

***Pdx1-P2A-iCre* Cas9-KI Strategy**

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Reviewer:

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Design Date:

2019-8-18

Project Overview

Project Name

Pdx1-P2A-iCre

Project type

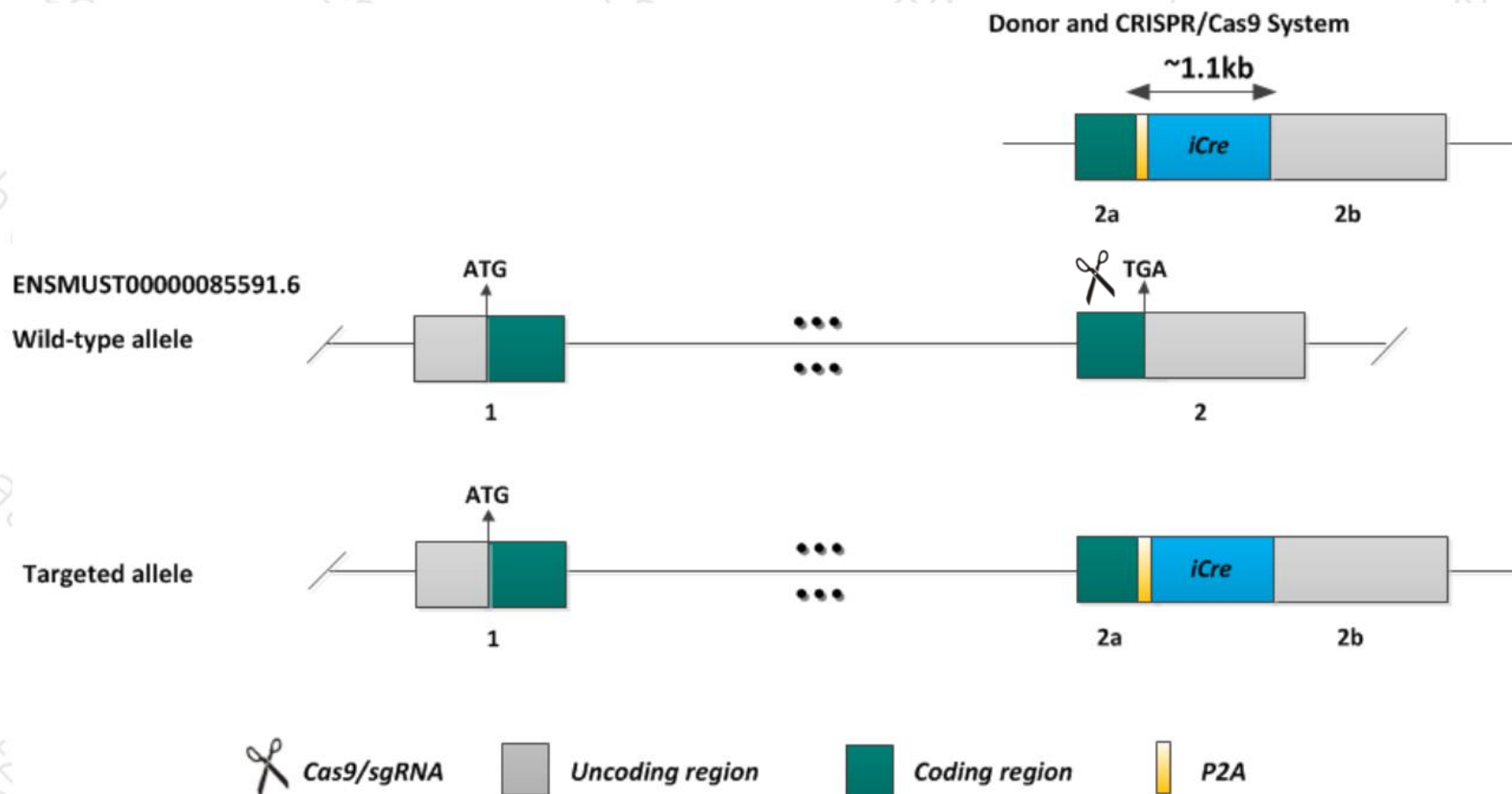
Cas9-KI

Strain background

C57BL/6J

Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Pdx1* gene. The schematic diagram is as follows:



- The *Pdx1* gene has 1 transcript. According to the structure of *Pdx1* gene, *Pdx1-201*(ENSMUST00000085591.6) is selected for presentation of the recommended strategy.
- *Pdx1-201* gene has 2 exons, with the ATG start codon in exon1 and TGA stop codon in exon2.
- We make *Pdx1*-IRES-iCre mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be coinjected into zygotes. sgRNA direct Cas9 endonuclease cleavage near the stop codon of *Pdx1* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in P2A-iCre inserted into exon2 of *Pdx1* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

- According to the existing MGI data, Mice homozygous for a knock-out allele exhibit postnatal lethality, abnormal pancreatic and liver development, and increased plasma glucose levels. Mice heterozygous for a knock-out allele exhibit abnormal pancreatic development and abnormal glucose homeostasis.
- The insertion site of this strategy is approximately 3.2 Kb from *Plut* gene and 5.8 Kb from *Gm27033* gene. The insertion fragment may disrupt the 5-terminal regulation of *Plut* and *Gm27033* gene.
- The *Pdx1* gene is located on the Chr5. 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.

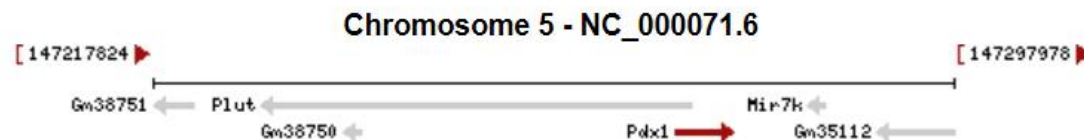
Gene information (NCBI)

Pdx1 pancreatic and duodenal homeobox 1 [*Mus musculus* (house mouse)]

Gene ID: 18609, updated on 13-Aug-2019

Summary

Official Symbol	Pdx1 provided by MGI
Official Full Name	pancreatic and duodenal homeobox 1 provided by MGI
Primary source	MGI:MGI:102851
See related	Ensembl:ENSMUSG00000029644
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	Ip1; IDX-1; IPF-1; Mody4; STF-1; pdx-1
Expression	Biased expression in duodenum adult (RPKM 36.7), small intestine adult (RPKM 27.4) and 1 other tissue See more
Orthologs	human all



Transcript information (Ensembl)

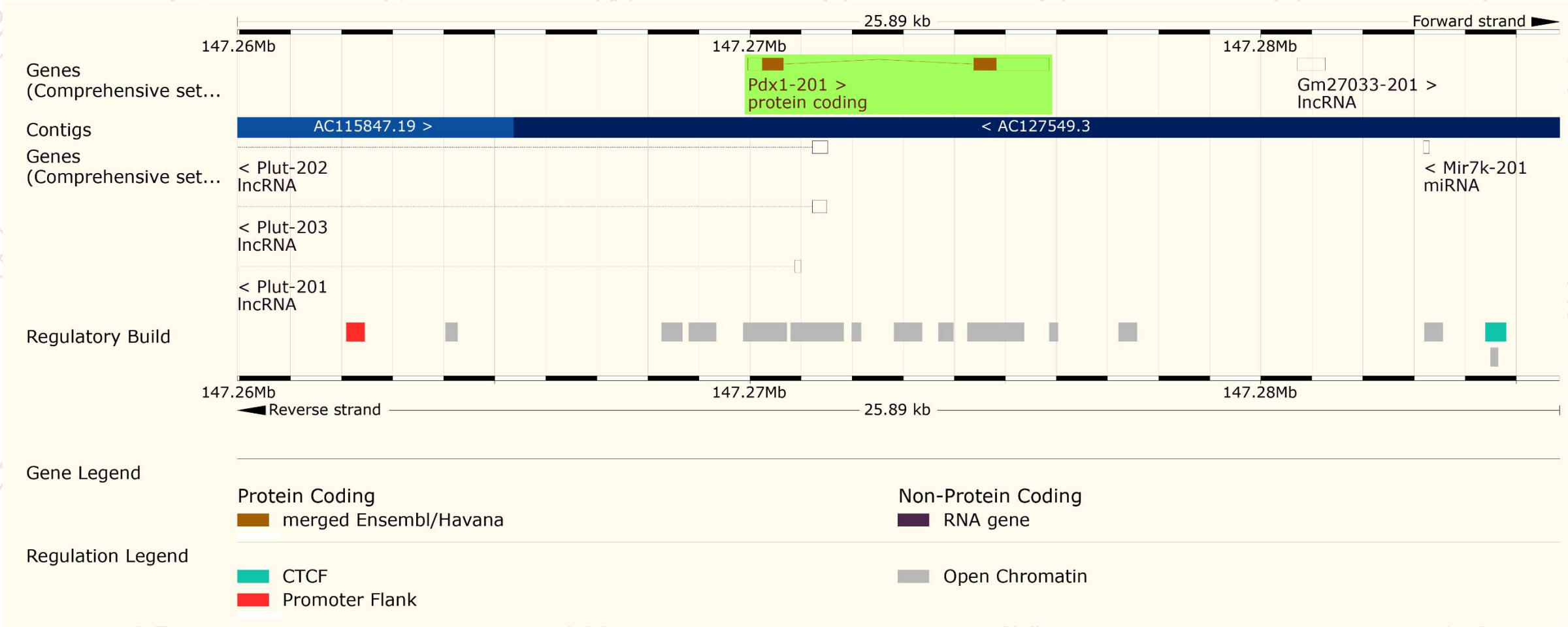
The gene has 1 transcript, and the transcript is shown below:

Name ▲	Transcript ID ▲	bp ▲	Protein ▲	Translation ID ▲	Biotype ▲	CCDS ▲	UniProt ▲	Flags ▲
Pdx1-201	ENSMUST00000085591.6	2158	284aa	ENSMUSP00000082729.5	Protein coding	CCDS39398	P52946	TSL:1 Gencode basic APPRIS P1

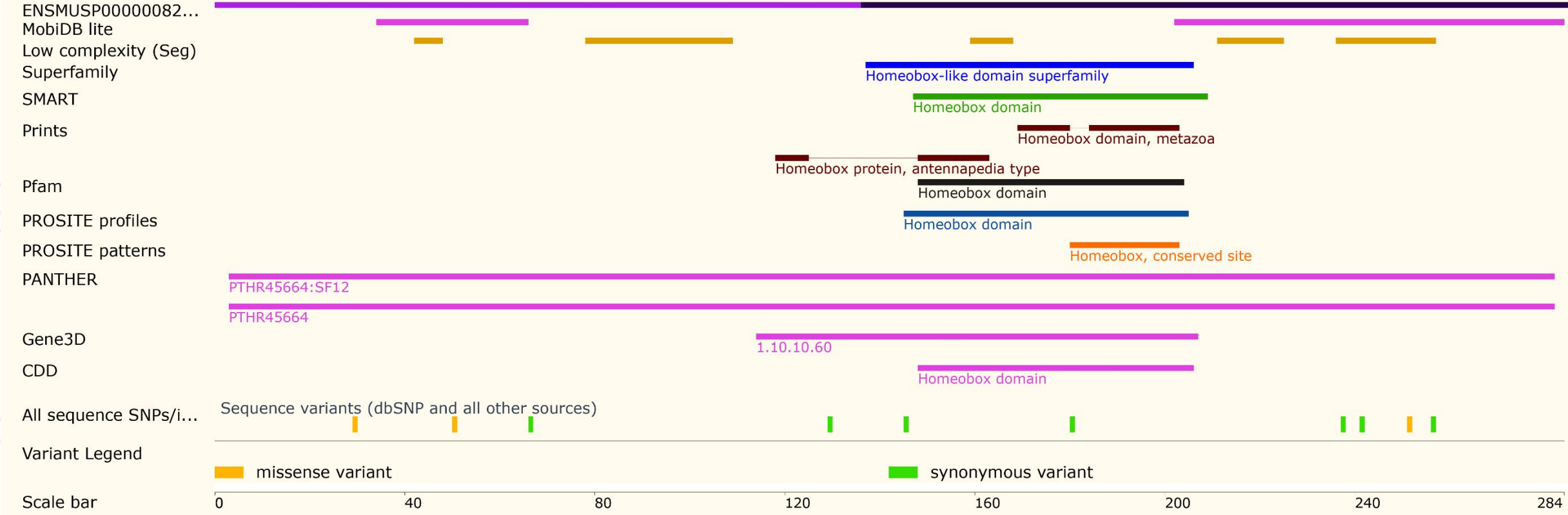
The strategy is based on the design of *Pdx1-201* transcript, The transcription is shown below



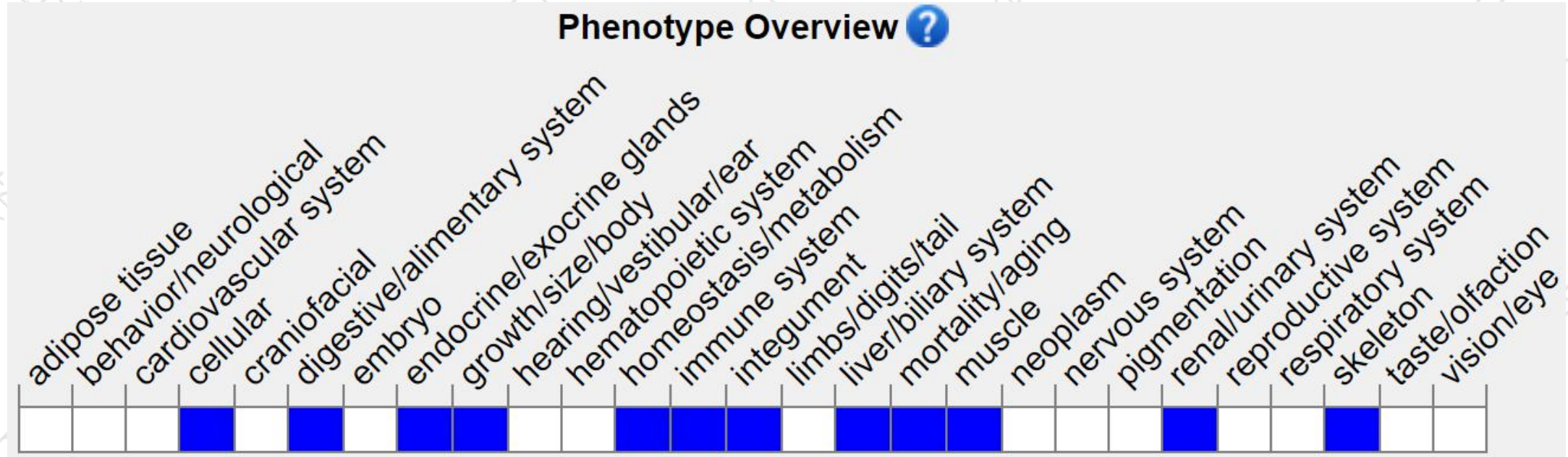
Genomic location distribution



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:102851>) .

According to the existing MGI data, Mice homozygous for a knock-out allele exhibit postnatal lethality, abnormal pancreatic and liver development, and increased plasma glucose levels. Mice heterozygous for a knock-out allele exhibit abnormal pancreatic development and abnormal glucose homeostasis.

iCre Sequence^[1] (1056bp)

ATGGTGCCCAAGAAGAAGAGGAAAGTCTCCAACCTGCTGACTGTGCACCAAAACCTGCCTGCCCTCCCTGTGGATGCCACCTCTGATGAAGTCAGGAAGA
ACCTGATGGACATGTTTCAGGGACAGGCAGGCCTTCTCTGAACACACCTGGAAGATGCTCCTGTCTGTGTGCAGATCCTGGGCTGCCTGGTGCAAGCTGAA
CAACAGGAAATGGTTCCCTGCTGAACCTGAGGATGTGAGGGACTACCTCCTGTACCTGCAAGCCAGAGGCCTGGCTGTGAAGACCATCCAACAGCACCTG
GGCCAGCTCAACATGCTGCACAGGAGATCTGGCCTGCCTCGCCCTTCTGACTCCAATGCTGTGTCCCTGGTGATGAGGAGAATCAGAAAGGAGAATGTGG
ATGCTGGGGAGAGAGCCAAGCAGGCCCTGGCCTTTGAACGCACTGACTTTGACCAAGTCAGATCCCTGATGGAGAACTCTGACAGATGCCAGGACATCAG
GAACCTGGCCTTCCTGGGCATTGCCTACAACACCCTGCTGCGCATTGCCGAAATTGCCAGAATCAGAGTGAAGGACATCTCCCGCACCGATGGTGGGAGA
ATGCTGATCCACATTGGCAGGACCAAGACCCTGGTGTCCACAGCTGGTGTGGAGAAGGCCCTGTCCCTGGGGGTTACCAAGCTGGTGGAGAGATGGATCT
CTGTGTCTGGTGTGGCTGATGACCCCAACAACCTGTTCTGCCGGGTCAGAAAGAATGGTGTGGCTGCCCCTTCTGCCACCTCCCAACTGTCCACCCG
GGCCCTGGAAGGGATCTTTGAGGCCACCCACCGCCTGATCTATGGTGCCAAGGATGACTCTGGGCAGAGATACCTGGCCTGGTCTGGCCACTCTGCCAGA
GTGGGTGCTGCCAGGGACATGGCCAGGGCTGGTGTGTCCATCCCTGAAATCATGCAGGCTGGTGGCTGGACCAATGTGAACATTGTGATGAACTACATCA
GAAACCTGGACTCTGAGACTGGGGCCATGGTGAGGCTGCTCGAGGATGGGGACTGA

Reference

[1] Shimshek DR, Kim J, Hübner MR, Spergel DJ. Codon-improved Cre recombinase (iCre) expression in the mouse. *Genetics*. 2002 Jan; 162(1):19-26.

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