Cldn10 Cas9-CKO Strategy

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Reviewer：   **Xiaojing Li**

Design Date: **2020-1-16**

Project Overview

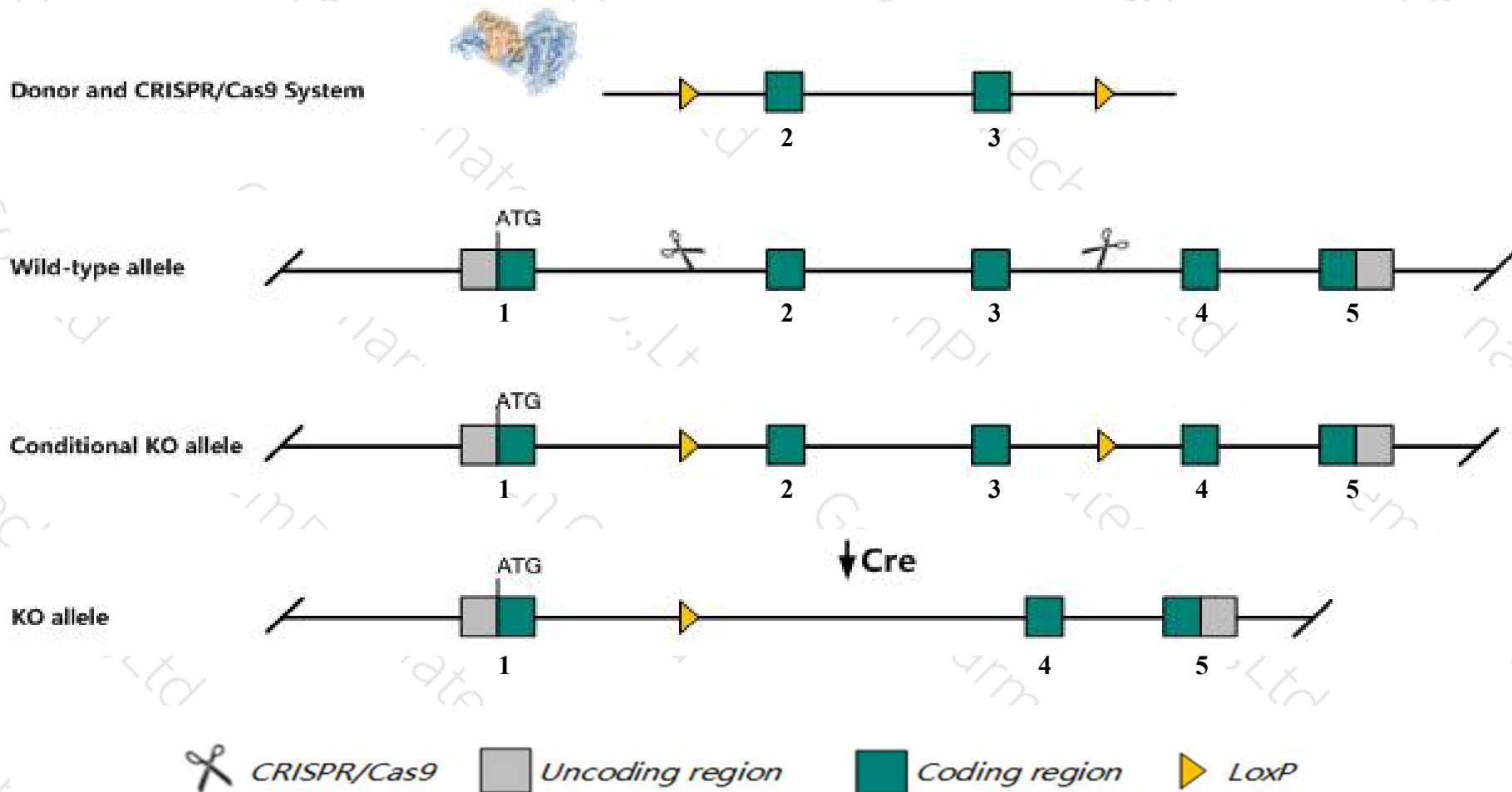|	
Project Name	*Cldn10*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cldn10* gene. The schematic diagram is as follows:

Technical routes

➤ The *Cldn10* gene has 3 transcripts. According to the structure of *Cldn10* gene, exon2-exon3 of *Cldn10-203* (ENSMUST00000100314.3) transcript is recommended as the knockout region.The region contains Ens 244bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Cldn10* 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, Mice lacking expression of this gene in the thick ascending limb of renal tubules display nephrocalcinosis, hypermagnesemia, and abnormalities in renal reabsorbtion.

➢ The *Cldn10* gene is located on the Chr14. 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）

GemPharmatech

Cldn10 claudin 10 [Mus musculus (house mouse)]

Gene ID: 58187, updated on 31-Jan-2019

Summary

|	
Official Symbol	Cldn10 provided by MGI
Official Full Name	claudin 10 provided by MGI
Primary source	MGI:MGI:1913101
See related	Ensembl:ENSMUSG00000022132
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	6720456I16Rik, Cldn10a, Cldn10b, D14Ertd728e
Summary	This intronless gene encodes a member of the claudin family. Claudins are integral membrane proteins and components of tight unction 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. Six alternatively spliced transcript variants have been identified, which encode different isoforms with distinct electric charge of the first extracellular loop and with or without the fourth transmembrane region. These isoforms exhibit distinct localization and function in paracellular anion or cation permeability. [provided by RefSeq, Aug 2010]
Expression	Biased expression in kidney adult (RPKM 74.8), genital fat pad adult (RPKM 19.6) and 7 other tissuesSee 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
Cldn10-203	ENSMUST00000100314.3	2128	231aa	Protein coding	CCDS37010	Q9Z0S6	TSL:1 GENCODE basic APPRIS P1
Cldn10-201	ENSMUST00000047761.12	1148	229aa	Protein coding	CCDS37009	Q9Z0S6	TSL:1 GENCODE basic
Cldn10-202	ENSMUST00000071546.13	1031	193aa	Protein coding	CCDS49566	Q9Z0S6	TSL:1 GENCODE basic

The strategy is based on the design of *Cldn10-203* transcript,The transcription is shown below

20.76 kb · Forward strand
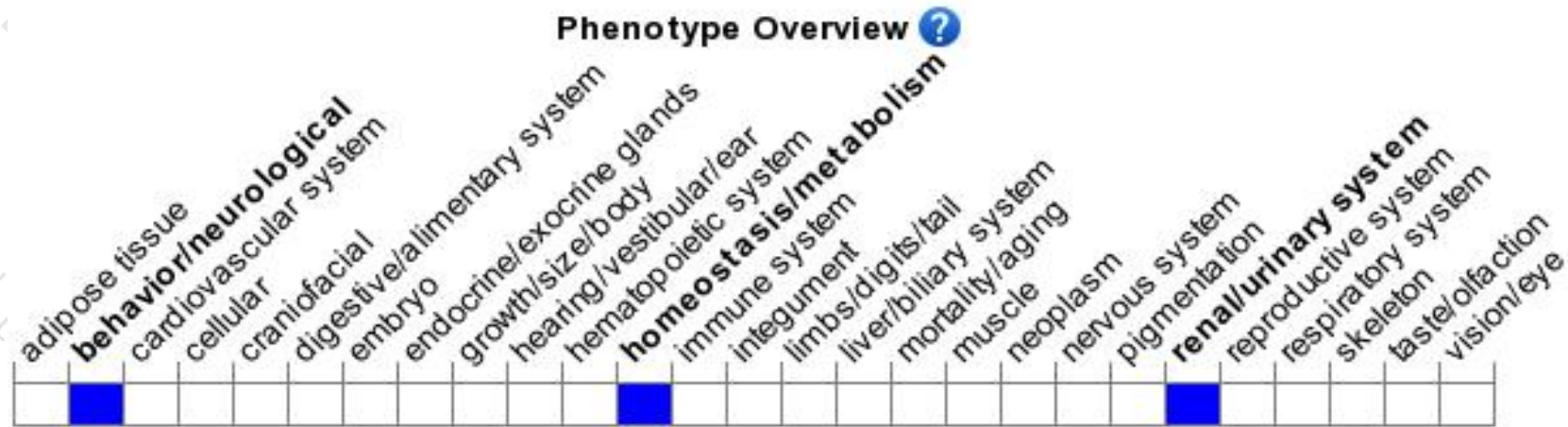
Cldn10-203 >
protein coding

Genomic location distribution
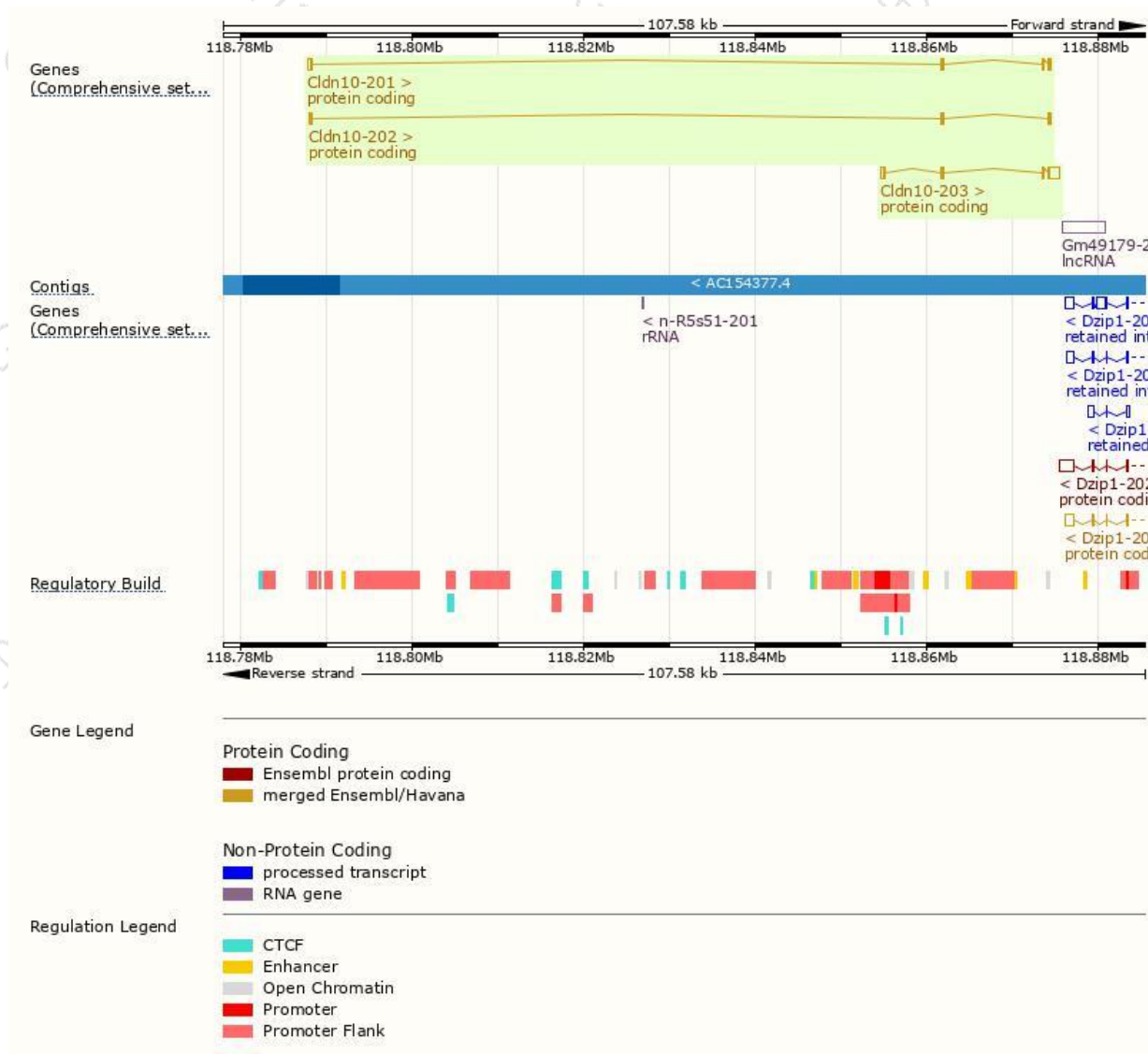
Protein domain
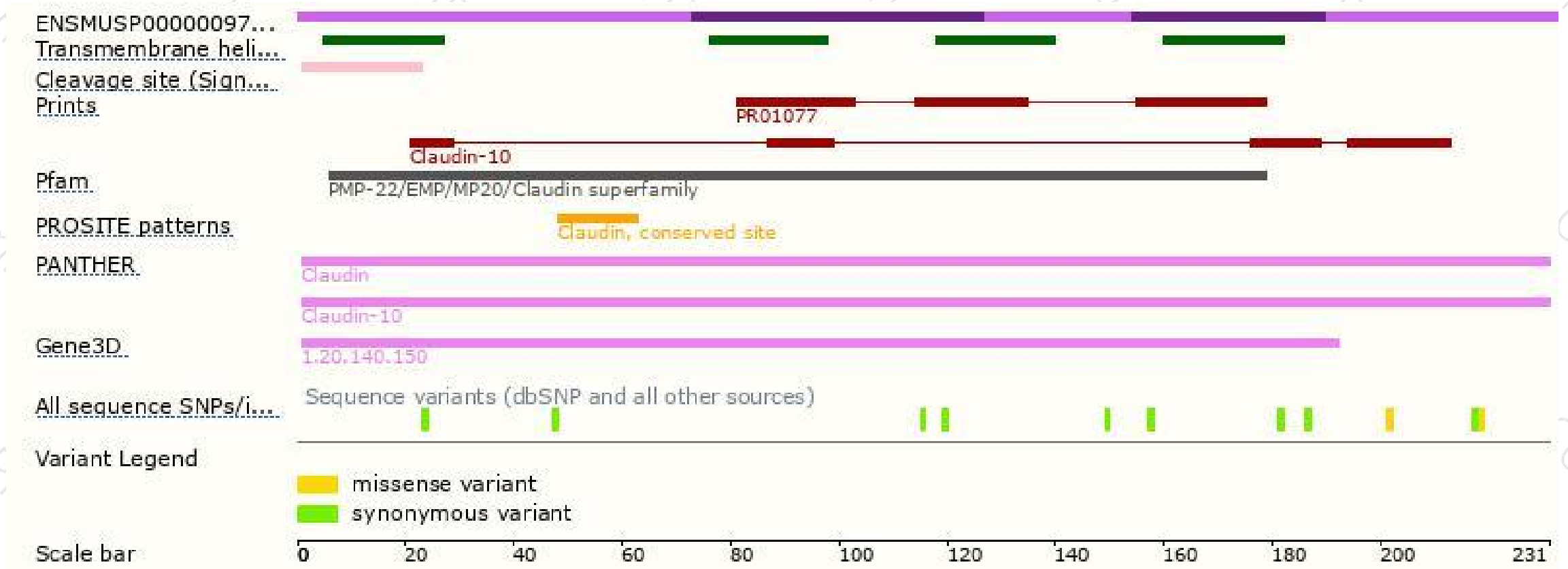
Mouse phenotype description(MGI)

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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