

Adra2b **Cas9-CKO Strategy**

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Project Overview

Project Name

Adra2b

Project type

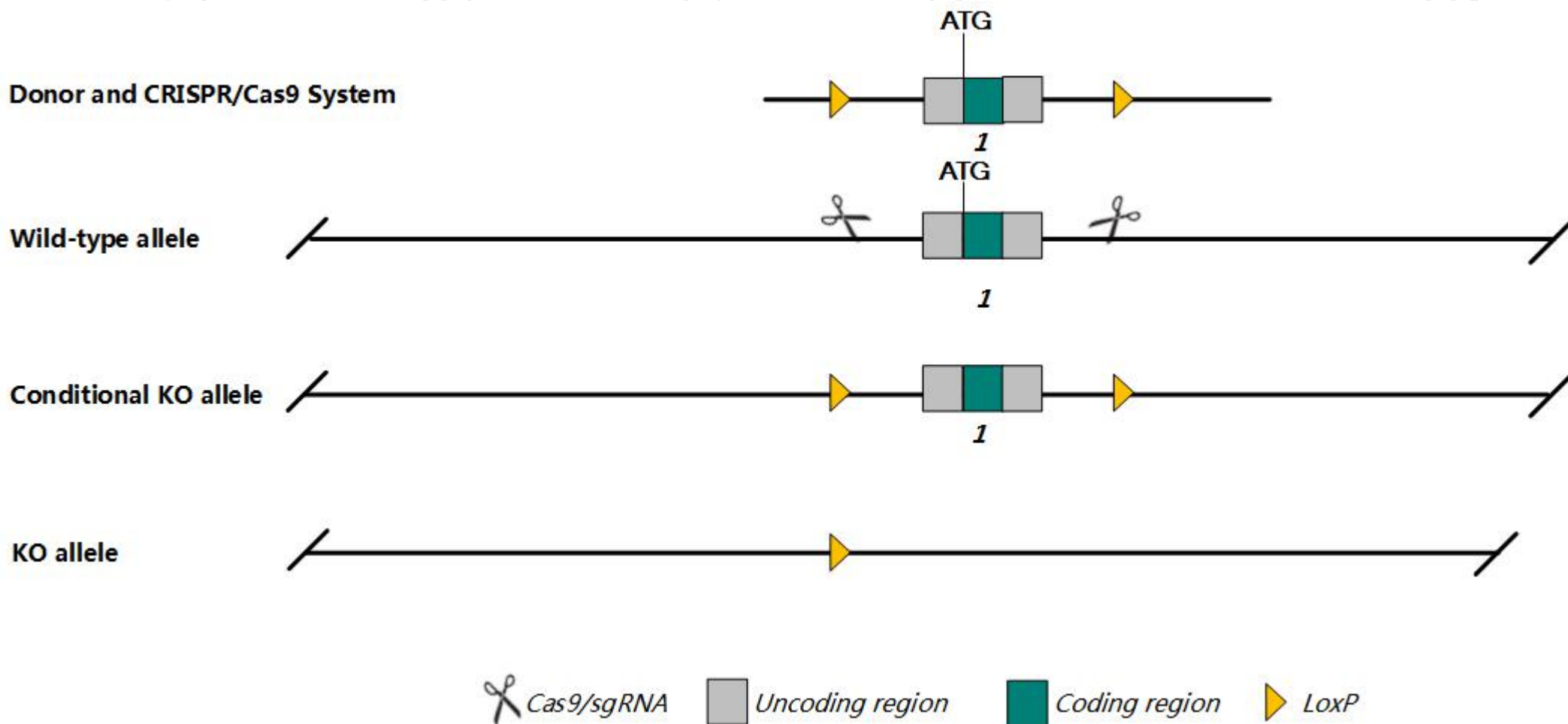
Cas9-CKO

Strain background

C57BL/6J

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Adra2b* gene. The schematic diagram is as follows:



Technical routes

- The *Adra2b* gene has 2 transcripts. According to the structure of *Adra2b* gene, exon1 of *Adra2b-201* (ENSMUST00000071902.4) transcript is recommended as the knockout region. The region contains all of the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Adra2b* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed. Cas9, sgRNA and Donor were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.
- The flox mice was 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.

- Homozygous null mice exhibit poor survival and breeding, lack the vasoconstrictor response to alpha2-adrenergic receptor agonists, and display background strain dependent postnatal respiratory failure. Heterozygotes show an attenuated hypertensive response to subtotal nephrectomy and salt loading.
- The *Adra2b* gene is located on the Chr2. 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)

Adra2b adrenergic receptor, alpha 2b [*Mus musculus* (house mouse)]

Gene ID: 11552, updated on 12-Aug-2019

Summary

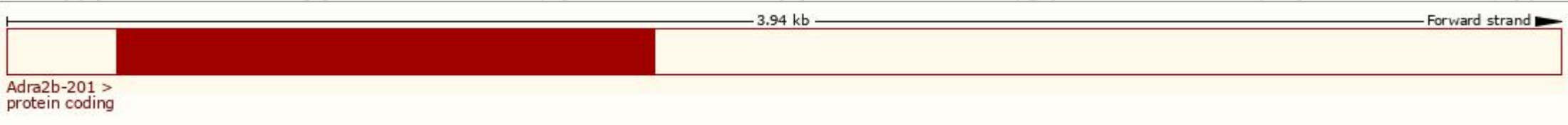
Official Symbol	Adra2b provided by MGI
Official Full Name	adrenergic receptor, alpha 2b provided by MGI
Primary source	MGI:MGI:87935
See related	Ensembl:ENSMUSG00000058620
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	[a]2B; a2b-AR; Adra-2b; alpha2B; alpha2-C2
Orthologs	human all

Transcript information (Ensembl)

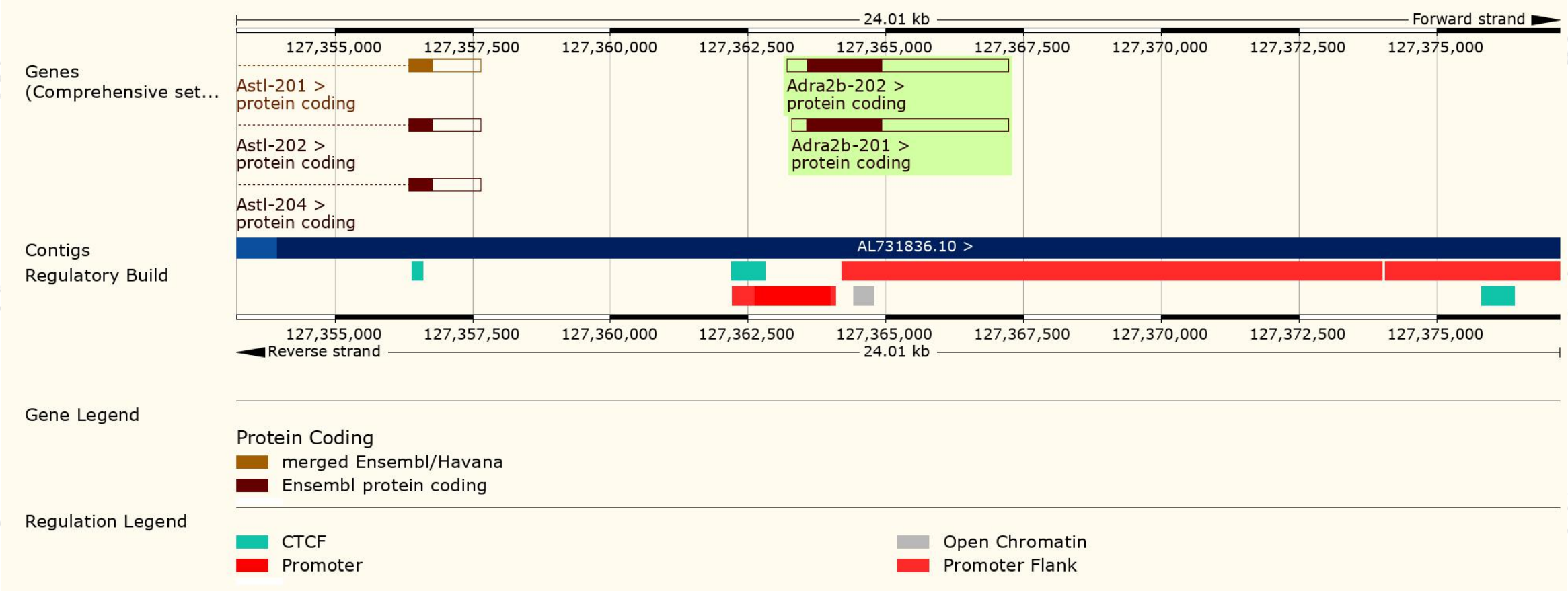
The gene has 2 transcripts, and the transcript is shown below:

Name ▲	Transcript ID ▲	bp ▲	Protein ▲	Biotype ▲	CCDS ▲	UniProt ▲	Flags ▲
Adra2b-201	ENSMUST00000071902.4	3936	453aa	Protein coding	CCDS16701	F8VQ23	TSL:NA GENCODE basic APPRIS P2
Adra2b-202	ENSMUST00000104934.1	4014	448aa	Protein coding	-	Q925K6	TSL:NA GENCODE basic APPRIS ALT2

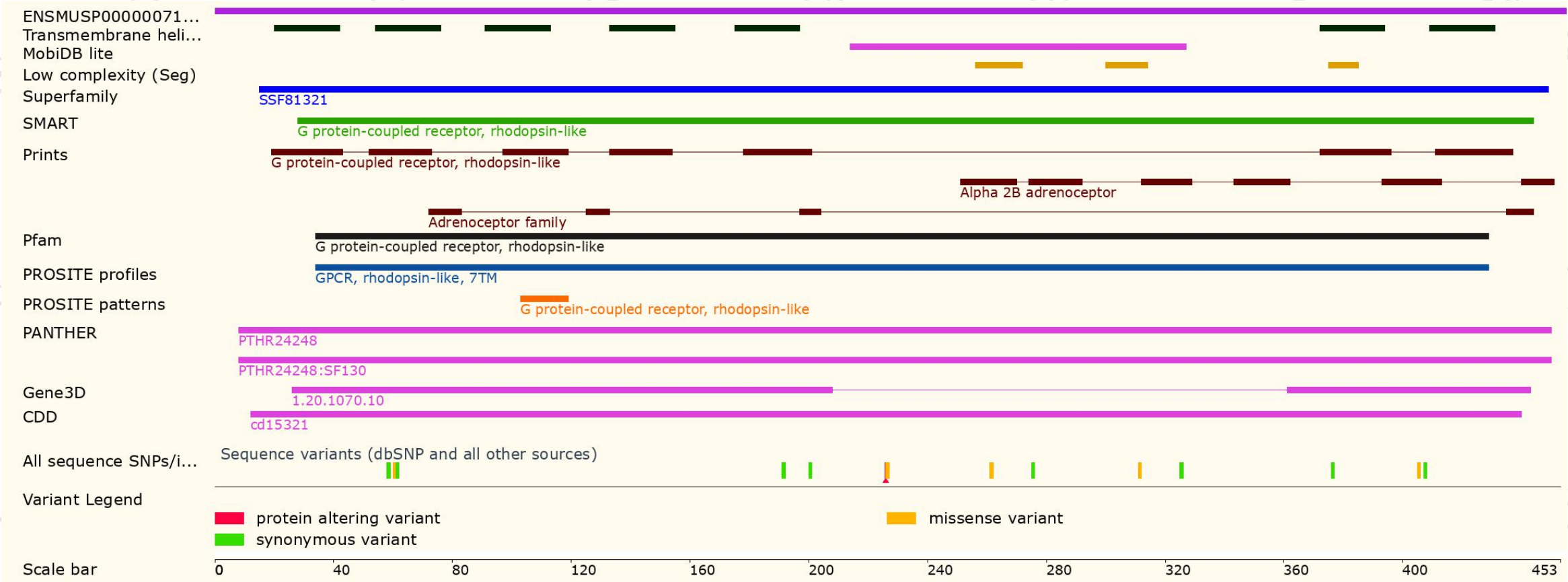
The strategy is based on the design of *Adra2b-201* transcript, The transcription is shown below



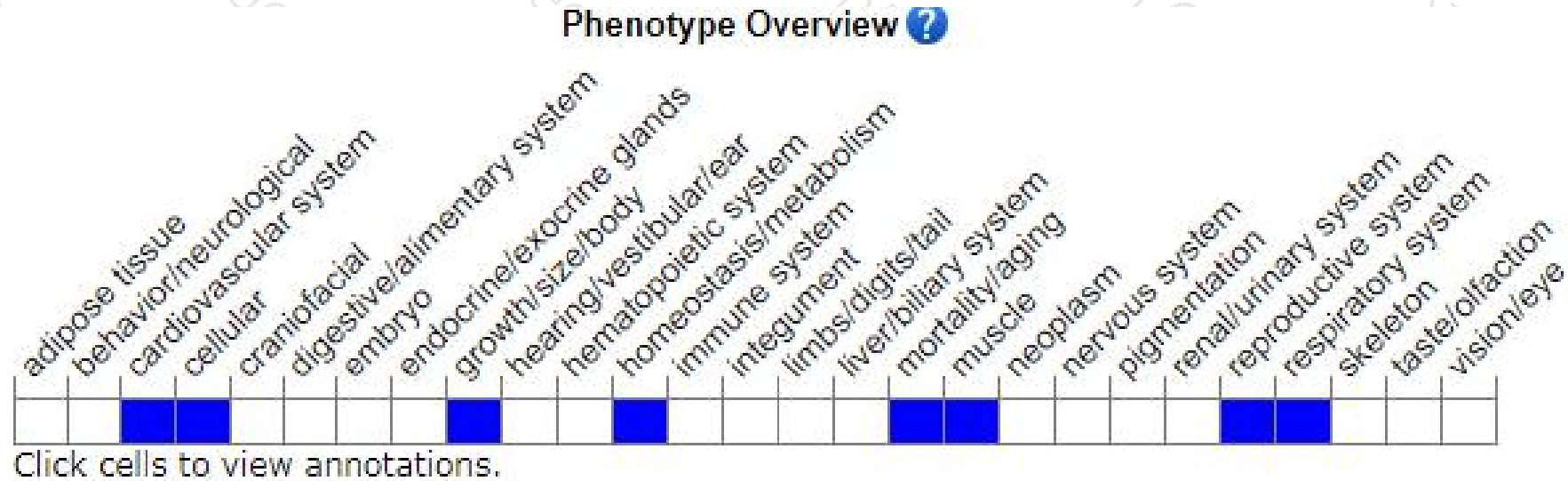
Genomic location distribution



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/>).

Homozygous null mice exhibit poor survival and breeding, lack the vasoconstrictor response to alpha2-adrenergic receptor agonists, and display background strain dependent postnatal respiratory failure. Heterozygotes show an attenuated hypertensive response to subtotal nephrectomy and salt loading.

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

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