集萃药康
GemPharmatech

Inpp4b Cas9-KO Strategy

Designer: Huan Wang

Reviewer: Yumeng Wang

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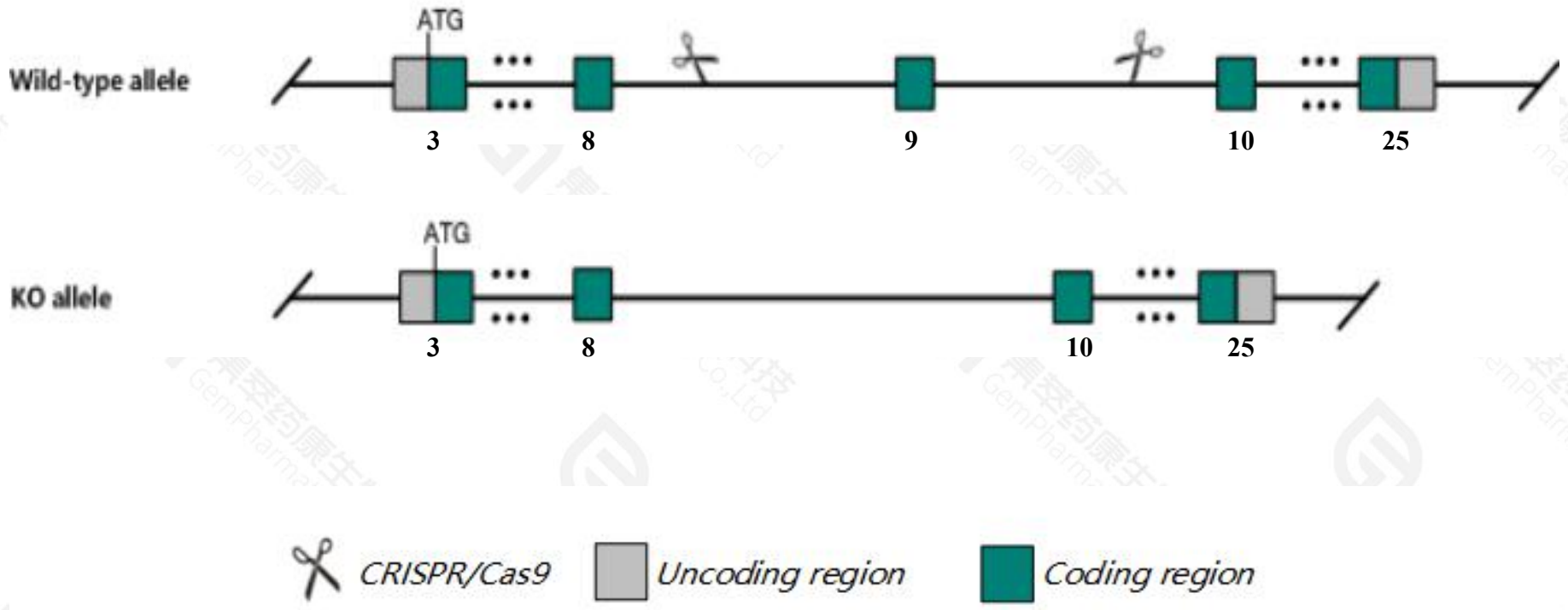
Project Overview

Project Name	***Inpp4b***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Inpp4b* gene. The schematic diagram is as follows:



Technical routes

➢ The *Inpp4b* gene has 15 transcripts. According to the structure of *Inpp4b* gene, exon9 of *Inpp4b-215*(ENSMUST00000217122.2) transcript is recommended as the knockout region.The region contains 112bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Inpp4b* gene. The brief process is as follows: CRISPR/Cas9 system 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,mice homozygous for a knock-out allele exhibit osteoporosis, reduced long bone length, increased osteoclast numbers and size, increased osteoblast numbers, and increased bone resorption and resorption.

➢ The *Inpp4b* gene is located on the Chr8. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

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Inpp4b inositol polyphosphate-4-phosphatase, type II [Mus musculus (house mouse)]

Gene ID: 234515, updated on 17-Feb-2021

Summary

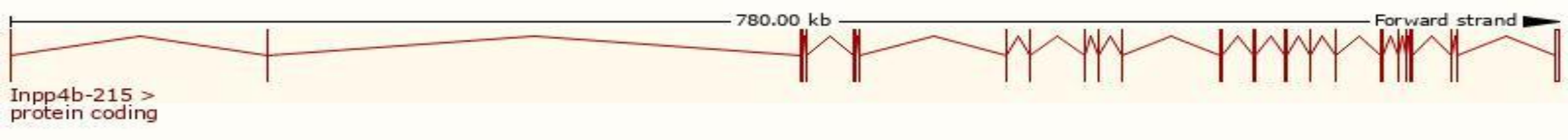
Official Symbol	Inpp4b provided by MGI
Official Full Name	inositol polyphosphate-4-phosphatase, type II provided byMGI
Primary source	MGI:MGI:2158925
See related	Ensembl:ENSMUSG00000037940
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	E130107I17Rik
Expression	Broad expression in heart adult (RPKM 1.6), cerebellum adult (RPKM 1.4) and 22 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

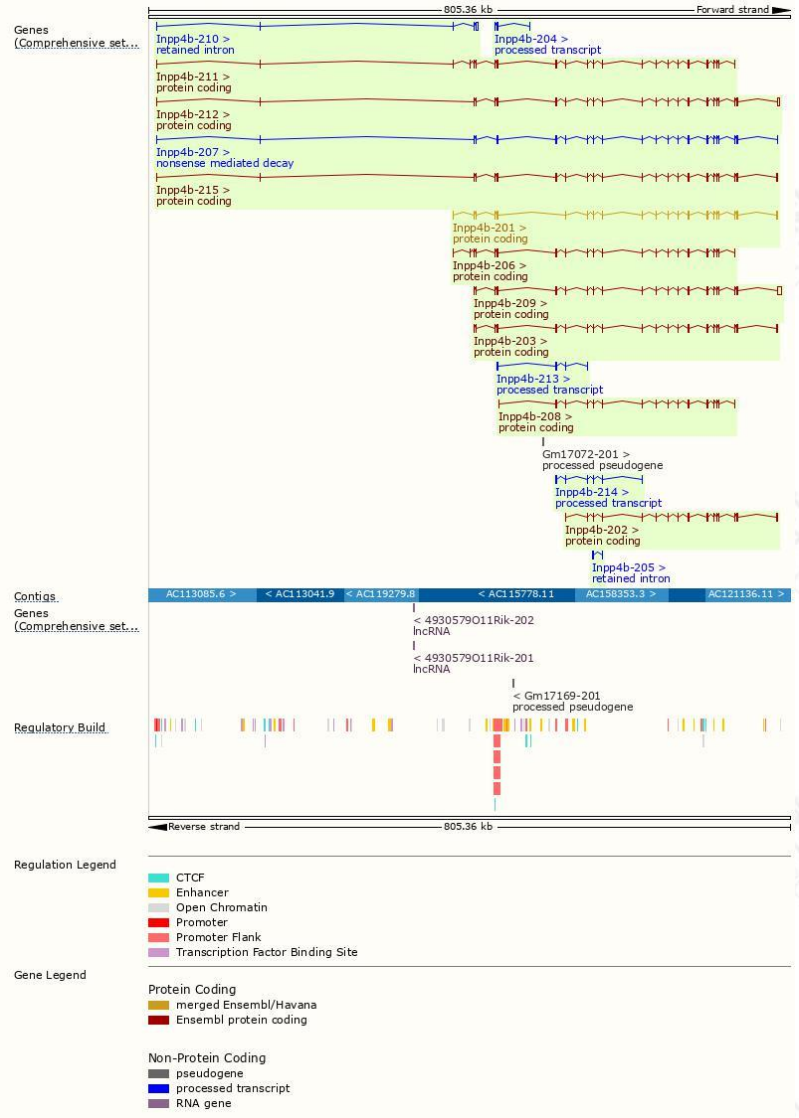
The gene has 15 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Inpp4b-209	ENSMUST00000172031.9	8067	927aa	Protein coding	CCDS80902		TSL:5 , GENCODE basic , APPRIS ALT2 ,
Inpp4b-212	ENSMUST00000215332.2	5486	927aa	Protein coding	CCDS80902		TSL:1 , GENCODE basic , APPRIS ALT2 ,
Inpp4b-215	ENSMUST00000217122.2	4392	941aa	Protein coding	CCDS80901		TSL:1 , GENCODE basic , APPRIS ALT2 ,
Inpp4b-201	ENSMUST00000042529.14	4120	924aa	Protein coding	CCDS22446		TSL:1 , GENCODE basic , APPRIS P3 ,
Inpp4b-203	ENSMUST00000109852.10	4095	941aa	Protein coding	CCDS80901		TSL:5 , GENCODE basic , APPRIS ALT2 ,
Inpp4b-202	ENSMUST00000109851.3	3906	809aa	Protein coding	-		TSL:1 , GENCODE basic , APPRIS ALT2 ,
Inpp4b-211	ENSMUST00000213285.2	3005	818aa	Protein coding	-		CDS 3' incomplete , TSL:1 ,
Inpp4b-206	ENSMUST00000169116.9	2702	818aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Inpp4b-208	ENSMUST00000170160.8	2108	633aa	Protein coding	-		CDS 3' incomplete , TSL:1 ,
Inpp4b-207	ENSMUST00000169387.10	3119	65aa	Nonsense mediated decay	-		TSL:1 ,
Inpp4b-214	ENSMUST00000216387.2	585	No protein	Processed transcript	-		TSL:5 ,
Inpp4b-213	ENSMUST00000216036.2	412	No protein	Processed transcript	-		TSL:3 ,
Inpp4b-204	ENSMUST00000164870.2	358	No protein	Processed transcript	-		TSL:5 ,
Inpp4b-210	ENSMUST00000172167.9	2705	No protein	Retained intron	-		TSL:1 ,
Inpp4b-205	ENSMUST00000165821.2	682	No protein	Retained intron	-		TSL:3 ,

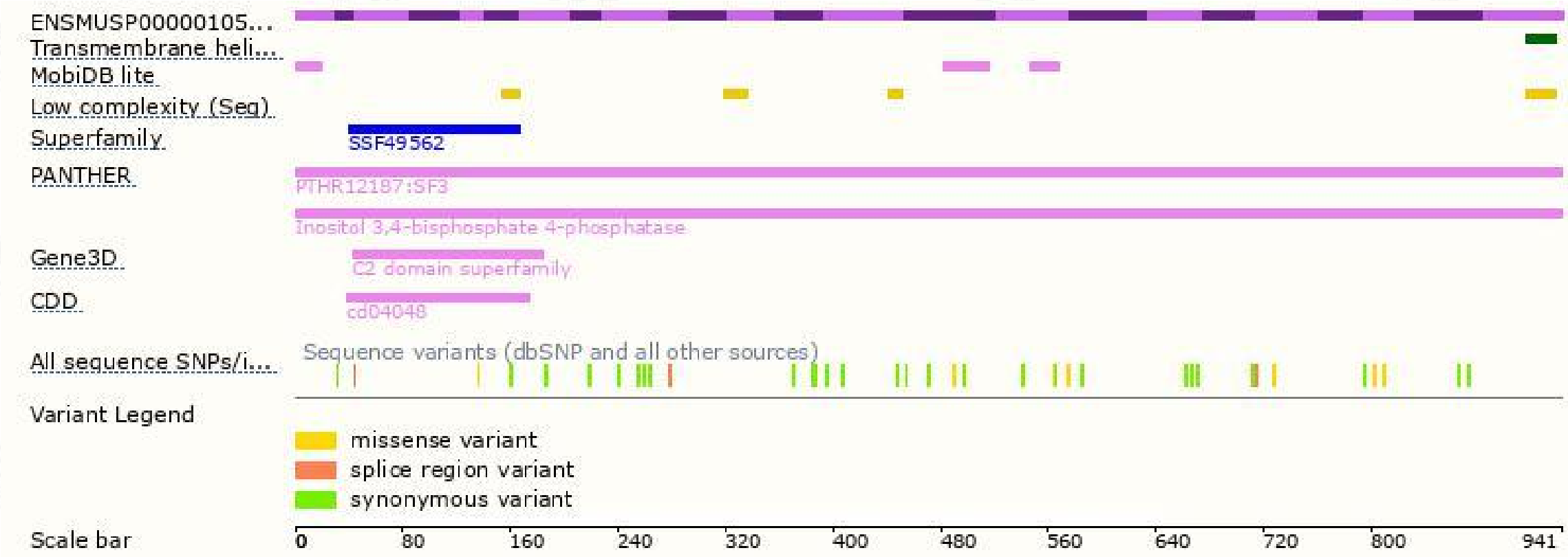
The strategy is based on the design of *Inpp4b-215* transcript,the transcription is shown below:



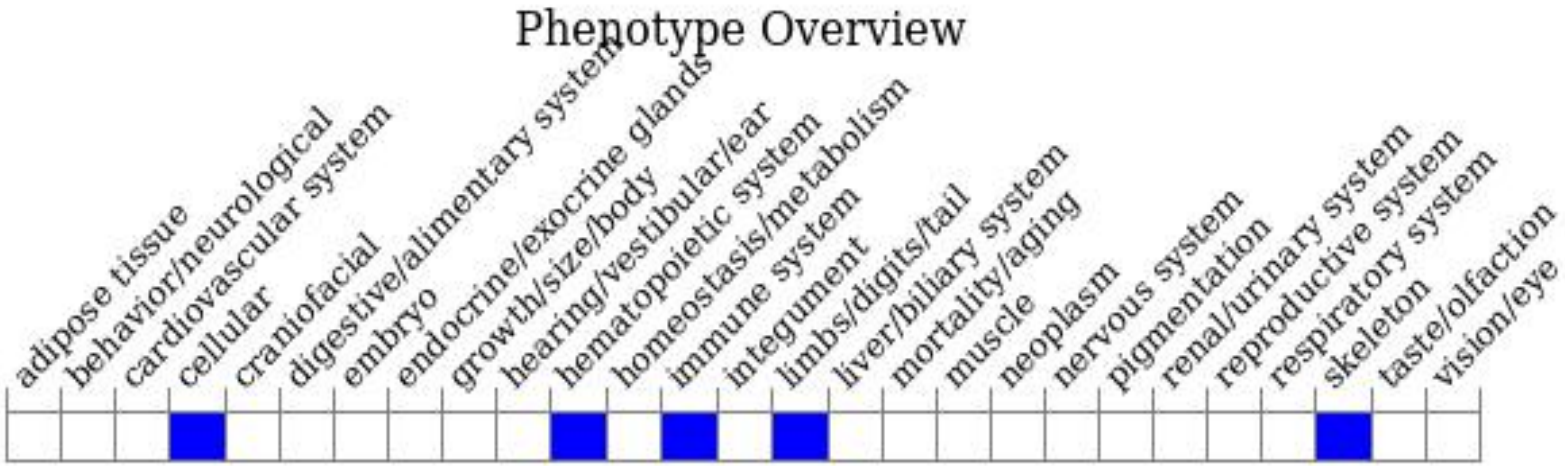
Genomic location distribution



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.
Tel: 400-9660890

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