Cldn5 Cas9-CKO Strategy

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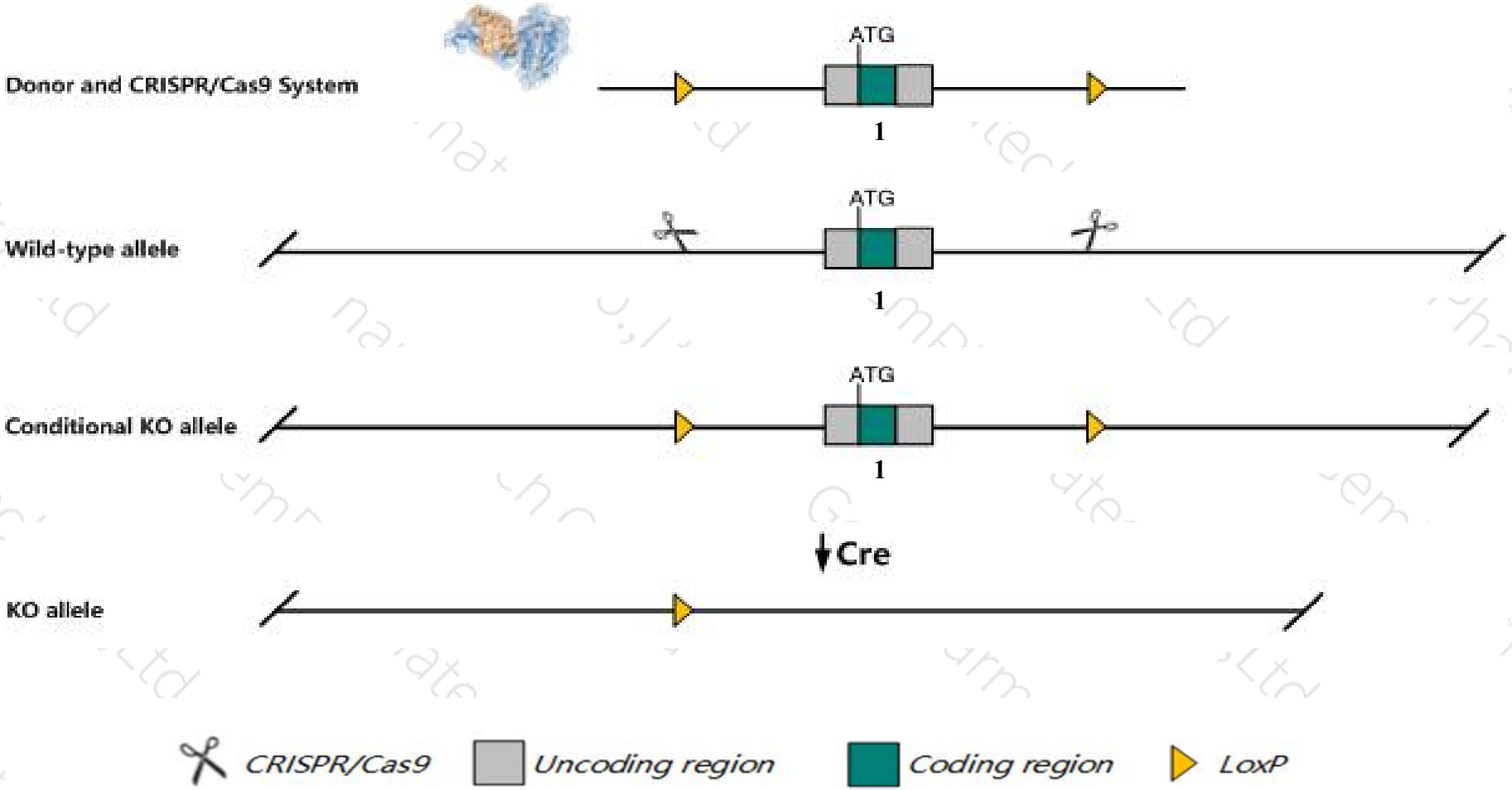
Project Overview

Project Name	***Cldn5***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cldn5* gene. The schematic diagram is as follows:



Technical routes

- The *Cldn5* gene has 1 transcript. According to the structure of *Cldn5* gene, exon1 of *Cldn5-201* (ENSMUST00000043577.2) 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 *Cldn5* 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,homozygous mutation of this gene results in size-selective loosening of the blood-brain barrier. homozygous mutant neonates gradually cease movement and die within 10 hours after birth.
- The *Cldn5* gene is located on the Chr16. 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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Cldn5 claudin 5 [Mus musculus (house mouse)]

Gene ID: 12741, updated on 13-Mar-2020

Summary

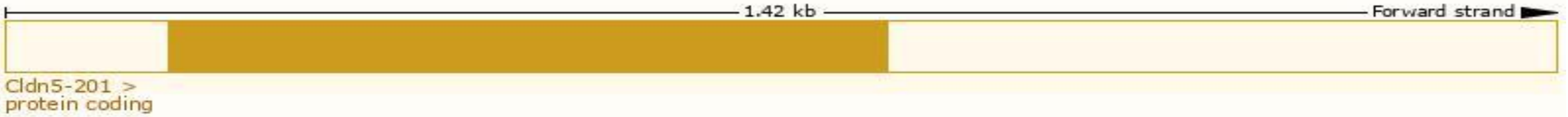
Official Symbol	Cldn5 provided by MGI
Official Full Name	claudin 5 provided byMGI
Primary source	MGI:MGI:1276112
See related	Ensembl:ENSMUSG00000041378
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI854493, MBEC1, Tmvcf
Summary	This gene encodes a member of the claudin family. Claudins are integral membrane proteins and components of tight junction strands. Tight junction strands serve as a physical barrier to prevent solutes and water from passing freely through the paracellular space between epithelial or endothelial cell sheets, and also play critical roles in maintaining cell polarity and signal transductions. The protein encoded by this gene is a critical component of endothelial tight junctions that control pericellular permeability. The knockout mice lacking this gene died within 10 h of birth and the blood-brain barrier in these mice against small molecules was selectively affected. This gene is expressed strongly in endothelium of normal lung and plays a regulation role during acrolein-induced acute lung injury. [provided by RefSeq, Aug 2010]
Orthologs	human all

Transcript information（Ensembl）

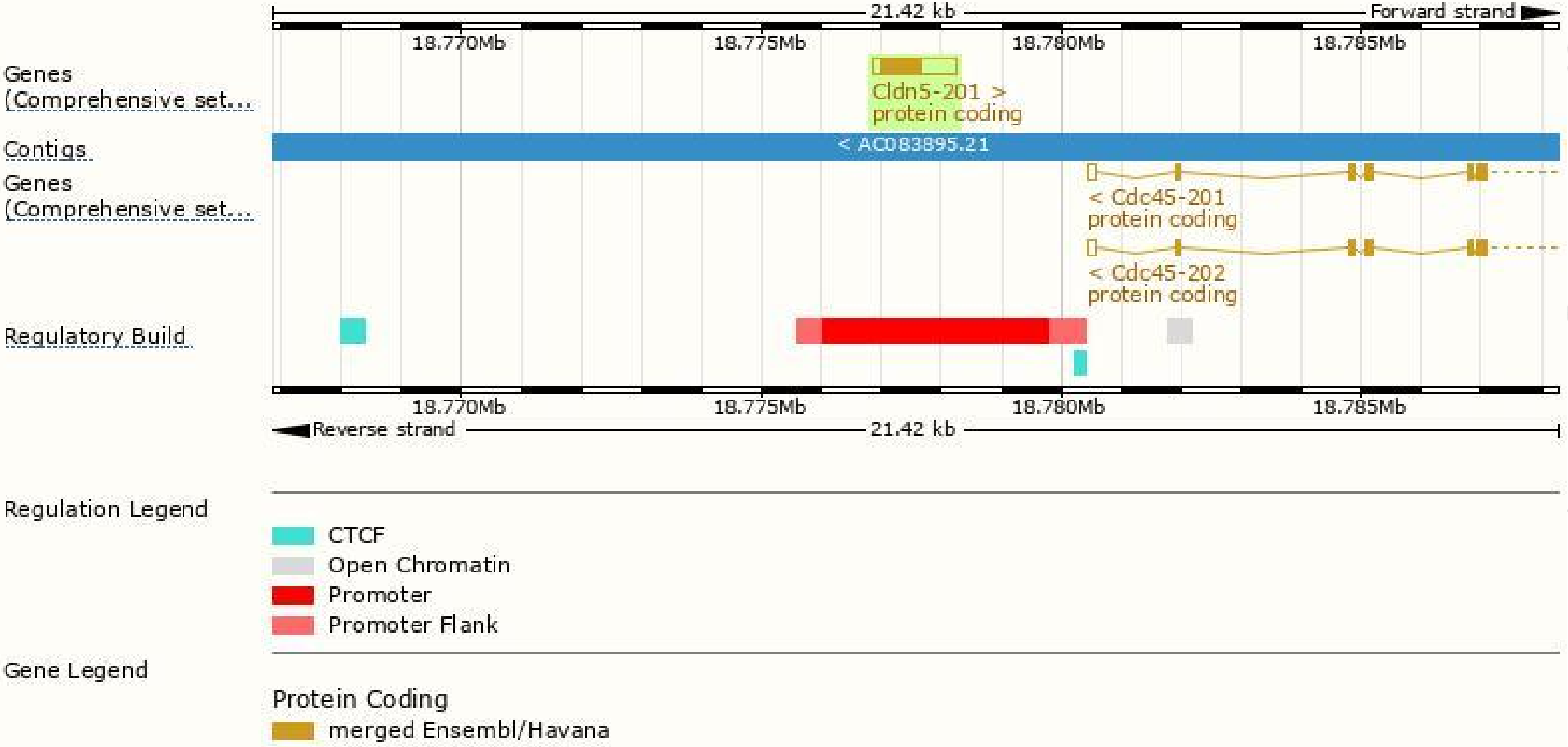
The gene has 1 transcript,and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cldn5-201	ENSMUST00000043577.2	1416	218aa	Protein coding	CCDS28026	O54942	TSL:NA GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1

The strategy is based on the design of *Cldn5-201* transcript,the transcription is shown below:

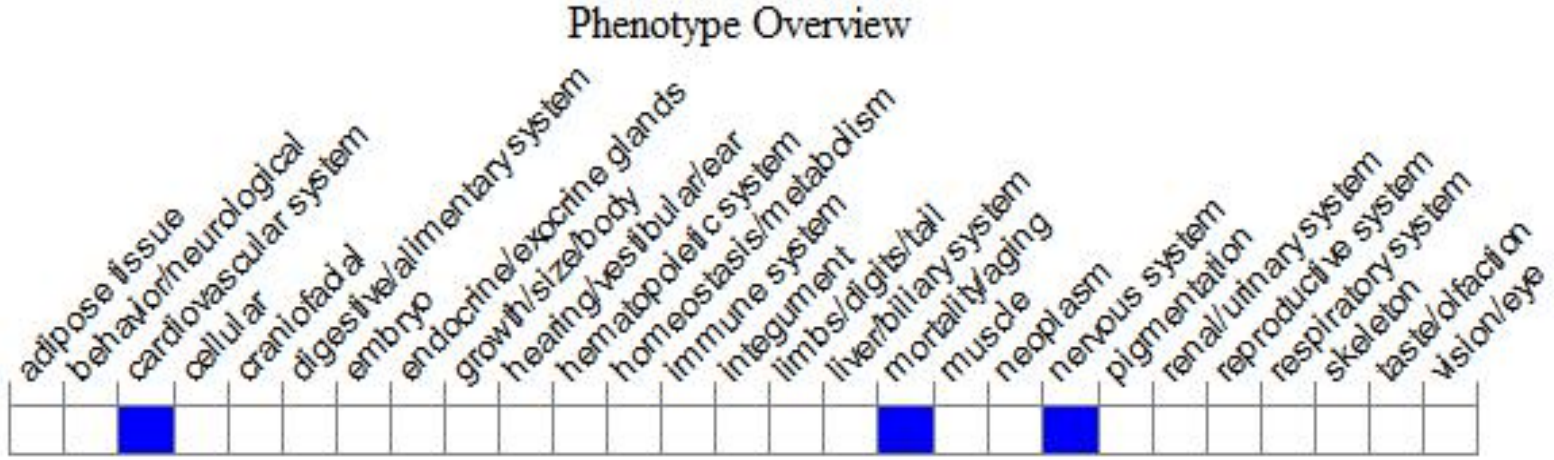


Genomic location distribution



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/).

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

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