

***Trpv6* Cas9-CKO Strategy**

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Design Date: 2019-9-12
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Project Overview

Project Name

Trpv6

Project type

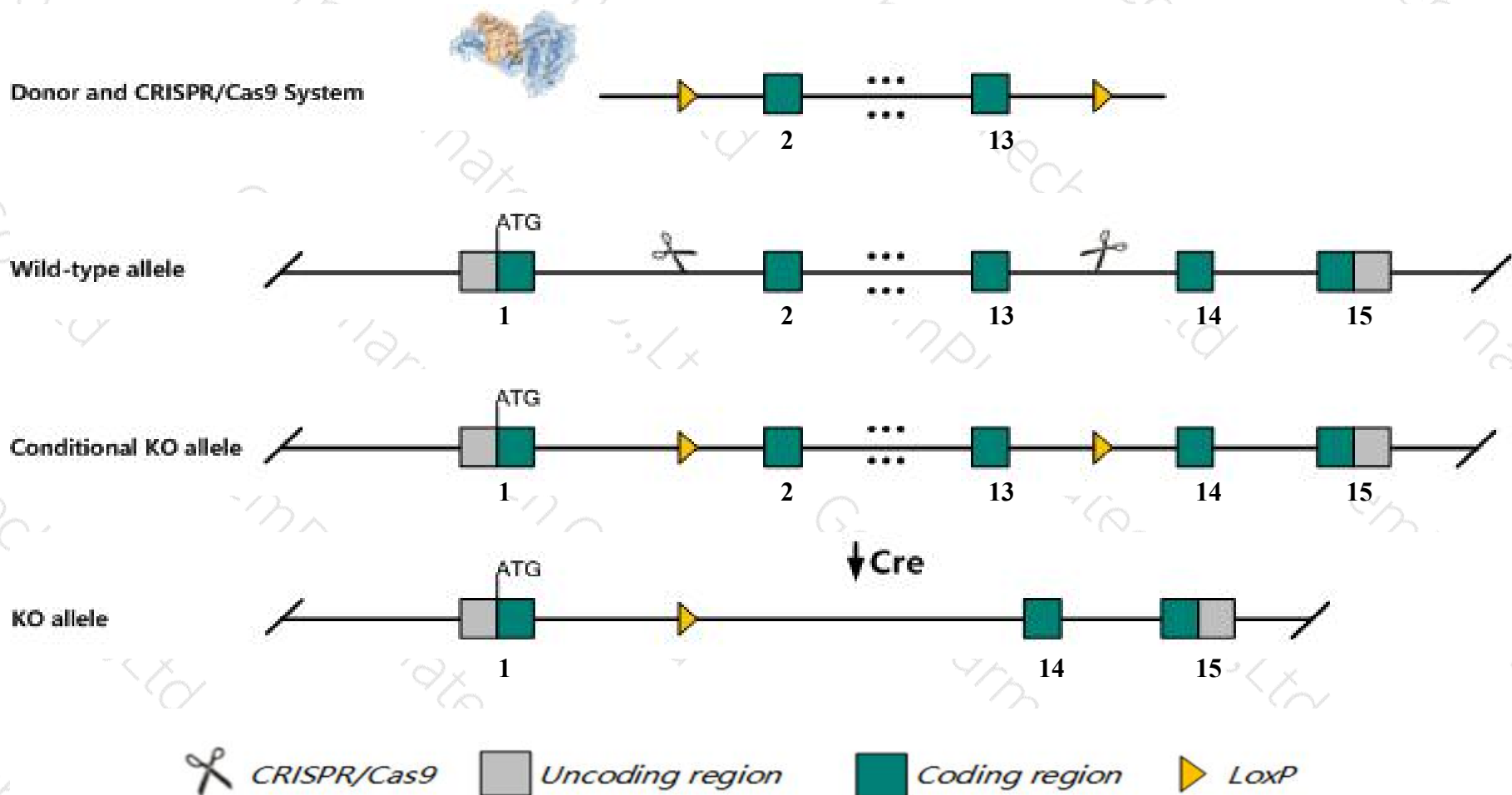
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Trpv6* gene. The schematic diagram is as follows:



Technical routes

- The *Trpv6* gene has 3 transcripts. According to the structure of *Trpv6* gene, exon2-exon13 of *Trpv6*-201 (ENSMUST00000031902.6) transcript is recommended as the knockout region. The region contains 1657bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Trpv6* 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 a knock-in allele exhibit impaired sperm motility and decreased fertilization by sperm.
- The *Trpv6* 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)

Trpv6 transient receptor potential cation channel, subfamily V, member 6 [Mus musculus (house mouse)]

Gene ID: 64177, updated on 19-Mar-2019

Summary



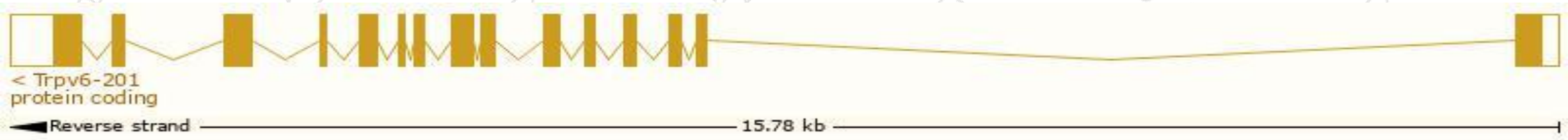
Official Symbol	Trpv6 provided by MGI
Official Full Name	transient receptor potential cation channel, subfamily V, member 6 provided by MGI
Primary source	MGI:MGI:1927259
See related	Ensembl:ENSMUSG00000029868
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	CAT, CaT1, Cac, Ecac2, Otrpc3
Expression	Biased expression in genital fat pad adult (RPKM 14.3), placenta adult (RPKM 5.0) and 13 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

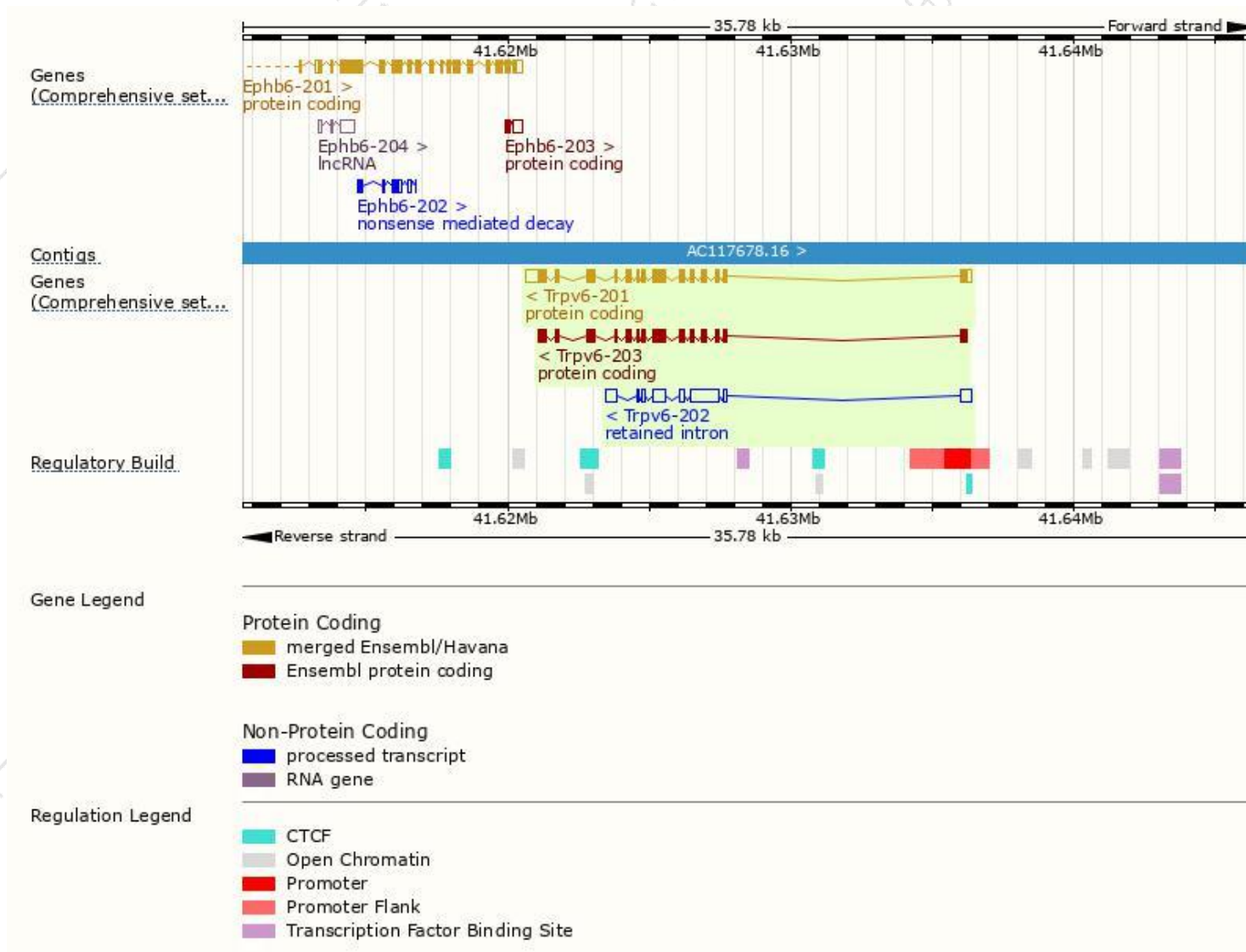
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Trpv6-201	ENSMUST00000031902.6	2923	767aa	Protein coding	CCDS20052	A0A0J9YMK6	TSL:1 GENCODE basic APPRIS P5
Trpv6-203	ENSMUST00000201471.4	2304	767aa	Protein coding	CCDS20052	Q91WD2	TSL:1 GENCODE basic APPRIS ALT2
Trpv6-202	ENSMUST00000194405.1	2633	No protein	Retained intron	-	-	TSL:2

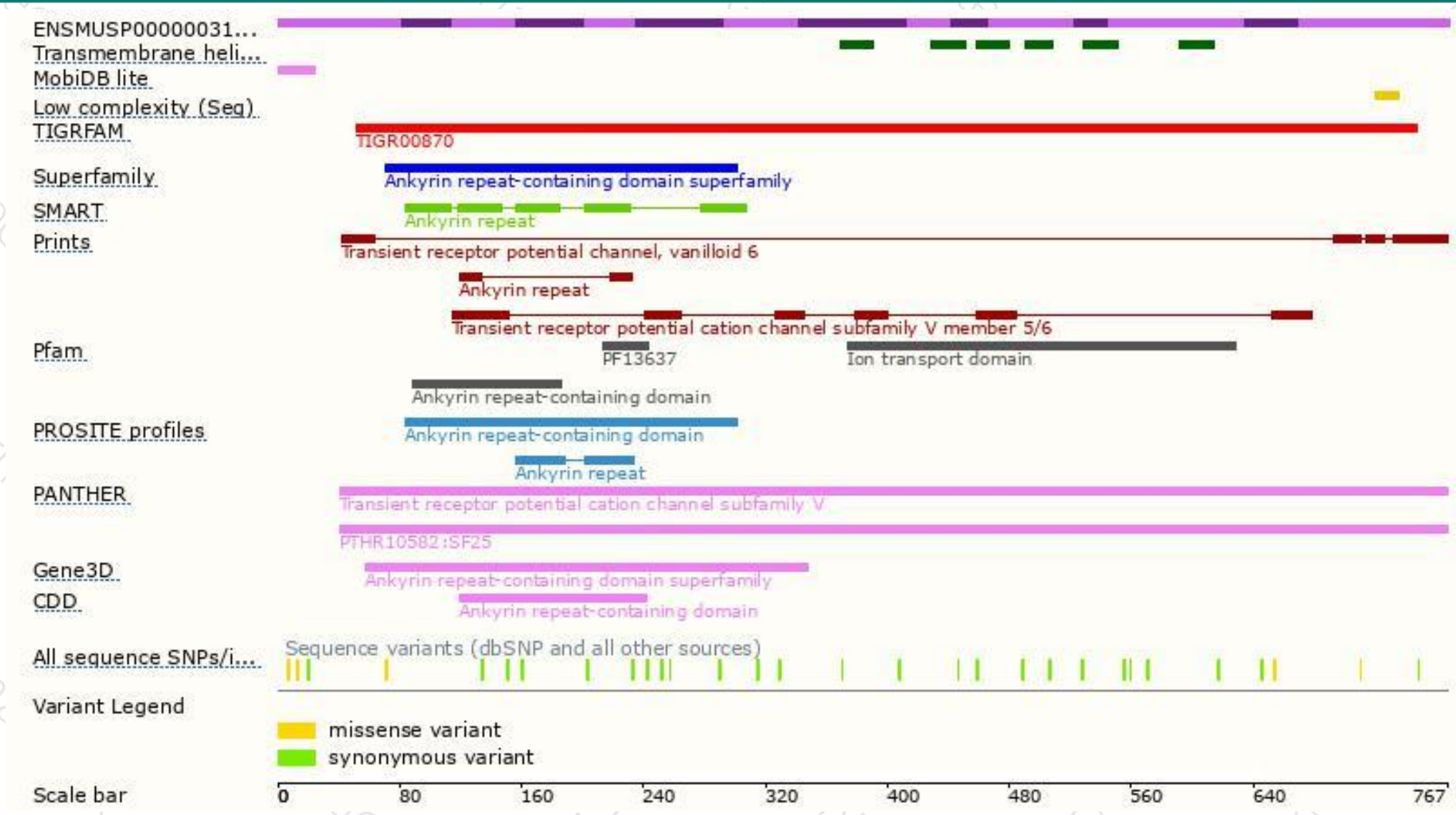
The strategy is based on the design of *Trpv6-201* transcript,The transcription is shown below



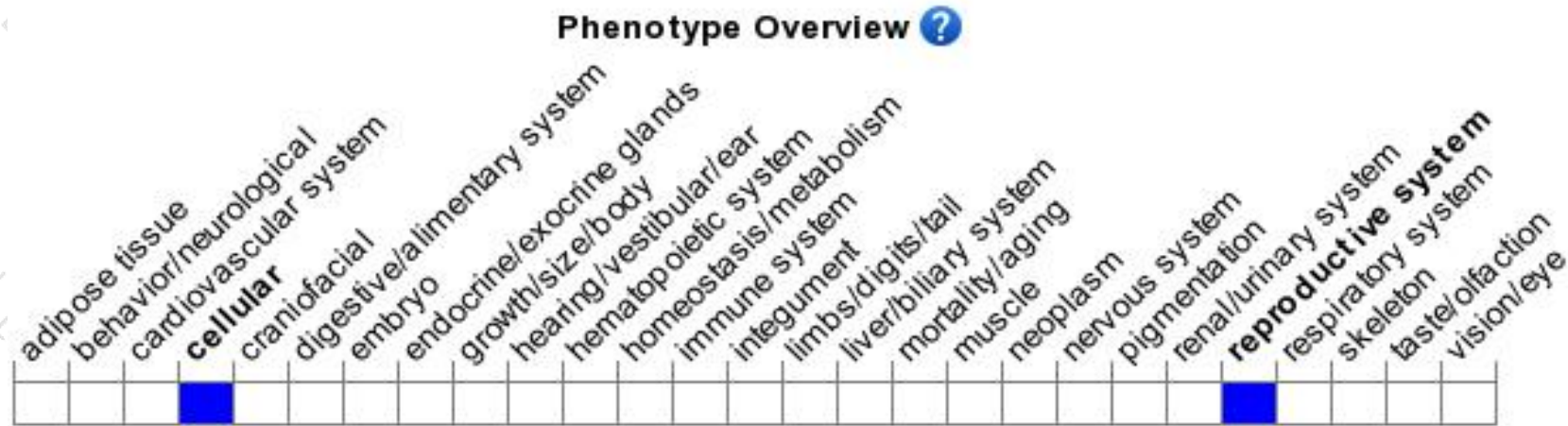
Genomic location distribution



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 a knock-in allele exhibit impaired sperm motility and decreased fertilization by sperm.

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

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