Cldn14 Cas9-CKO Strategy

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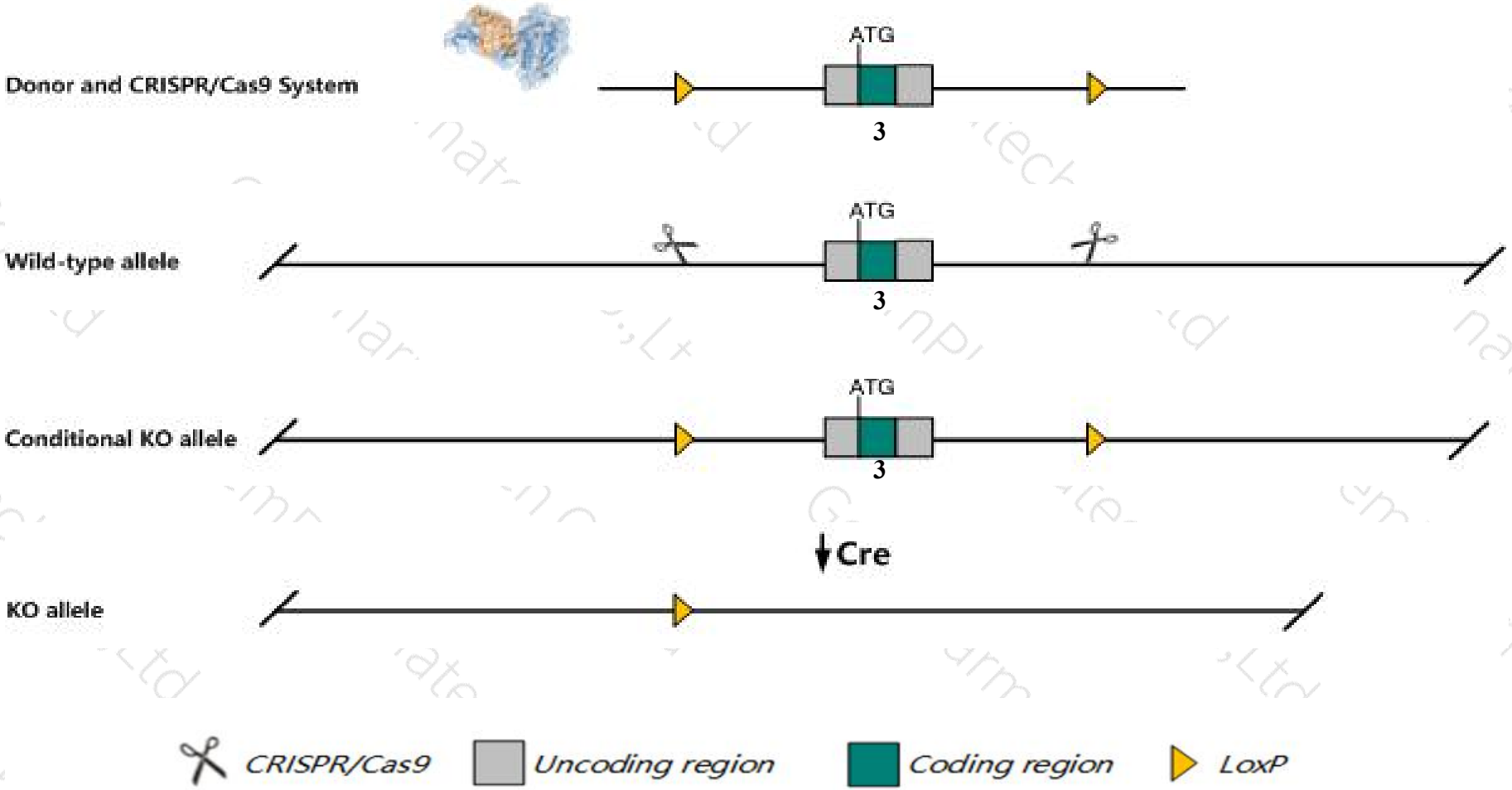
Project Overview

Project Name	***Cldn14***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cldn14* gene. The schematic diagram is as follows:



Technical routes

- The *Cldn14* gene has 6 transcripts. According to the structure of *Cldn14* gene, exon3 of *Cldn14-201* (ENSMUST00000050962.4) 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 *Cldn14* 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 mutant mice have a normal endocochlear potential but are deaf due to cochlear hair cell degeneration within the first 3 weeks of age.

➢ The *Cldn14* 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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Cldn14 claudin 14 [*Mus musculus* (house mouse)]

Gene ID: 56173, updated on 26-Nov-2019

Summary

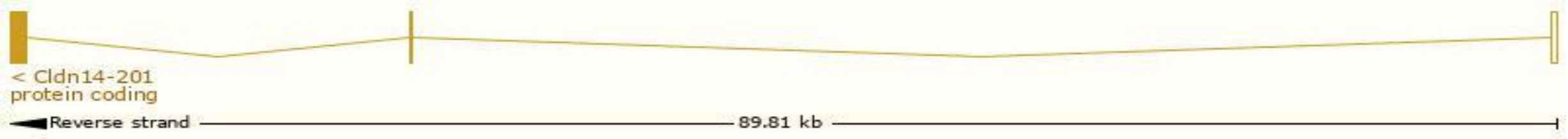
Official Symbol	Cldn14 provided by MGI
Official Full Name	claudin 14 provided by MGI
Primary source	MGI:MGI:1860425
See related	Ensembl:ENSMUSG00000047109
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI851731
Summary	This gene encodes a member of the claudin family of tight junction proteins. The encoded protein is an integral membrane protein that may function in maintaining apical membrane polarization in tight junctions located between outer hair cells and supporting cells. Loss of function of this gene is associated with hearing problems. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Oct 2009]
Expression	Biased expression in genital fat pad adult (RPKM 18.8), liver adult (RPKM 9.9) and 3 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

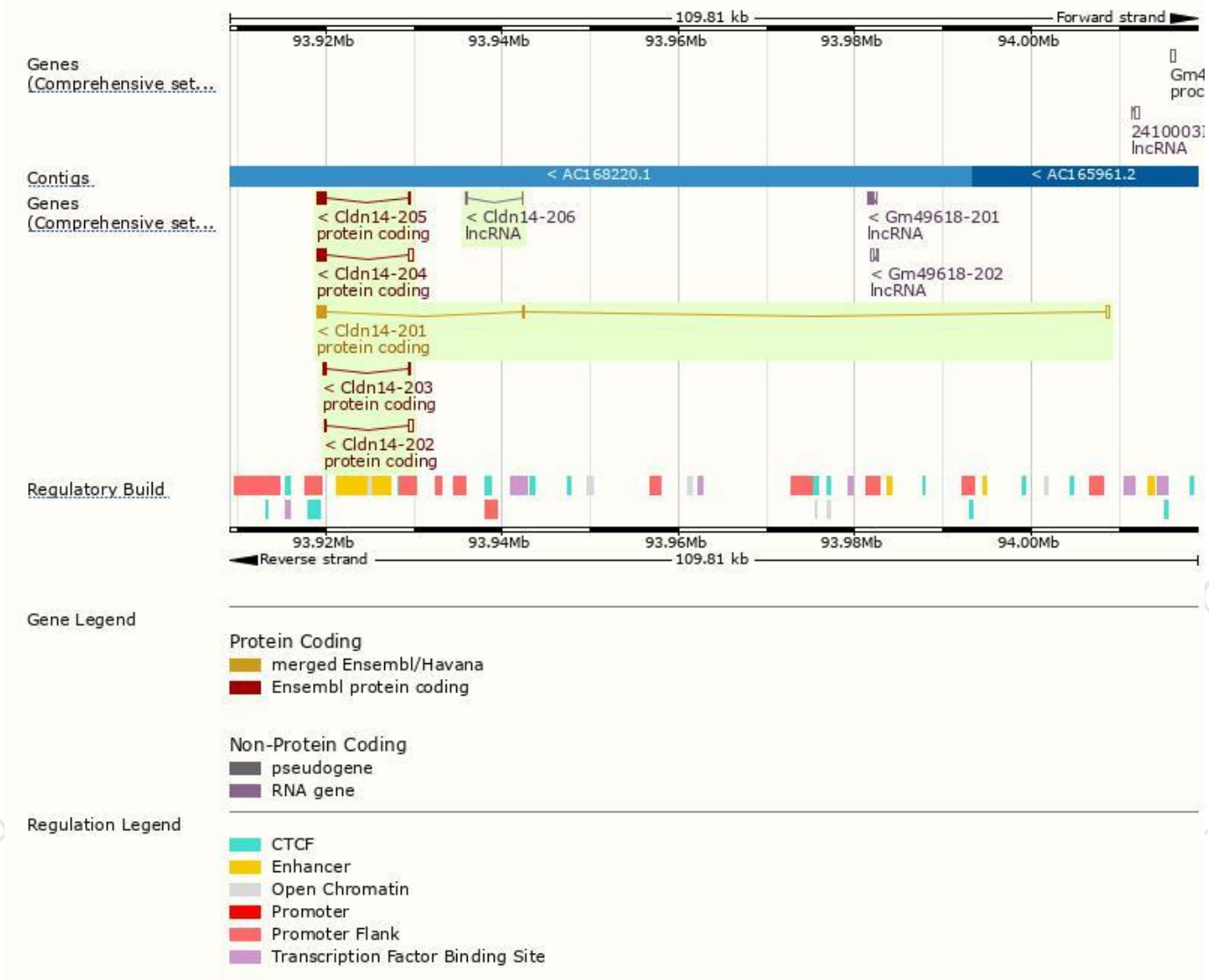
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cldn14-202	ENSMUST00000137163.1	680	50aa	Protein coding	-	A0A338P6L6	CDS 3' incomplete TSL:2
Cldn14-203	ENSMUST00000142083.1	548	69aa	Protein coding	-	A0A338P6X8	CDS 3' incomplete TSL:2
Cldn14-201	ENSMUST00000050962.4	1457	239aa	Protein coding	CCDS28345	A2RSP0 Q9Z0S3	TSL:1 GENCODE basic APPRIS P1
Cldn14-204	ENSMUST00000169391.7	1456	239aa	Protein coding	CCDS28345	A2RSP0 Q9Z0S3	TSL:2 GENCODE basic APPRIS P1
Cldn14-205	ENSMUST00000177648.7	1266	239aa	Protein coding	CCDS28345	A2RSP0 Q9Z0S3	TSL:2 GENCODE basic APPRIS P1
Cldn14-206	ENSMUST00000232406.1	279	No protein	Processed transcript	-	-	-

The strategy is based on the design of *Cldn14-201* transcript,The transcription is shown below

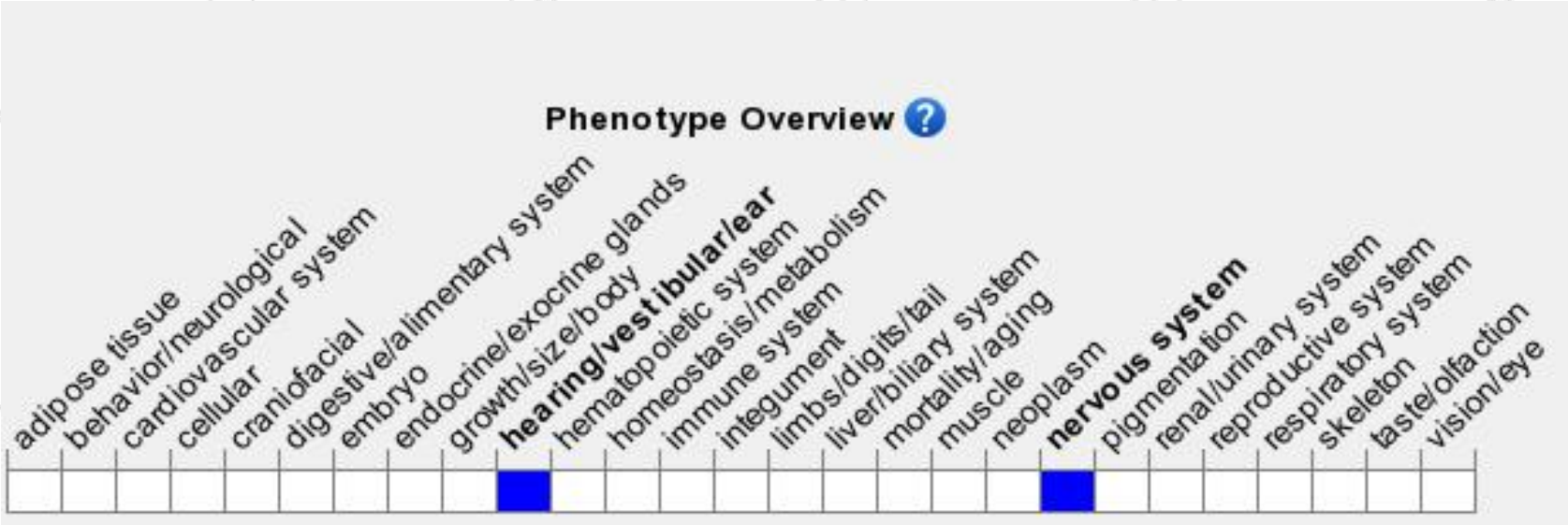


Genomic location distribution



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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