Gja10-iCre Cas9-KI Strategy

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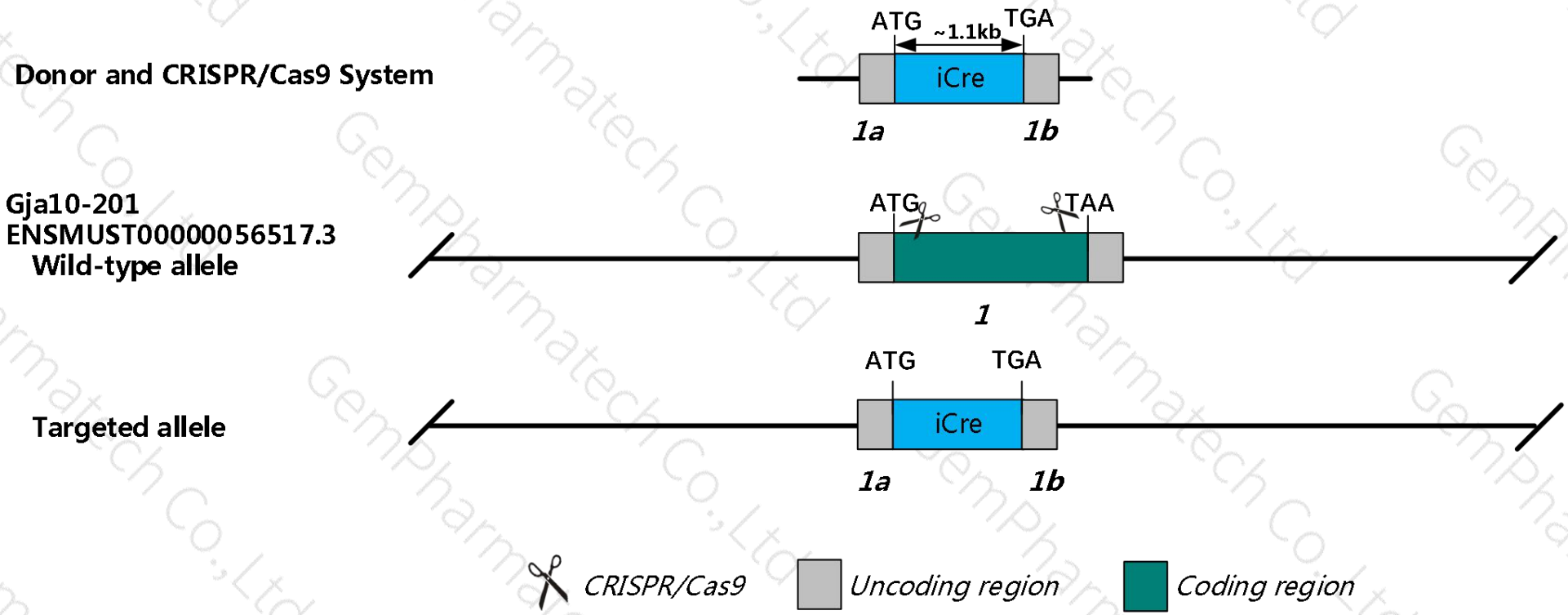
Design Date:	**2021-9-10**

Project Overview

Project Name	***Gja10-iCre***
Project type	**Cas9-KI**
Strain background	**C57BL/6JGpt**

Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Gja10* gene. The schematic diagram is as follows:



Technical routes

- The *Gja10* gene has 2 transcripts.According to the structure of *Gja10* gene,*Gja10-201*(ENSMUST00000056517.3)transcript is selected for this strategy.The transcript of *Gja10-201* contains gene has 1 exon,with the ATG start codon in exon1 and TAA stop codon in exon1.

- We constructed CRISPR/Cas9 system targeting mouse *Gja10* gene and donor vector,iCre will replace the entire coding region of the *Gja10* gene.The iCre will be expressed under the direction of endogenous regulatory mechanism.

- The project will use CRISPR/Cas9 technology to modify *Gja10* 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.

Notice

- According to the existing MGI data,homozygous null mice are fertile with no obvious anatomical or behavioral abnormalities,some models have abnormal seminal vesicle morphology and absent testes.
- Insertion of iCre directly destroys the expression of target gene.
- Expression of *Gja10*-Cre may be mainly expressed in the horizontal cells.
- The *Gja10* gene is located on the Chr4. 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.
- This strategy is designed based on genetic information in existing databases. Due to the complexity of gene transcription and translation processes, all risks cannot be predicted under existing information.

Coding Sequence of Codon-Optimized Cre Gene[1]

ATGGTGCCCAAGAAGAAGAGGAAAGTCTCCAACCTGCTGACTGTGCACCAAAACCTGCCTGCCCTCCCTGTGG
ATGCCACCTCTGATGAAGTCAGGAAGAACCTGATGGACATGTTCAGGGACAGGCAGGCCTTCTCTGAACACAC
CTGGAAGATGCTCCTGTCTGTGTGCAGATCCTGGGCTGCCTGGTGCAAGCTGAACAACAGGAAATGGTTCCCTG
CTGAACCTGAGGATGTGAGGGACTACCTCCTGTACCTGCAAGCCAGAGGCCTGGCTGTGAAGACCATCCAACA
GCACCTGGGCCAGCTCAACATGCTGCACAGGAGATCTGGCCTGCCTCGCCCTTCTGACTCCAATGCTGTGTCCC
TGGTGATGAGGAGAATCAGAAAGGAGAATGTGGATGCTGGGGAGAGAGCCAAGCAGGCCCTGGCCTTTGAAC
GCACTGACTTTGACCAAGTCAGATCCCTGATGGAGAACTCTGACAGATGCCAGGACATCAGGAACCTGGCCTTC
CTGGGCATTGCCTACAACACCCTGCTGCGCATTGCCGAAATTGCCAGAATCAGAGTGAAGGACATCTCCCGCAC
CGATGGTGGGAGAATGCTGATCCACATTGGCAGGACCAAGACCCTGGTGTCCACAGCTGGTGTGGAGAAGGCC
CTGTCCCTGGGGGTTACCAAGCTGGTGGAGAGATGGATCTCTGTGTCTGGTGTGGCTGATGACCCCAACAACTA
CCTGTTCTGCCGGGTCAGAAAGAATGGTGTGGCTGCCCCTTCTGCCACCTCCCAACTGTCCACCCGGGCCCTGG
AAGGGATCTTTGAGGCCACCCACCGCCTGATCTATGGTGCCAAGGATGACTCTGGGCAGAGATACCTGGCCTGG
TCTGGCCACTCTGCCAGAGTGGGTGCTGCCAGGGACATGGCCAGGGCTGGTGTGTCCATCCCTGAAATCATGCA
GGCTGGTGGCTGGACCAATGTGAACATTGTGATGAACTACATCAGAAACCTGGACTCTGAGACTGGGGCCATGG
TGAGGCTGCTCGAGGATGGGGACTGA

Target gene

Gene name	mouse *Gja10*
Gene ID(NCBI)	14610
Gene link(NCBI)	https://www.ncbi.nlm.nih.gov/gene/14610
Gene link(Ensembl)	http://asia.ensembl.org/Mus_musculus/Gene/Summary?g=ENSMUSG00000051056;r=4:32596960-32602760
chromosome location	Chr4

Gene information （NCBI）

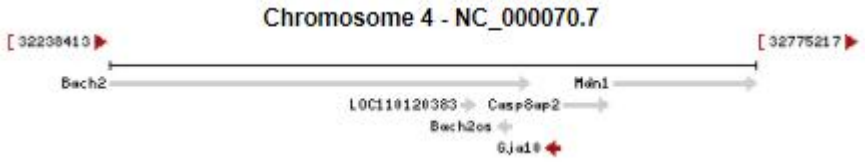
集萃药康 GemPharmatech

Gja10 gap junction protein, alpha 10 [*Mus musculus* (house mouse)]

Gene ID: 14610, updated on 3-Jul-2021

Summary

Official Symbol	Gja10 provided by MGI
Official Full Name	gap junction protein, alpha 10 provided by MGI
Primary source	MGI:MGI:1339969
See related	Ensembl:ENSMUSG00000051056
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Cx59; Cxnn; cx57; Cx-57; connex
Orthologs	human all



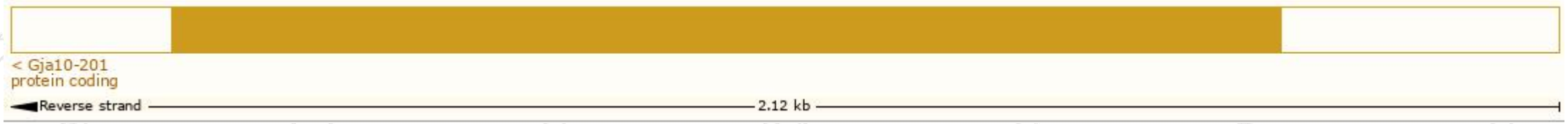
Transcript information（Ensembl）

The gene has 2 transcripts, and all transcripts are shown below:

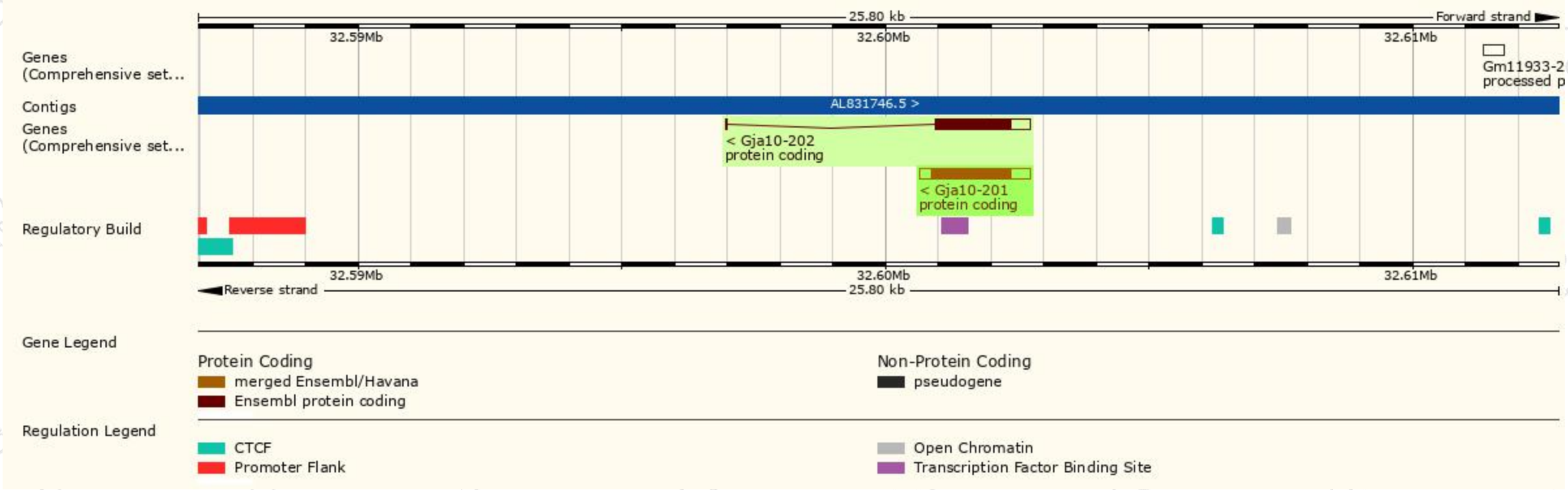
Show/hide columns (1 hidden) Filter

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Gja10-201	ENSMUST00000056517.3	2117	505aa	Protein coding	CCDS18015	Q9WUS4-1	GENCODE basic APPRIS P2 TSL:NA
Gja10-202	ENSMUST00000219644.2	1857	492aa	Protein coding	-	Q9WUS4-2	GENCODE basic APPRIS ALT2 TSL:5

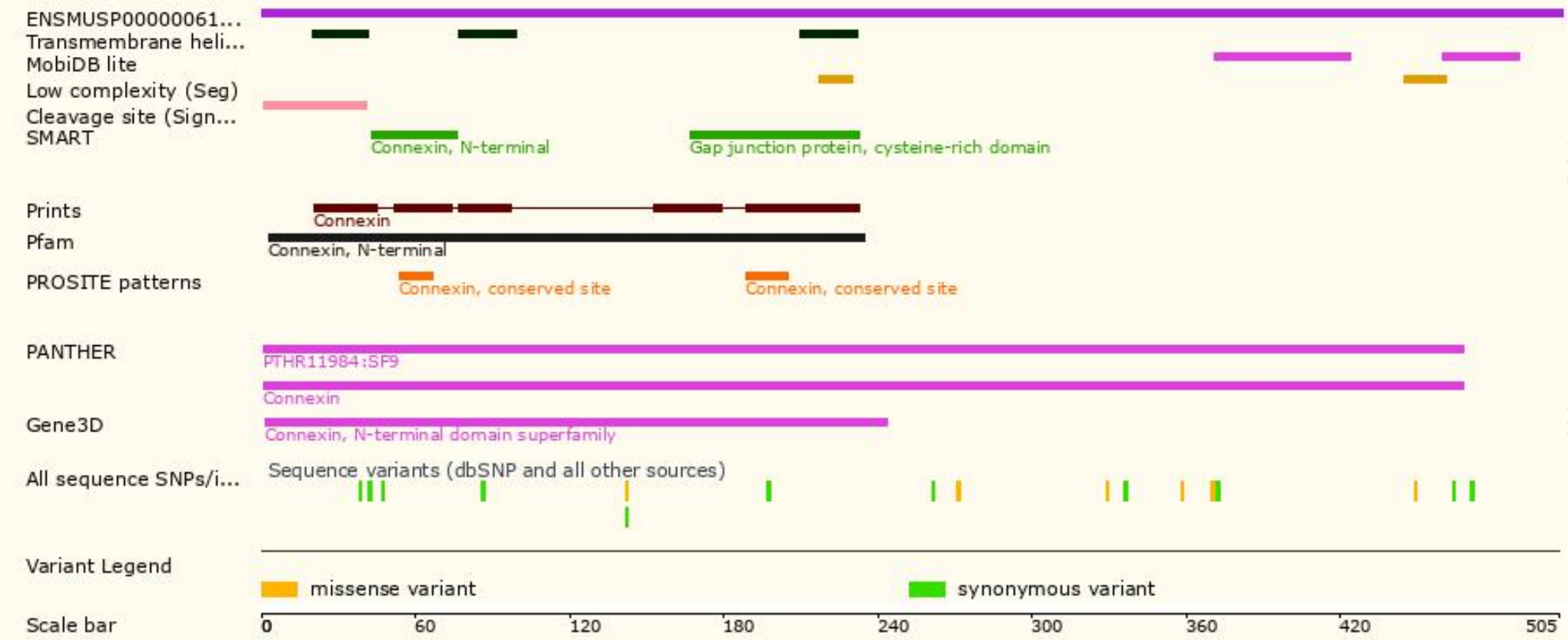
The strategy is based on the design of *Gja10-201* transcript,The transcription is shown below:



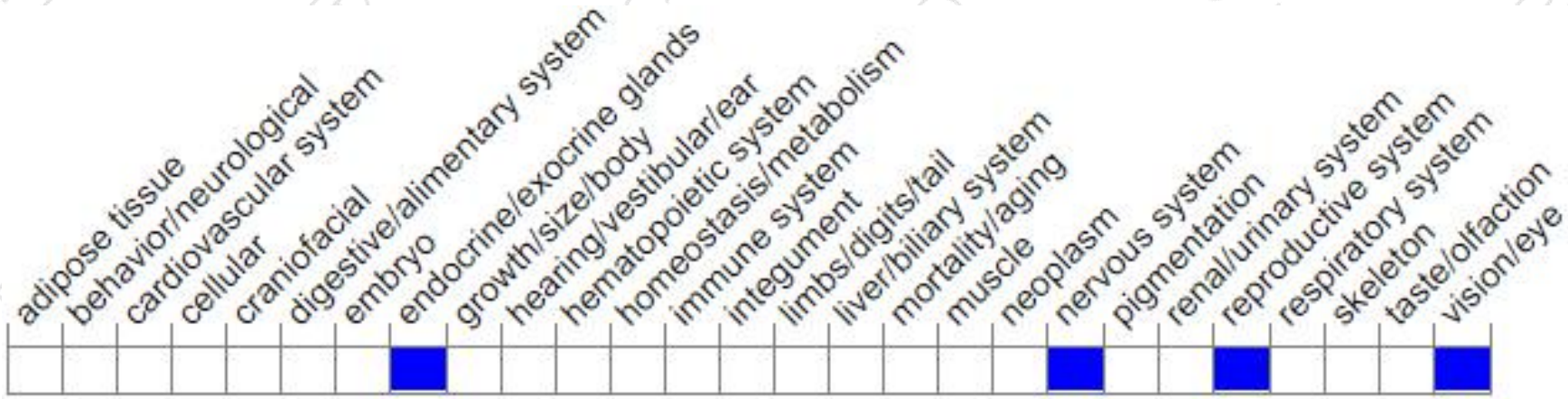
Genomic location distribution



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:1339969) .

Homozygous null mice are fertile with no obvious anatomical or behavioral abnormalities.

Existing Model Reporting[2]

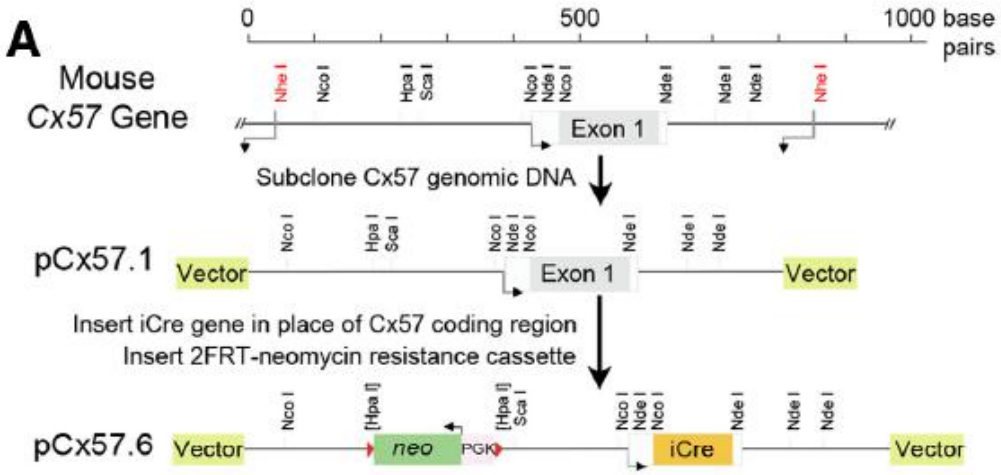


Figure 1. Genetic engineering of *Cx57-iCre* knock-in transgenic mice. ***A***, The Cx57 coding region was removed and replaced with an in-frame, codon-optimized, improved *iCre* gene to produce the mouse Cx57-iCre targeting construct pCx57.6. ***B–D***, In *Cx57-iCre::*

Generation of the targeting vector

The genomic DNA clone of the mouse connexin 57 (*Cx57, Gja10*) gene was isolated from adult 129/SvJae mouse liver genomic DNA. A Sau3AI restriction endonuclease fragments encoding the *Gja10* gene (GenBank accession #NM_010289) was obtained from a mouse strain 129S4/SvJae genomic DNA library (Stratagene) and subcloned into the vector λ FIX II (catalog #248211, Stratagene). Three genomic clones (MG801, MG806, MG811) containing the Cx57 coding sequence (CDS) were sequenced to determine the physical map. A 8133 bp NheI restriction endonuclease fragment of MG801 (15,946 bp insert) was subcloned into pBS SK[−] (Stratagene) to produce the pCx57.1 construct.

The protein-coding region of the Cx57 gene was replaced precisely by NcoI and NdeI restriction endonuclease digestion with the improved Cre recombinase gene (where the codon usage has been optimized for expression in mammalian cells; Shimshek et al., 2002). The *iCre* gene was obtained from pBOB-CAG-iCRE-SD (plasmid ID no. 12336; Addgene). Finally, a positive selection phosphoglycerate kinase (PGK) promoter–neomycin (neo) resistance cassette flanked by two Flp recombinase recognition (FRT) sites was inserted upstream of the iCre gene. The 2FRT-PGK neo cassette was obtained from ploxP-2FRT-PGKneo (originally a gift from S. Fiering, Dartmouth College, Hanover, NH). This construct was subcloned into the targeting vector pKO-Select DT (Lex-

References

[1] Shimshek DR, Kim J, Hübner MR, Spergel DJ. Codon-improved Cre recombinase (iCre) expression in the mouse. GeAlbis.2002 Jan.32(1):19-26.

[2] Hirano AA; Liu X; Boulter J; Grove J; Perez de Sevilla Muller L; Barnes S; Brecha NC. Targeted Deletion of Vesicular GABA Transporter from Retinal Horizontal Cells Eliminates Feedback Modulation of Photoreceptor Calcium Channels. eNeuro;2016

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