

Atp4b-iCre cas9-ki Strategy

Designer:

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Reviewer

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Design Date:

2021-6-3

Project Overview

Project Name

Atp4b-iCre

Project type

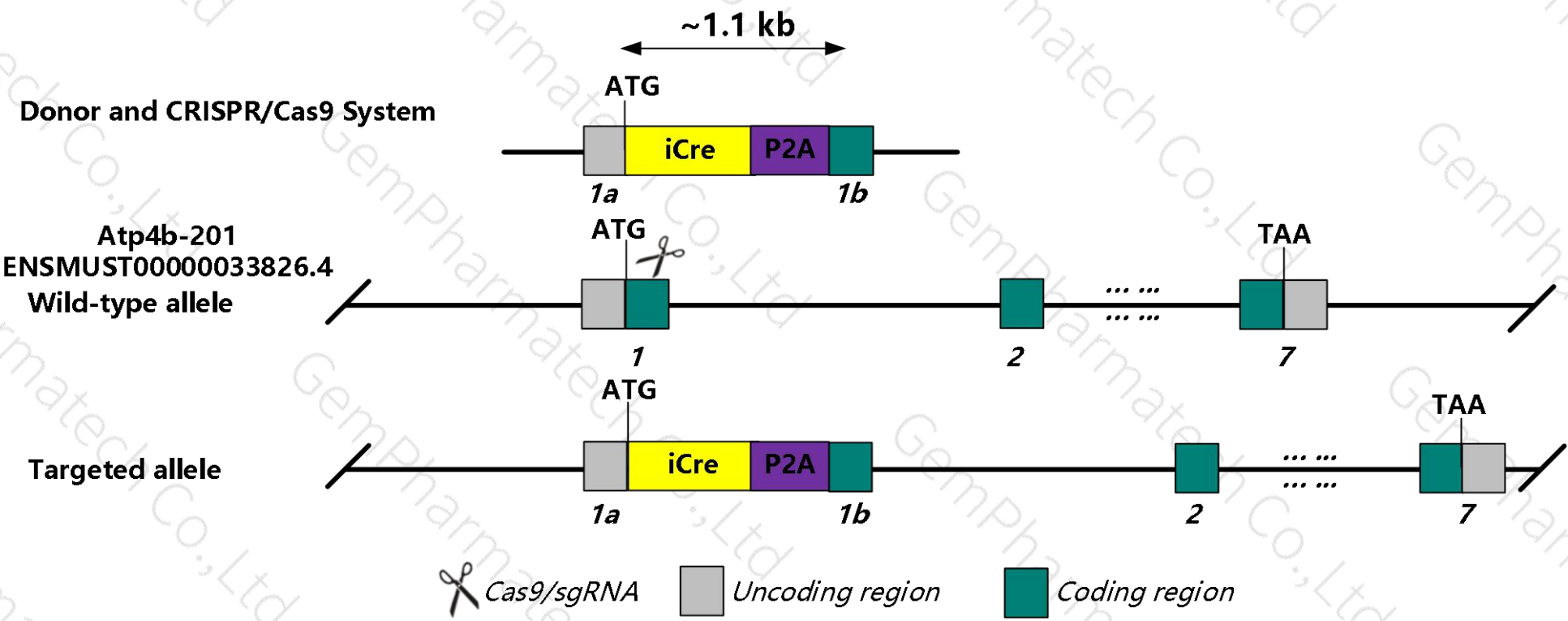
cas9-ki

Strain background

C57BL/6JGpt

Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Atp4b* gene. The schematic diagram is as follows:



- The *Atp4b* gene has 2 transcripts. According to the reference and structure of *Atp4b* gene, *Atp4b-201*(ENSMUST00000033826.4) is selected for presentation of the recommended strategy.
- *Atp4b-201* gene has 7 exons, with the ATG start codon in exon1 and TAA stop codon in exon7.
- We make *Atp4b-P2A-iCre* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage near start codon(ATG) of *Atp4b* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in iCre-P2A after start codon(ATG) of *Atp4b* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

- According to the existing MGI data, mice homozygous for disruptions in this gene are superficially normal but have hypertrophied stomach mucosa, neutral luminal pH in the stomach, and hypergastrinemia.
- The P2A-linked gene drives expression in the same promoter and is cleaved at the translational level. The gene expression levels are consistent, and the before of P2A expressing gene carries the P2A-translated polypeptide.
- Insertion of iCre- P2A may affect the regulation of the 5' end of the *Atp4b* gene.
- There may be base mutations in the modeling process because of the repetitive sequences upstream of the insertion site.
- The insertion site is about 8 kb away from the 5 end of the *Grk1* gene, and the influence is unknown.
- There may be 1 to 2 amino acid synonymous mutation in exon1 of *Atp4b* gene in this strategy.
- The *Atp4b* gene is located on the Chr8. 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.

Coding Sequence of Codon-Optimized *Cre* Gene ^[1](*iCre* :1056 bp)

ATGGTGCCCAAGAAGAAGAGGAAAGTCTCCAACCTGCTGACTGTGCACCAAAACCTGCCTGCCCTCCCTGTGGATGC
CACCTCTGATGAAGTCAGGAAGAACCTGATGGACATGTTTCAGGGACAGGCAGGCCTTCTCTGAACACACCTGGAAG
ATGCTCCTGTCTGTGTGCAGATCCTGGGCTGCCTGGTGCAAGCTGAACAACAGGAAATGGTTCCCTGCTGAACCTGA
GGATGTGAGGGACTACCTCCTGTACCTGCAAGCCAGAGGCCTGGCTGTGAAGACCATCCAACAGCACCTGGGGCCAG
CTCAACATGCTGCACAGGAGATCTGGCCTGCCTCGCCCTTCTGACTCCAATGCTGTGTCCCTGGTGATGAGGAGAATC
AGAAAGGAGAATGTGGATGCTGGGGAGAGAGCCAAGCAGGCCCTGGCCTTTGAACGCACTGACTTTGACCAAGTCA
GATCCCTGATGGAGAACTCTGACAGATGCCAGGACATCAGGAACCTGGCCTTCCTGGGCATTGCCTACAACACCCTG
CTGCGCATTGCCGAAATTGCCAGAATCAGAGTGAAGGACATCTCCCGCACCGATGGTGGGAGAATGCTGATCCACAT
TGGCAGGACCAAGACCCTGGTGTCCACAGCTGGTGTGGAGAAGGCCCTGTCCCTGGGGGGTTACCAAGCTGGTGGAG
AGATGGATCTCTGTGTCTGGTGTGGCTGATGACCCCAACAACACTACCTGTTCTGCCGGGTCAGAAAGAATGGTGTGGCT
GCCCCTTCTGCCACCTCCCAACTGTCCACCCGGGGCCCTGGAAGGGATCTTTGAGGCCACCCACCGCCTGATCTATGGT
GCCAAGGATGACTCTGGGCAGAGATACCTGGCCTGGTCTGGCCACTCTGCCAGAGTGGGTGCTGCCAGGGACATGGC
CAGGGCTGGTGTGTCCATCCCTGAAATCATGCAGGCTGGTGGCTGGACCAATGTGAACATTGTGATGAACTACATCAG
AAACCTGGACTCTGAGACTGGGGCCATGGTGAGGCTGCTCGAGGATGGGGACTGA

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

Gene information (NCBI)

Atp4b ATPase, H⁺/K⁺ exchanging, beta polypeptide [*Mus musculus* (house mouse)]

[Download Datasets](#)

Gene ID: 11945, updated on 6-May-2021

Summary

Official Symbol

Atp4b provided by [MGI](#)

Official Full Name

ATPase, H⁺/K⁺ exchanging, beta polypeptide provided by [MGI](#)

Primary source

[MGI:MGI:88114](#)

See related

[Ensembl:ENSMUSG000000031449](#)

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

AV080843

Expression

Restricted expression toward stomach adult (RPKM 1152.3) [See more](#)

Orthologs

[human](#) [all](#)

NEW

[Try the new Gene table](#)

[Try the new Transcript table](#)

Genomic context

Location: 8 A1.1; 8 5.89 cM

See Atp4b in [Genome Data Viewer](#)

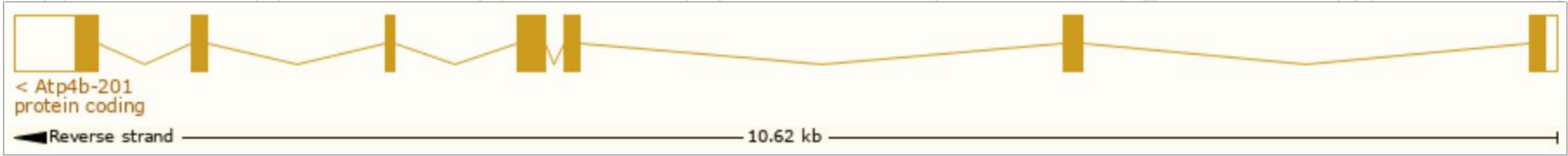
Exon count: 7

Transcript information (Ensembl)

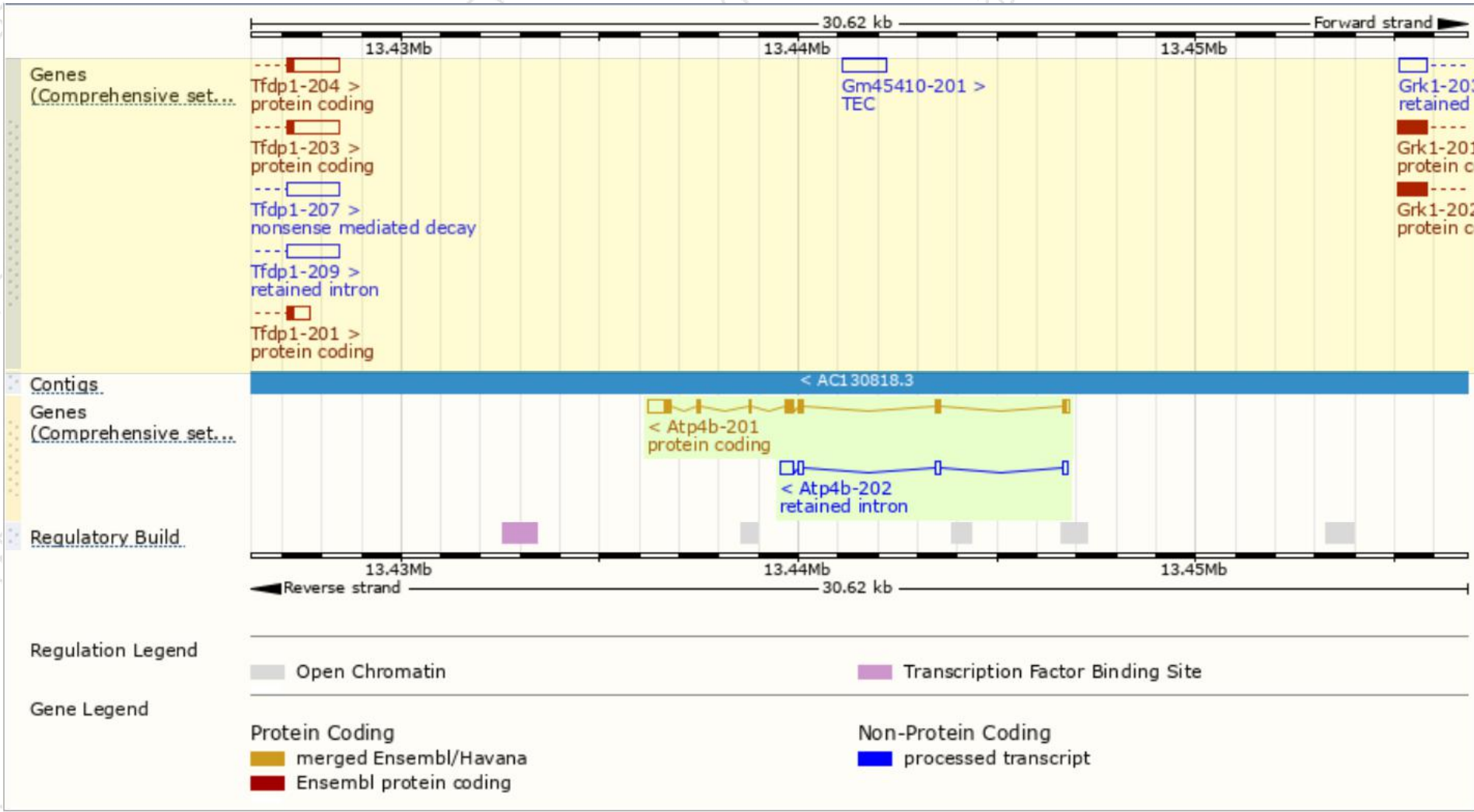
The gene has 2 transcripts, and all transcripts are shown below :

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Atp4b-201	ENSMUST00000033826.4	1378	294aa	Protein coding	CCDS22111	P50992 Q0VBB6	GENCODE basic APPRIS P1 TSL:1
Atp4b-202	ENSMUST00000210491.2	709	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Atp4b-201* transcript,the transcription is shown below



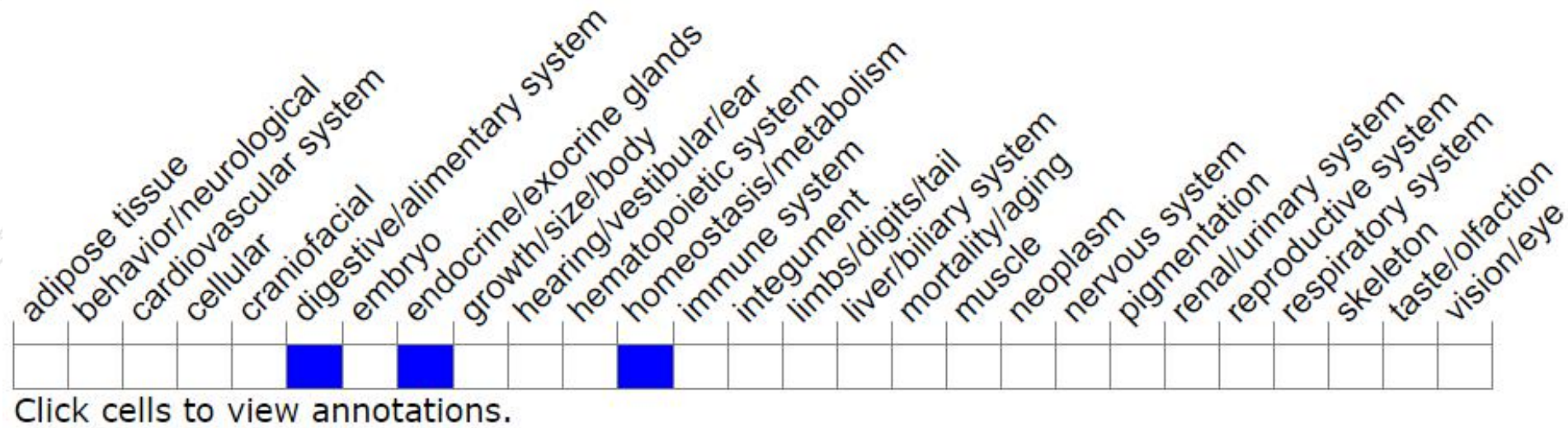
Genomic location distribution



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:88114>) .

Mice homozygous for disruptions in this gene are superficially normal but have hypertrophied stomach mucosa, neutral luminal pH in the stomach, and hypergastrinemia.

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