Cldn3 Cas9-CKO Strategy

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Project Overview

Project Name	***Cldn3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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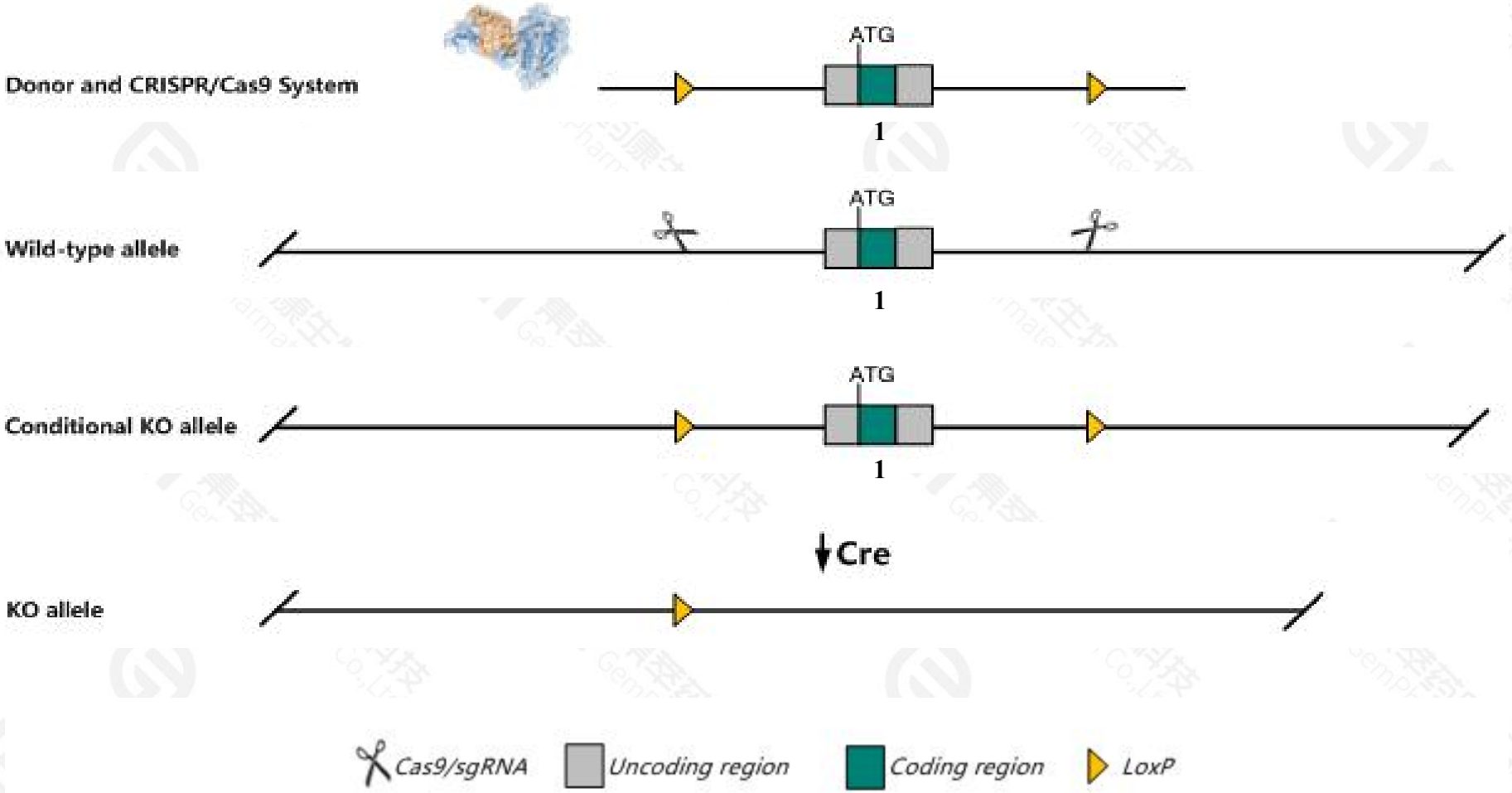
This model will use CRISPR/Cas9 technology to edit the *Cldn3* gene. The schematic diagram is as follows:



Technical routes

➢ The *Cldn3* gene has 1 transcript. According to the structure of *Cldn3* gene, exon1 of *Cldn3-201*(ENSMUST00000094245.3) 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 *Cldn3* 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/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 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.

Notice

➢ According to the existing MGI data,mice homozygous for a null allele are fertile with mutant males exhibiting normal spermatogenesis and fully functional Sertoli cell tight junctions.

➢ The KO region contains functional region of the *Wbscr25* gene. Knockout the region may affect the function of *Wbscr25* gene.

➢ The *Cldn3* gene is located on the Chr5. 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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Cldn3 claudin 3 [Mus musculus (house mouse)]

Gene ID: 12739, updated on 13-Mar-2020

Summary

Official Symbol	Cldn3 provided by MGI
Official Full Name	claudin 3 provided by MGI
Primary source	MGI:MGI:1329044
See related	Ensembl:ENSMUSG00000070473
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	AI182374, Cpetr2, mRVP1
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 low-affinity receptor for clostridium perfringens enterotoxin (CPE) produced by the bacterium Clostridium perfringens, and the interaction with CPE results in increased membrane permeability by forming small pores in plasma membrane. This protein is highly overexpressed in uterine carcinosarcoma. This protein is also predominantly present in brain endothelial cells, where it plays a specific role in the establishment and maintenance of blood brain barrier tight junction morphology. [provided by RefSeq, Aug 2012]
Orthologs	human all

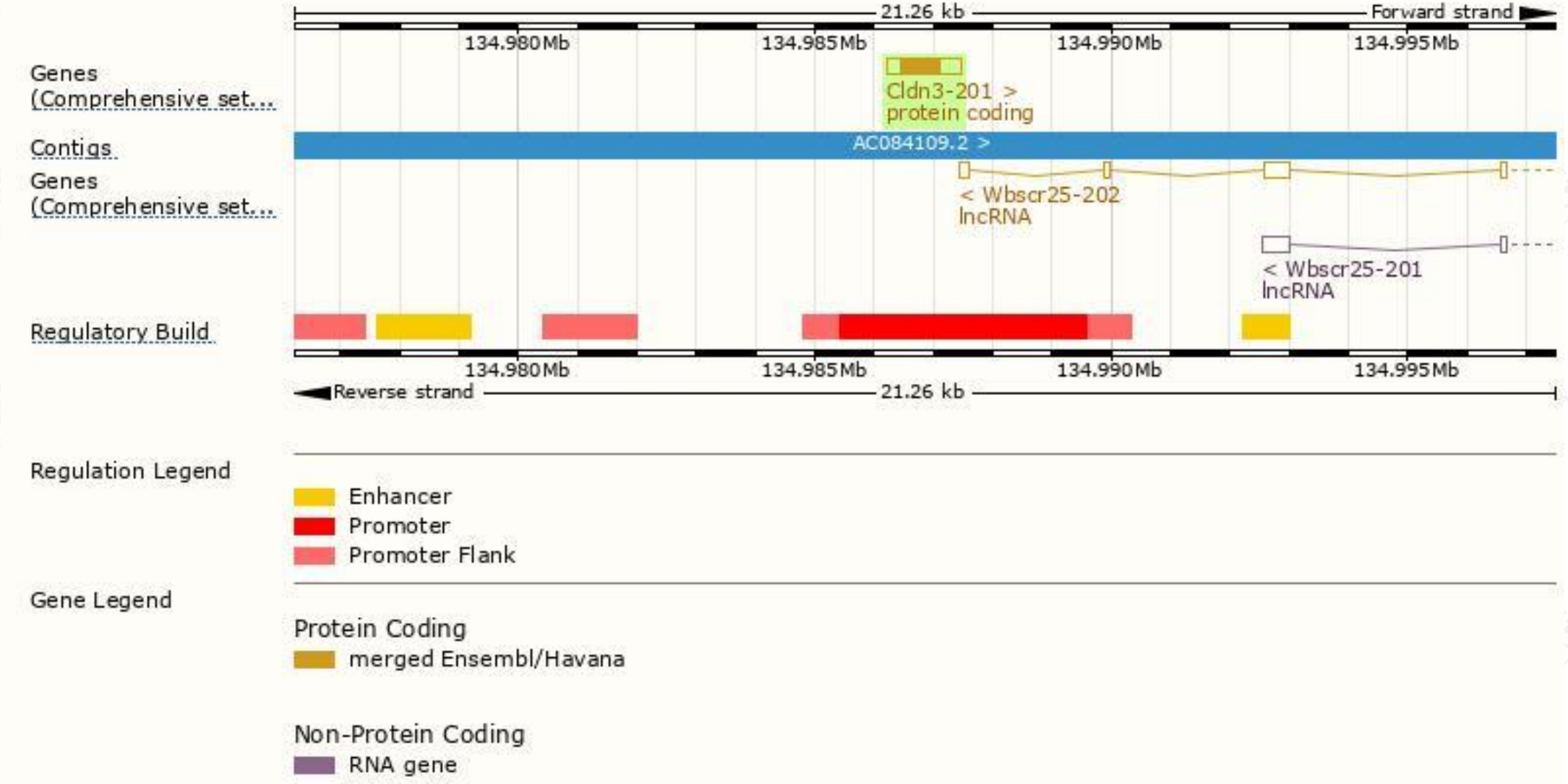
Transcript information（Ensembl）

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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cldn3-201	ENSMUST00000094245.3	1259	219aa	Protein coding	CCDS19729	Q545A5 Q9Z0G9	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 *Cldn3-201* transcript,the transcription is shown below:

Genomic location distribution



Protein domain

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

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