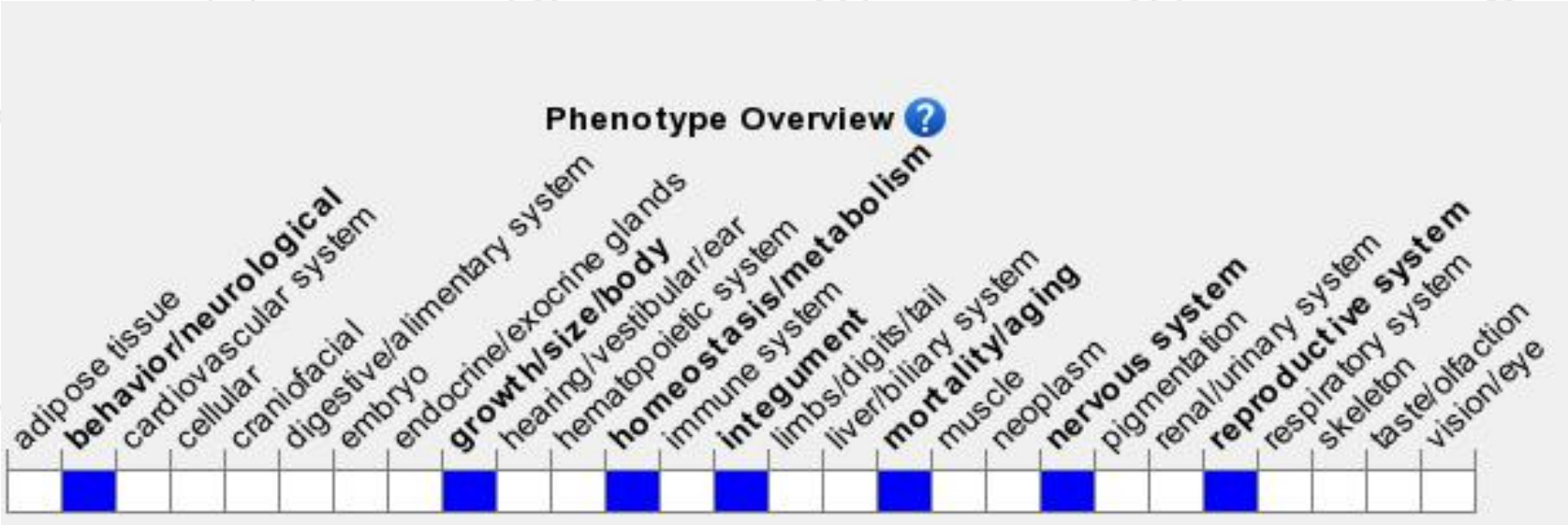
Genomic location distribution



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/).

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

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