Atp6v1d Cas9-CKO Strategy

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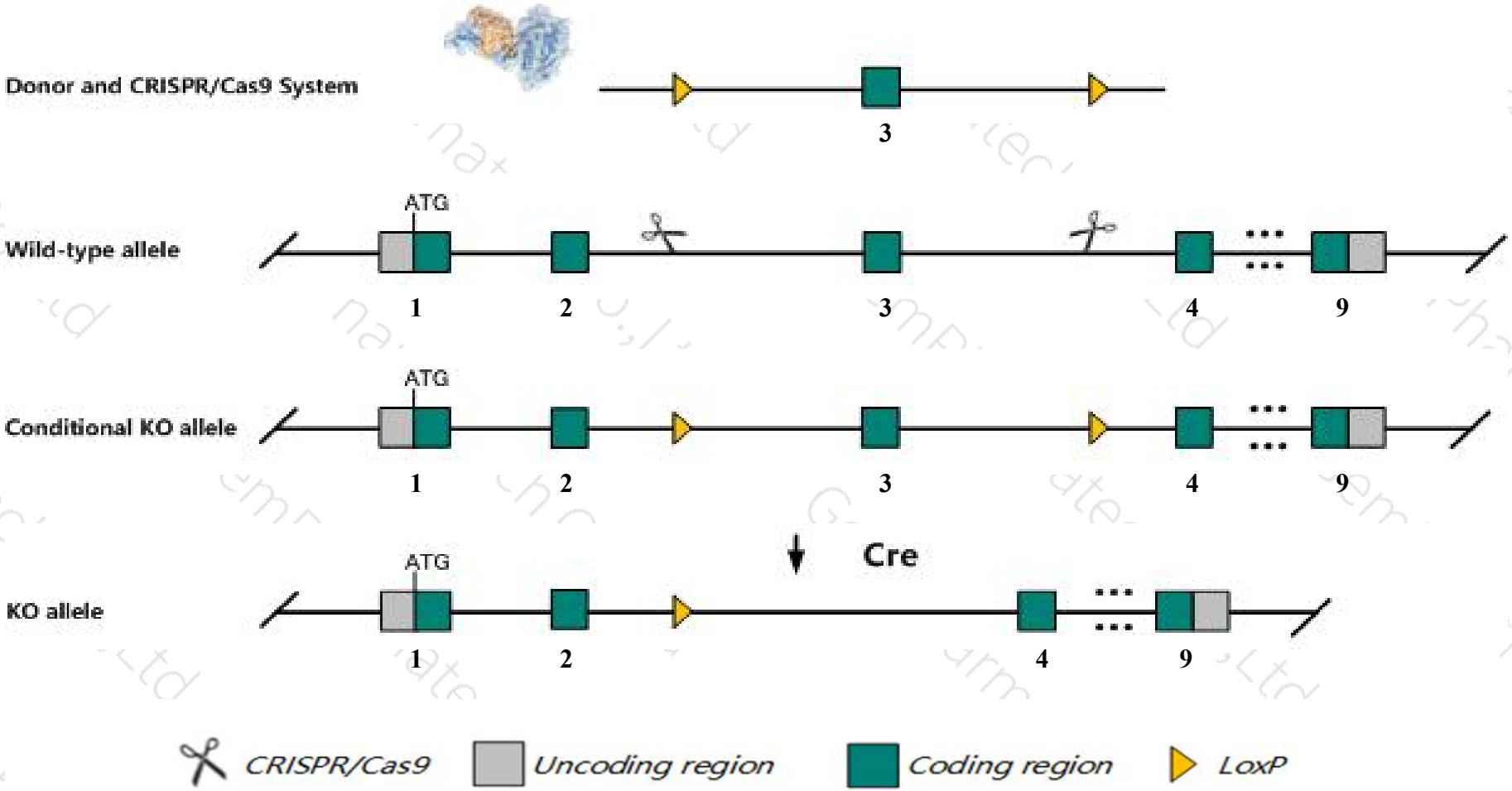
Project Overview

Project Name	***Atp6v1d***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Atp6v1d* gene. The schematic diagram is as follows:



Technical routes

➢ The *Atp6v1d* gene has 4 transcripts. According to the structure of *Atp6v1d* gene, exon3 of *Atp6v1d-201*(ENSMUST00000021536.8) transcript is recommended as the knockout region.The region contains 80bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Atp6v1d* 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

➢ The *Atp6v1d* gene is located on the Chr12. 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）

集萃药康 GemPharmatech

Atp6v1d ATPase, H+ transporting, lysosomal V1 subunit D [Mus musculus (house mouse)]

Gene ID: 73834, updated on 13-Mar-2020

Summary

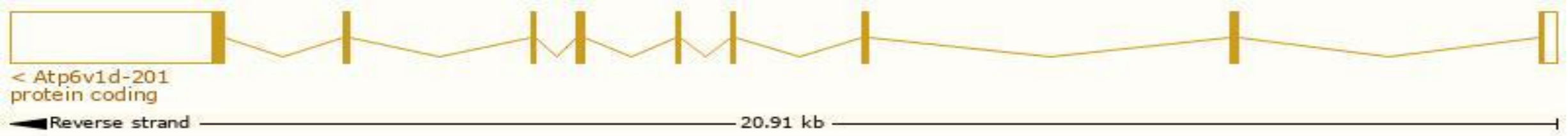
Official Symbol	Atp6v1d provided by MGI
Official Full Name	ATPase, H+ transporting, lysosomal V1 subunit D provided byMGI
Primary source	MGI:MGI:1921084
See related	Ensembl:ENSMUSG00000021114
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1110004P10Rik, Atp6m, VATD, Vma8
Expression	Broad expression in cortex adult (RPKM 56.2), frontal lobe adult (RPKM 53.1) and 22 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

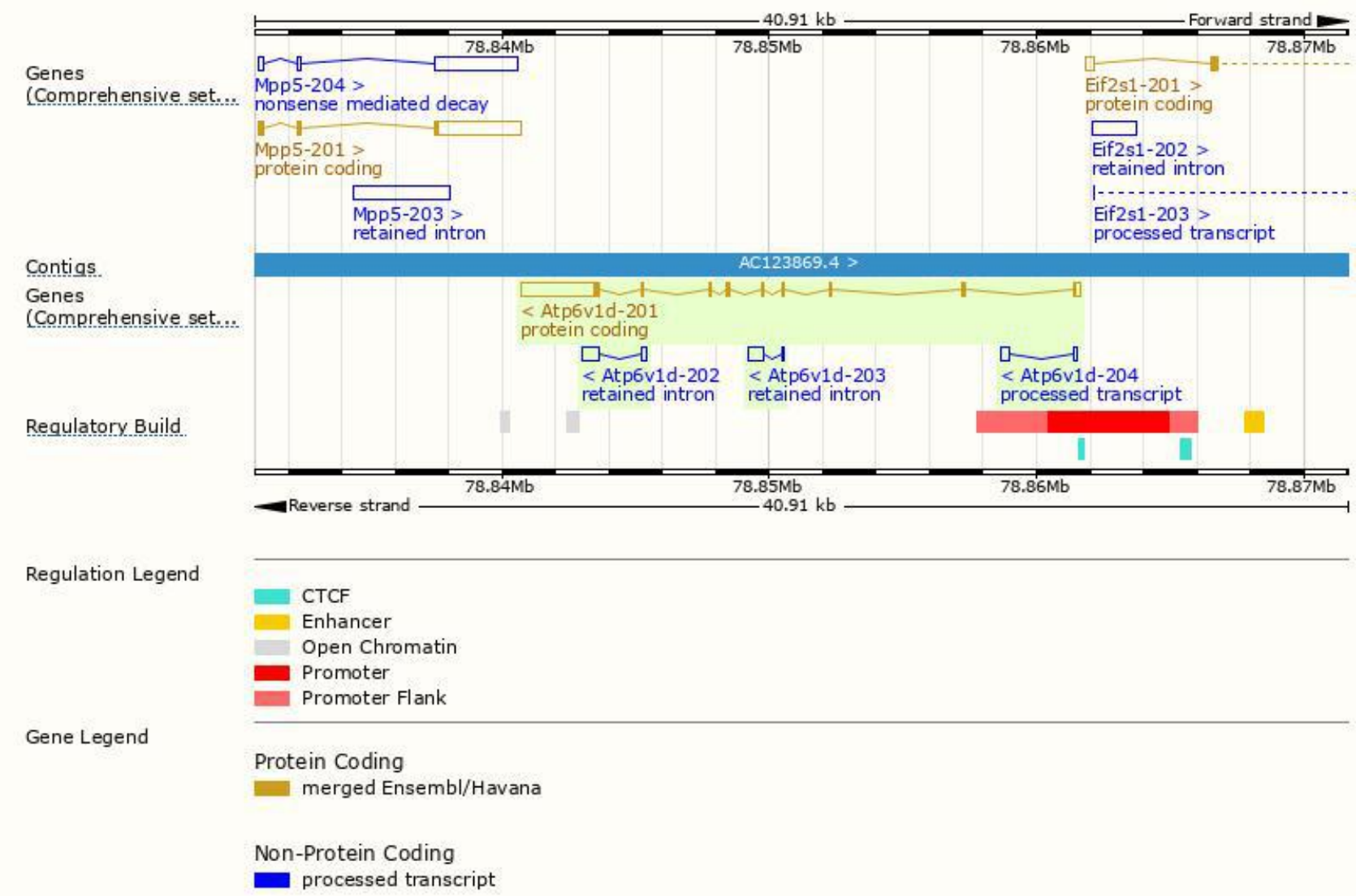
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Atp6v1d-201	ENSMUST00000021536.8	3667	247aa	Protein coding	CCDS26002	P57746 Q3U861	TSL:1 GENCODE basic APPRIS P1
Atp6v1d-204	ENSMUST00000219891.1	381	No protein	Processed transcript	-	-	TSL:2
Atp6v1d-202	ENSMUST00000219316.1	775	No protein	Retained intron	-	-	TSL:2
Atp6v1d-203	ENSMUST00000219377.1	634	No protein	Retained intron	-	-	TSL:2

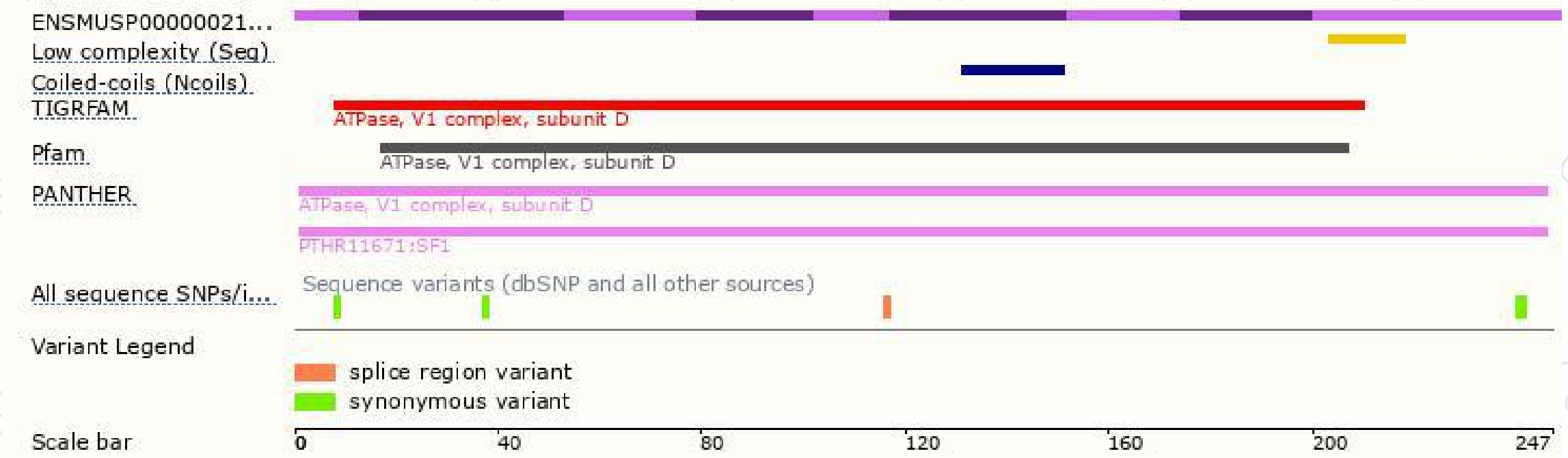
The strategy is based on the design of *Atp6v1d-201* transcript,the transcription is shown below:



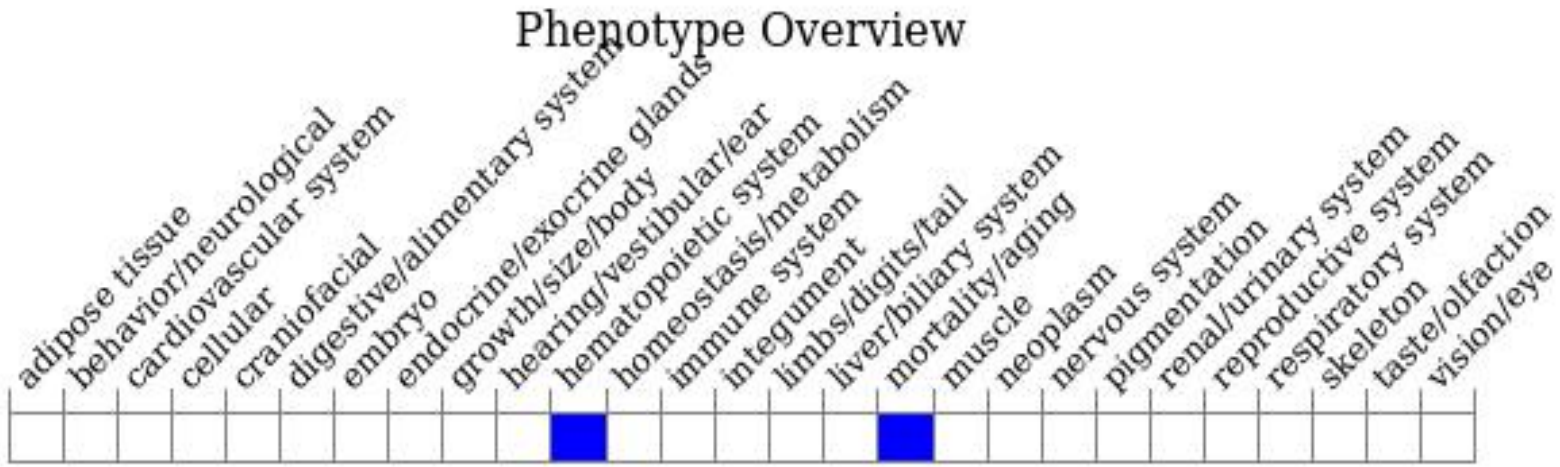
Genomic location distribution



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/).

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

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