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GemPharmatech

Arf6 Cas9-CKO Strategy

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Project Overview

Project Name	***Arf6***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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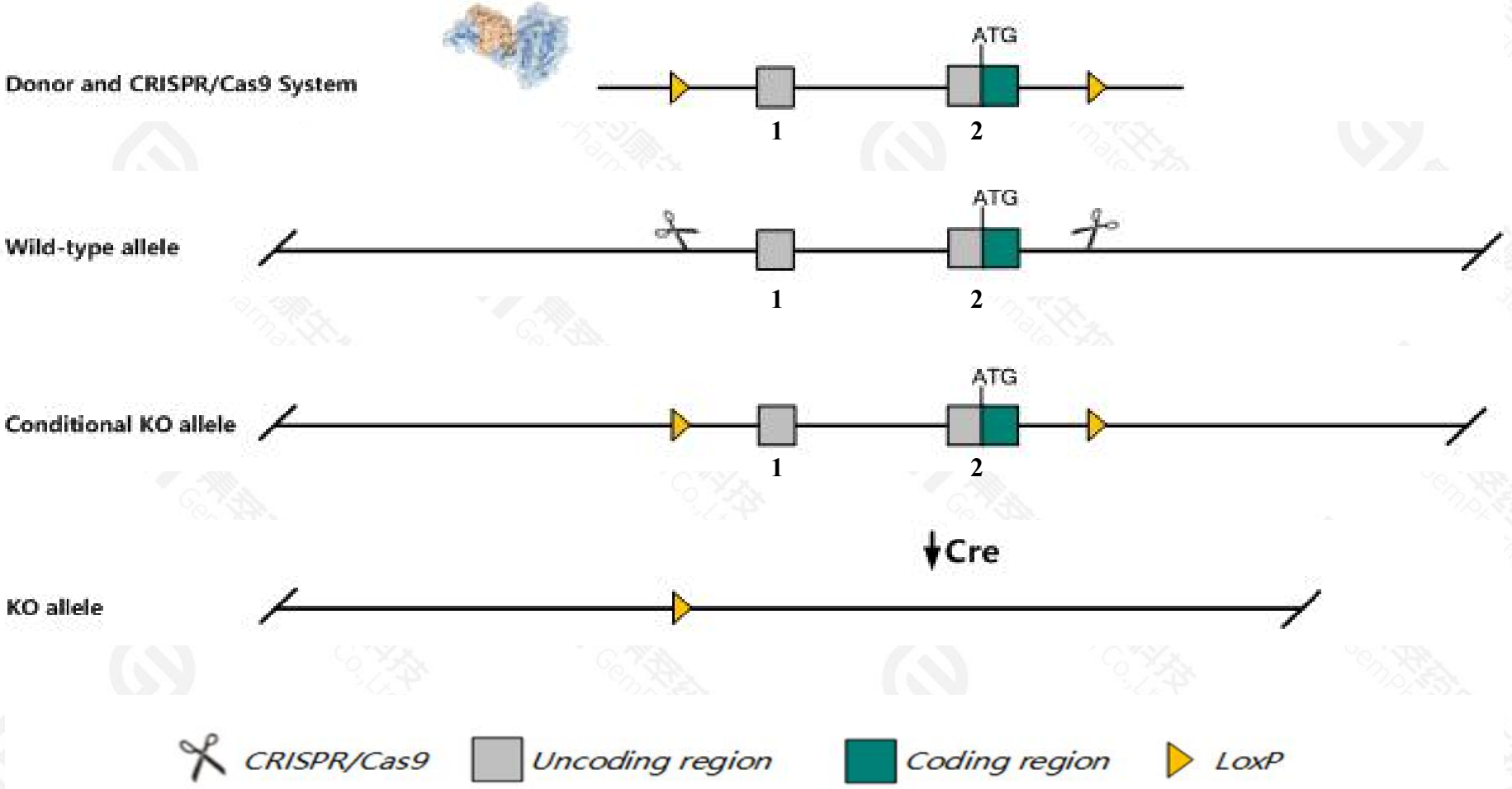
This model will use CRISPR/Cas9 technology to edit the *Arf6* gene. The schematic diagram is as follows:



Technical routes

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➢ The *Arf6* gene has 1 transcript. According to the structure of *Arf6* gene, exon1-exon2 of *Arf6-201*(ENSMUST00000050063.9) 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 *Arf6* 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

➢ Gm18113、Gm9887 gene will be deleted.

➢ According to the existing MGI data,mice homozygous for a null allele display embryonic and perinatal lethality beginning around mid-gestation, impaired liver development, and edema.

➢ The *Arf6* 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）

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Arf6 ADP-ribosylation factor 6 [Mus musculus (house mouse)]

Gene ID: 11845, updated on 14-Feb-2021

Summary

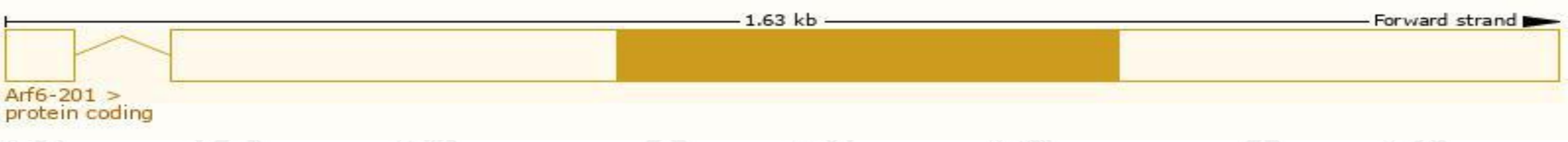
Official Symbol	Arf6 provided by MGI
Official Full Name	ADP-ribosylation factor 6 provided byMGI
Primary source	MGI:MGI:99435
See related	Ensembl:ENSMUSG00000044147
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI788669, AW496366
Expression	Ubiquitous expression in duodenum adult (RPKM 90.2), colon adult (RPKM 84.0) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

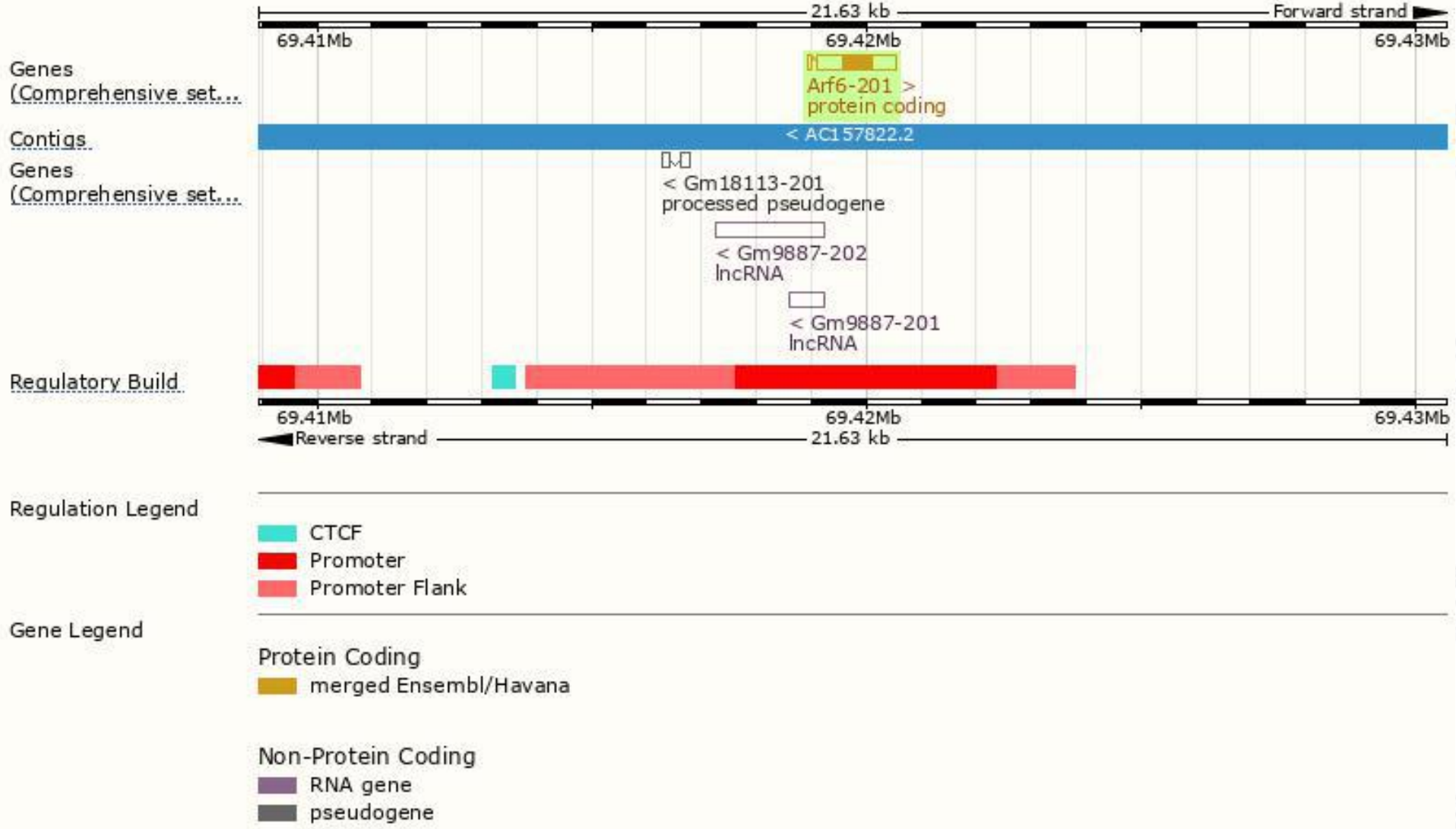
The gene has 1 transcript,and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Arf6-201	ENSMUST00000050063.9	1534	175aa	Protein coding	CCDS25952		TSL:1 , GENCODE basic , APPRIS P1 ,

The strategy is based on the design of *Arf6-201* transcript,the transcription is shown below:

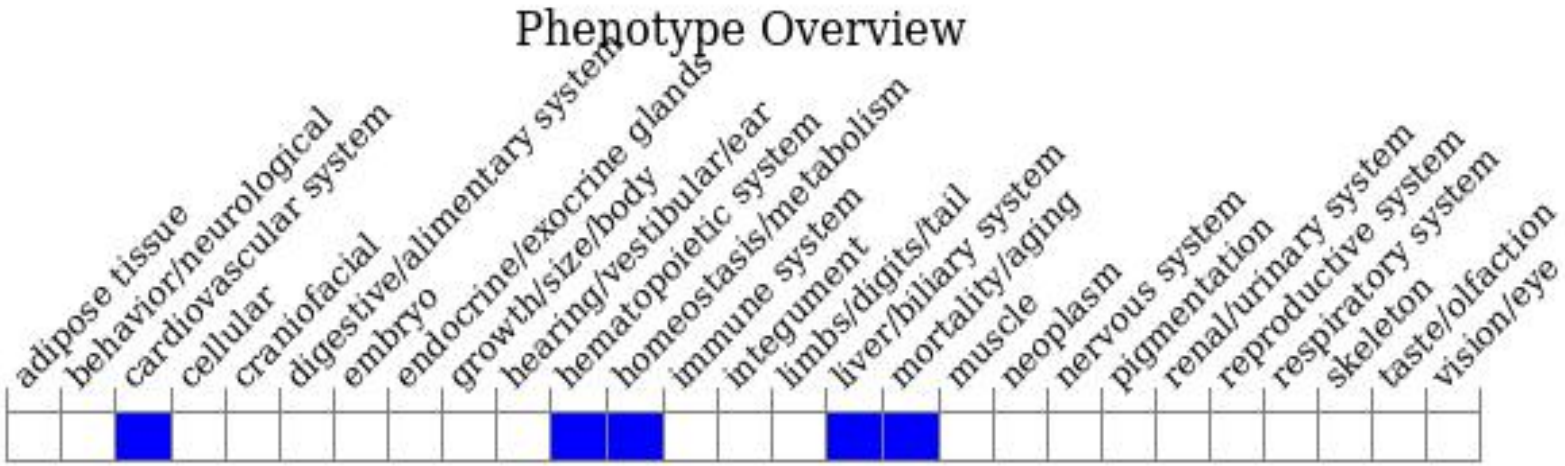


Genomic location distribution



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 null allele display embryonic and perinatal lethality beginning around mid-gestation, impaired liver development, and edema.

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