Trpv4 Cas9-CKO Strategy

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Project Overview

Project Name	***Trpv4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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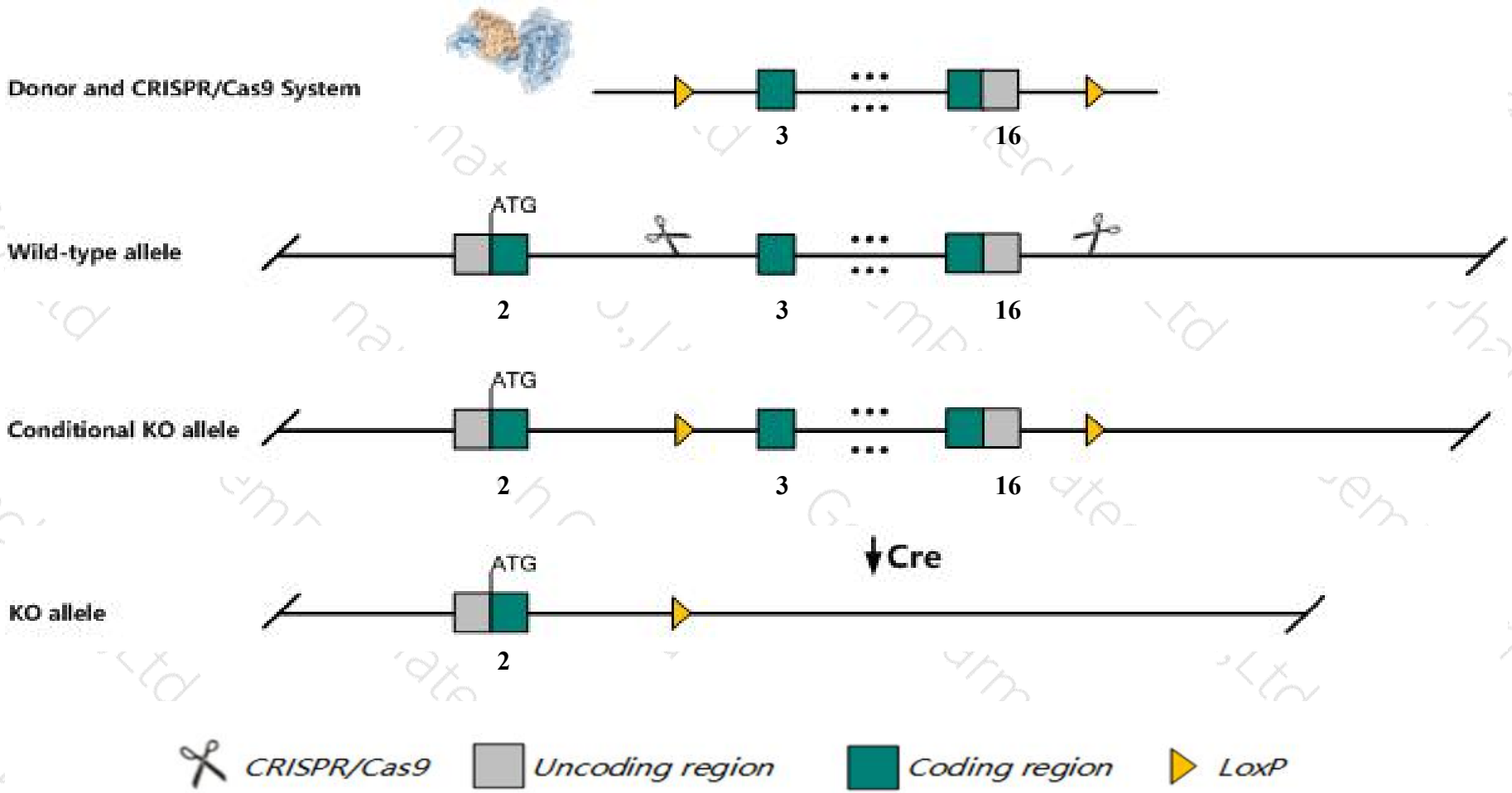
This model will use CRISPR/Cas9 technology to edit the *Trpv4* gene. The schematic diagram is as follows:



Technical routes

➢ The *Trpv4* gene has 8 transcripts. According to the structure of *Trpv4* gene, exon3-exon16 of *Trpv4-201*(ENSMUST00000071968.8) transcript is recommended as the knockout region.The region contains 2230bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Trpv4* 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,homozygotes for a null allele show abnormal touch/ nociception and late-onset hearing loss. Homozygotes for a different null allele show impaired bladder voiding, abnormalities in touch/ nociception, osmotic regulation and vasodilation, ocular hypertension but no hearing or vestibular deficits.

➢ The *Trpv4* gene is located on the Chr5. 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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Trpv4 transient receptor potential cation channel, subfamily V, member 4 [Mus musculus (house mouse)]

Gene ID: 63873, updated on 6-Apr-2019

Summary

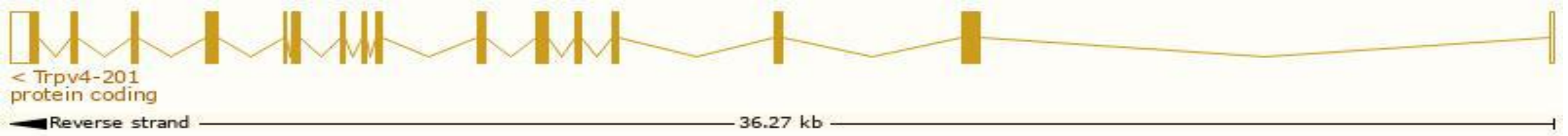
Official Symbol	Trpv4 provided by MGI
Official Full Name	transient receptor potential cation channel, subfamily V, member 4 provided by MGI
Primary source	MGI:MGI:1926945
See related	Ensembl:ENSMUSG00000014158
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	0610033B08Rik, OTRPC4, Trp12, VR-OAC, VRL-2, VROAC
Expression	Biased expression in kidney adult (RPKM 53.0), bladder adult (RPKM 22.4) and 7 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

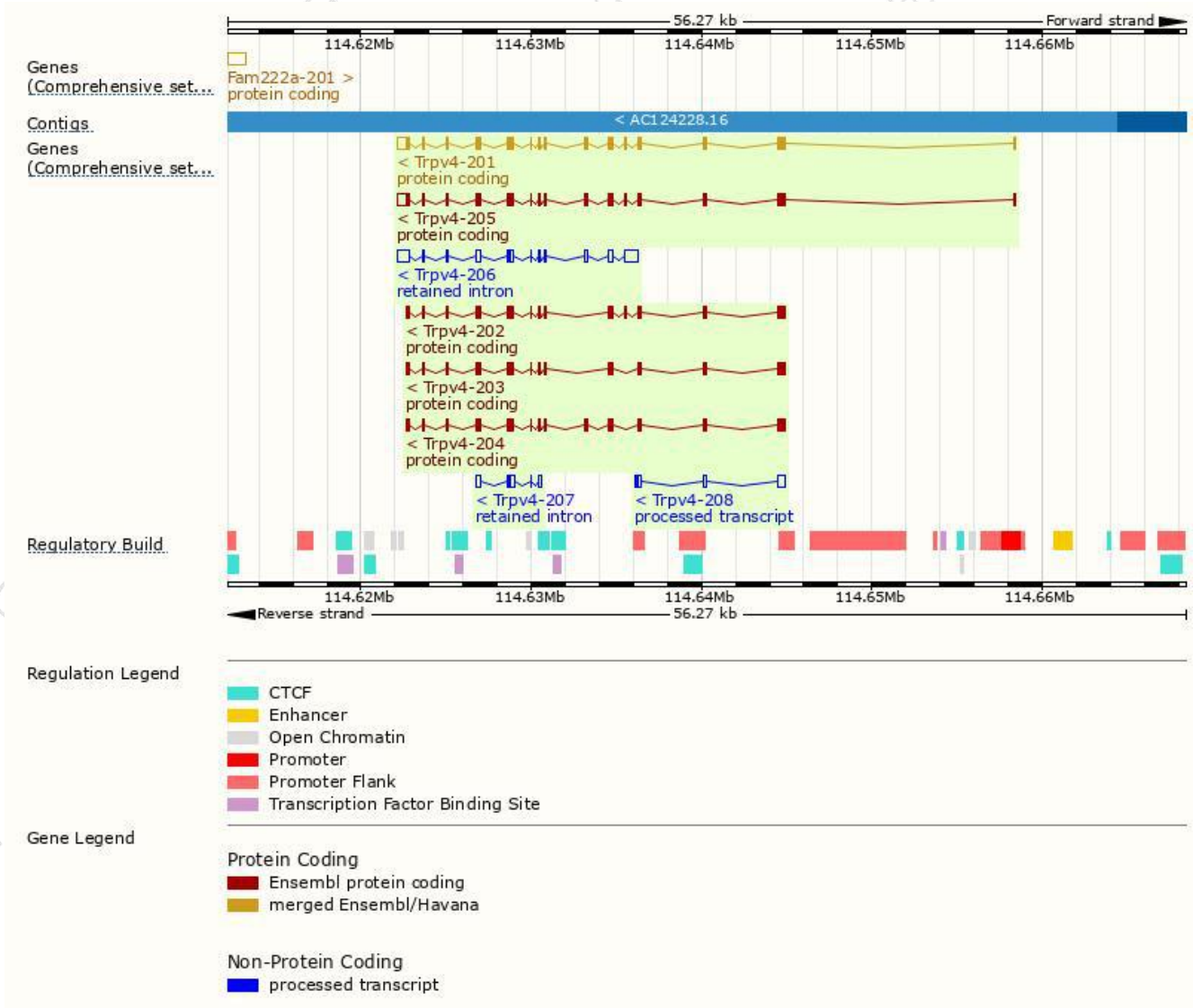
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Trpv4-201	ENSMUST00000071968.8	3228	871aa	Protein coding	CCDS19568	Q9EPK8	TSL:1 GENCODE basic APPRIS P1
Trpv4-205	ENSMUST00000112225.7	3226	871aa	Protein coding	CCDS19568	Q9EPK8	TSL:5 GENCODE basic APPRIS P1
Trpv4-204	ENSMUST00000112222.7	2475	824aa	Protein coding	-	E9Q7L7	TSL:5 GENCODE basic
Trpv4-202	ENSMUST00000112217.1	2436	811aa	Protein coding	-	D3Z1H7	TSL:5 GENCODE basic
Trpv4-203	ENSMUST00000112219.7	2295	764aa	Protein coding	-	D3Z1H6	TSL:5 GENCODE basic
Trpv4-208	ENSMUST00000212469.1	771	No protein	Processed transcript	-	-	TSL:5
Trpv4-206	ENSMUST00000133019.7	3074	No protein	Retained intron	-	-	TSL:2
Trpv4-207	ENSMUST00000141828.1	888	No protein	Retained intron	-	-	TSL:3

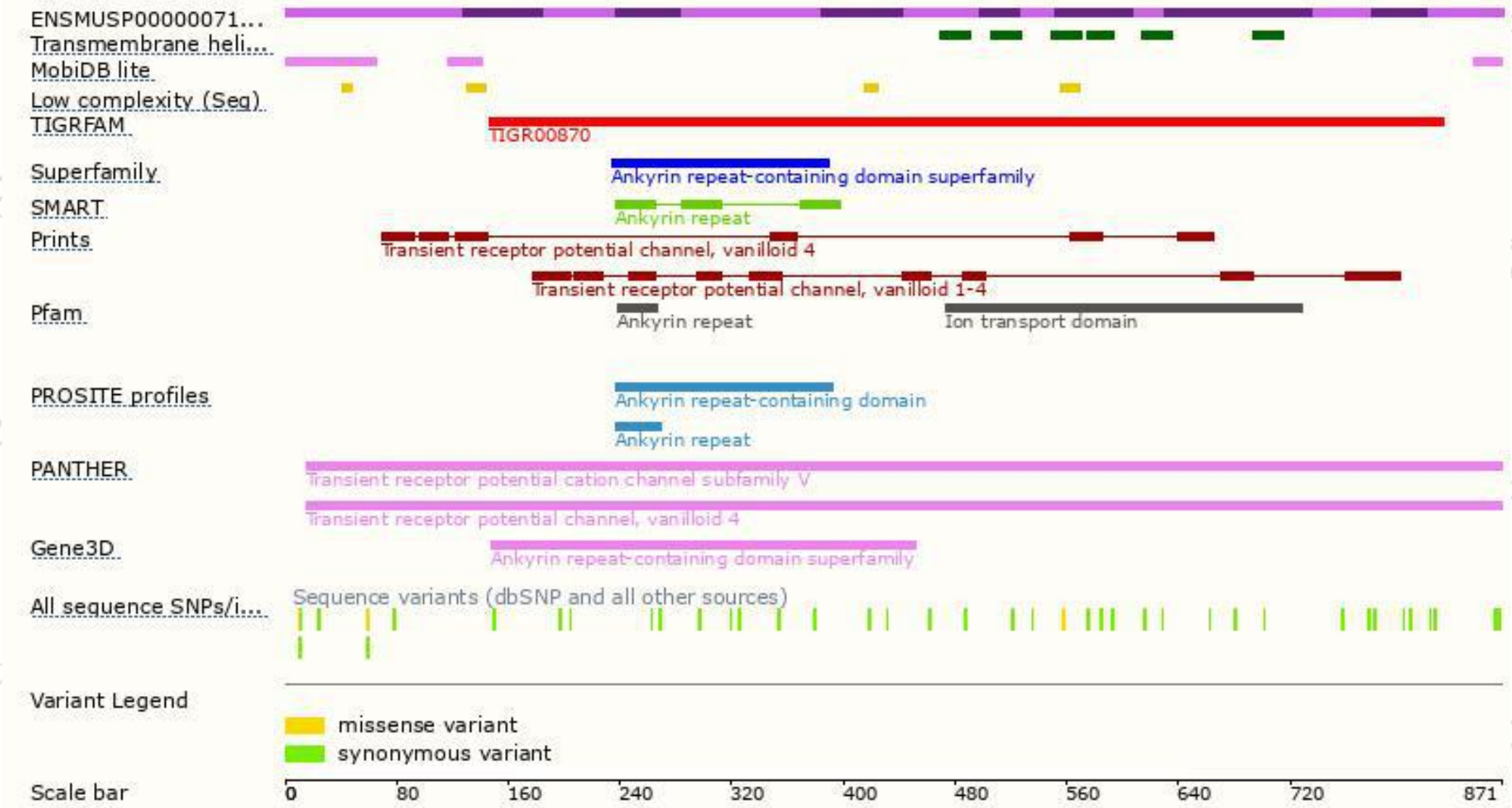
The strategy is based on the design of *Trpv4-201* transcript,the transcription is shown below:



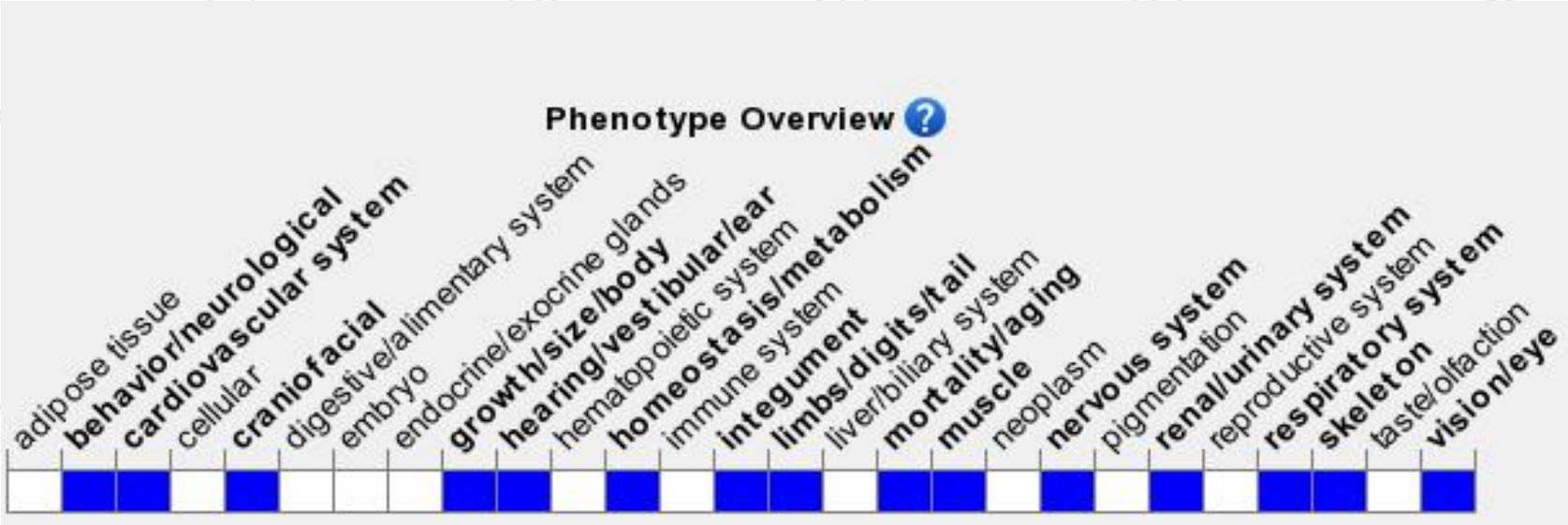
Genomic location distribution



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,homozygotes for a null allele show abnormal touch/ nociception and late-onset hearing loss. Homozygotes for a different null allele show impaired bladder voiding, abnormalities in touch/ nociception, osmotic regulation and vasodilation, ocular hypertension but no hearing or vestibular deficits.

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

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