Cldn18-IRES-CreERT2 Cas9-KI Strategy

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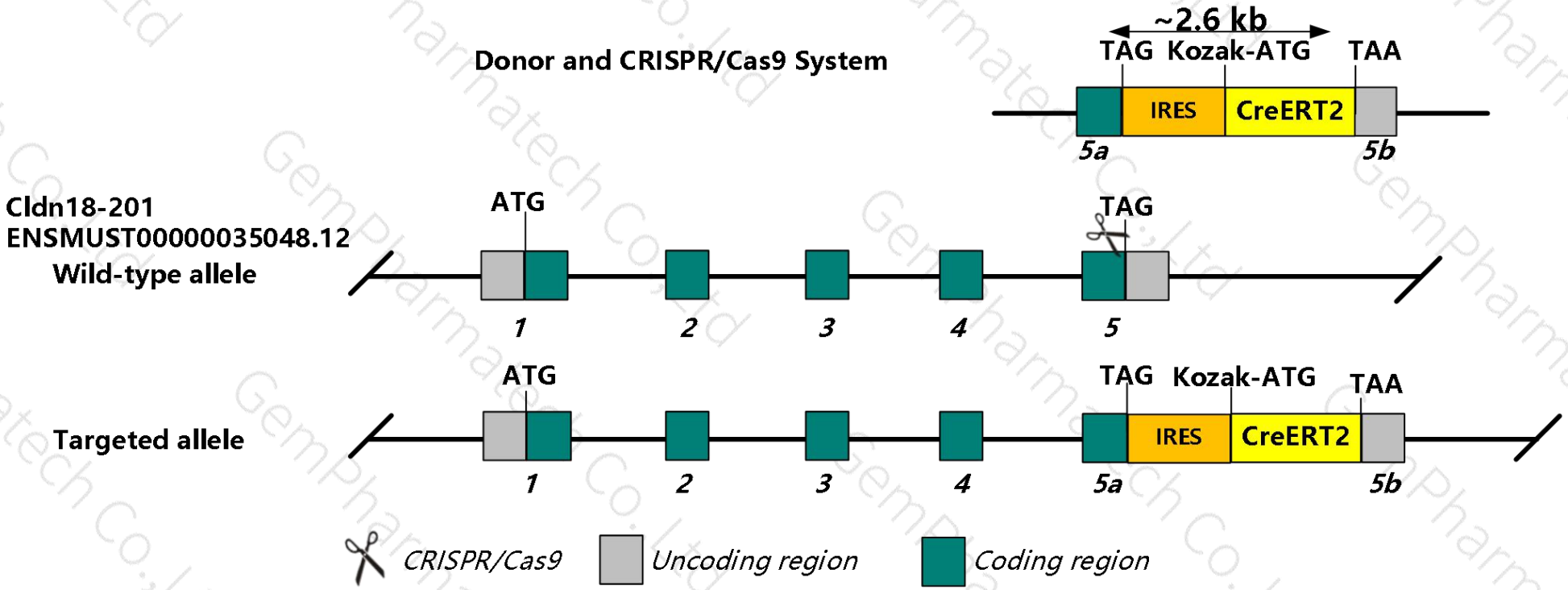
Design Date:	**2021-12-21**

Project Overview

Project Name	**Cldn18-IRES-CreERT2**
Project type	**Cas9-KI**
Strain background	**C57BL/6JGpt**

Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Cldn18* gene. The schematic diagram is as follows:



Technical routes

- The *Cldn18* gene has 4 transcripts.According to the structure of *Cldn18* gene, *Cldn18-201*(ENSMUST00000035048.12) is selected for presentation of the recommended strategy.

- *Cldn18-201* gene has 5 exons, with the ATG start codon in exon1 and TAG stop codon in exon5.

- We make *Cldn18-IRES-CreERT2* knockin mice via CRISPR/Cas9 system. CRISPR/Cas9 system and donor will be co-injected into zygotes. Cas9 endonuclease cleavage near stop codon(TAG) of exon5 of *Cldn18* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in *IRES-CreERT2* after stop coding(TAG) of *Cldn18* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

Notice

- According to the existing MGI data, mice homozygous for a knock-out allele exhibit increased bone resorption and osteoclast differentiation. Homozygotes for another knock-out allele have impaired alveolarization and alveolar epithelial barrier function.
- If the two genes are linked with IRES,and they will be transcripted together and then be translated two protein separately. Otherwise, IRES may produce lower amounts of the downstream protein in relation to the upstream protein.
- Insertion of IRES-CreERT2 may affect the regulation of the 3' end of the *Cldn18* gene.
- The effect of transcripts 203, 204 is unknowm.
- There may be 1 to 2 amino acid synonymous mutation in exon5 of *Cldn18* gene in this strategy.
- The *Cldn18* gene is located on the Chr9. If the knockin mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- The scheme is designed according to the genetic information in the existing database. Inserting a foreign gene after the gene coding region may affect the expression of endogenous and foreign genes. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

Gene information (NCBI)

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Cldn18 claudin 18 [*Mus musculus* (house mouse)]

Download Datasets

Gene ID: 56492, updated on 27-Jul-2021

Summary

Official Symbol	Cldn18 provided by MGI
Official Full Name	claudin 18 provided by MGI
Primary source	MGI:MGI:1929209
See related	Ensembl:ENSMUSG00000032473
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
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. This gene is a downstream target gene regulated by the T/EBP/NKX2.1 homeodomain transcription factor. Four alternatively spliced transcript variants resulted from alternative promoters and alternative splicing have been identified, which encode two lung-specific isoforms and two stomach-specific isoforms respectively. This gene is also expressed in colons, inner ear and skin, and its expression is increased in both experimental colitis and ulcerative colitis. [provided by RefSeq, Aug 2010]
Expression	Biased expression in lung adult (RPKM 177.1), stomach adult (RPKM 118.5) and 1 other tissue See more
Orthologs	human all

NEW Try the new Gene table

Try the new Transcript table

Genomic context

Location: 9; 9 E3.3

See Cldn18 in Genome Data Viewer

Exon count: 6

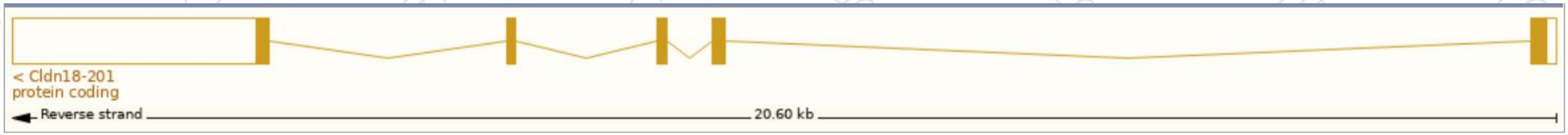
Chromosome 9 - NC_000075.7

Transcript information （Ensembl）

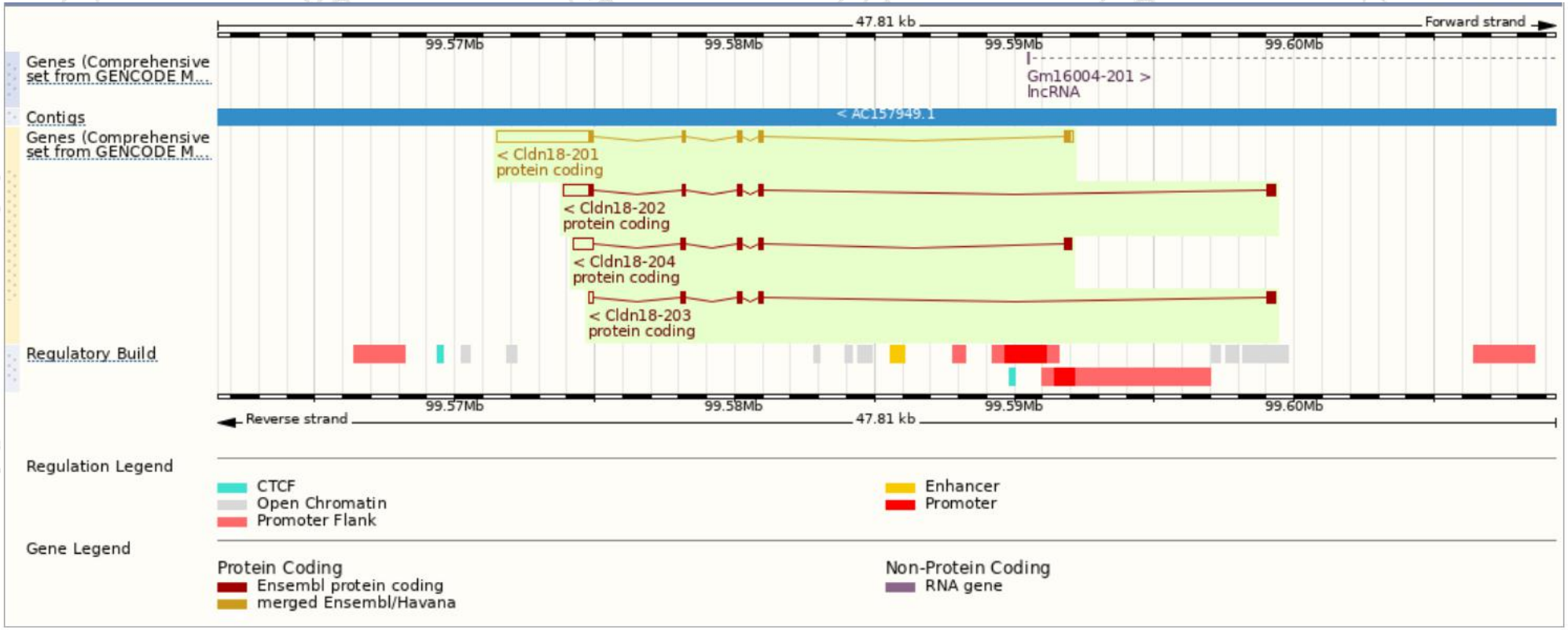
The gene has 4 transcripts, and all transcripts are shown below：

Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000035048.12	Cldn18-201	4172	264aa	Protein coding	CCDS23437	P56857-1	GENCODE basic APPRIS P3 TSL:1
ENSMUST00000112882.9	Cldn18-202	1750	264aa	Protein coding	CCDS57694	P56857-3	GENCODE basic APPRIS ALT1 TSL:1
ENSMUST00000136429.8	Cldn18-204	1409	208aa	Protein coding	CCDS57692	P56857-2	GENCODE basic TSL:1
ENSMUST00000131922.2	Cldn18-203	860	208aa	Protein coding	CCDS57693	P56857-4	GENCODE basic TSL:1

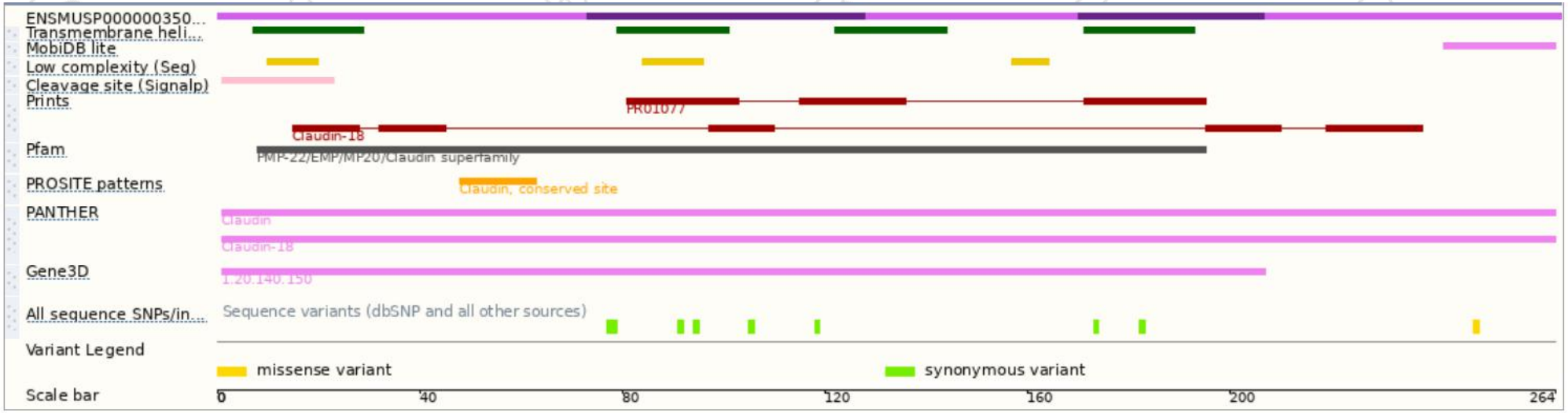
The strategy is based on the design of *Cldn18-201* transcript,the transcription is shown below



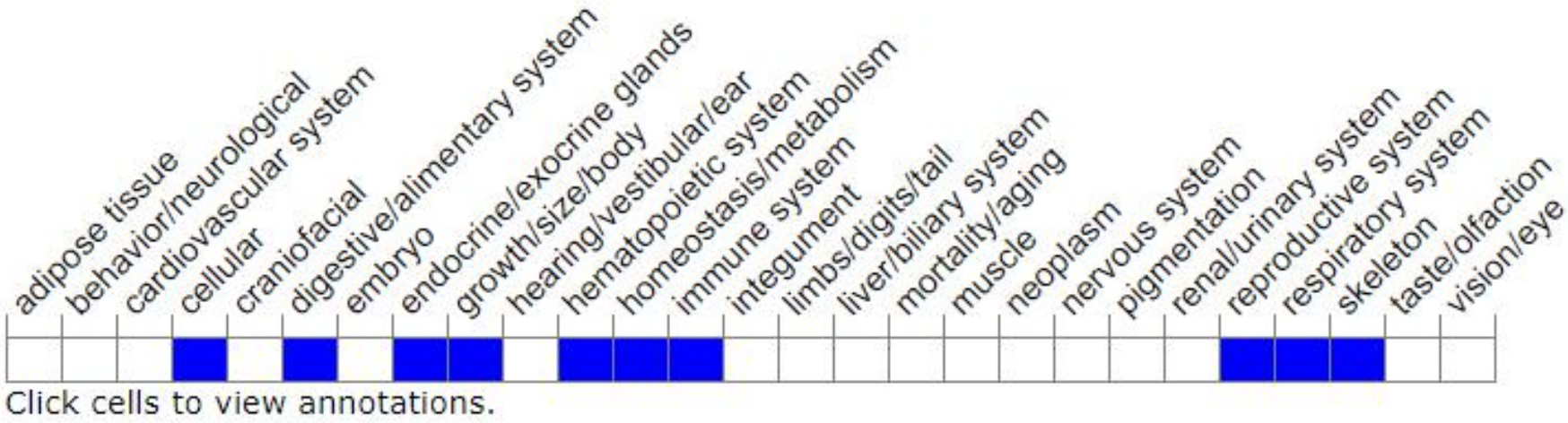
Genomic location distribution



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/marker/MGI:1929209) .

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Homozygotes for another knock-out allele have impaired alveolarization and alveolar epithelial barrier function.

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

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