Ucp2 Cas9-CKO Strategy

Designer: Huan Wang

Reviewer: Fengjuan Wang

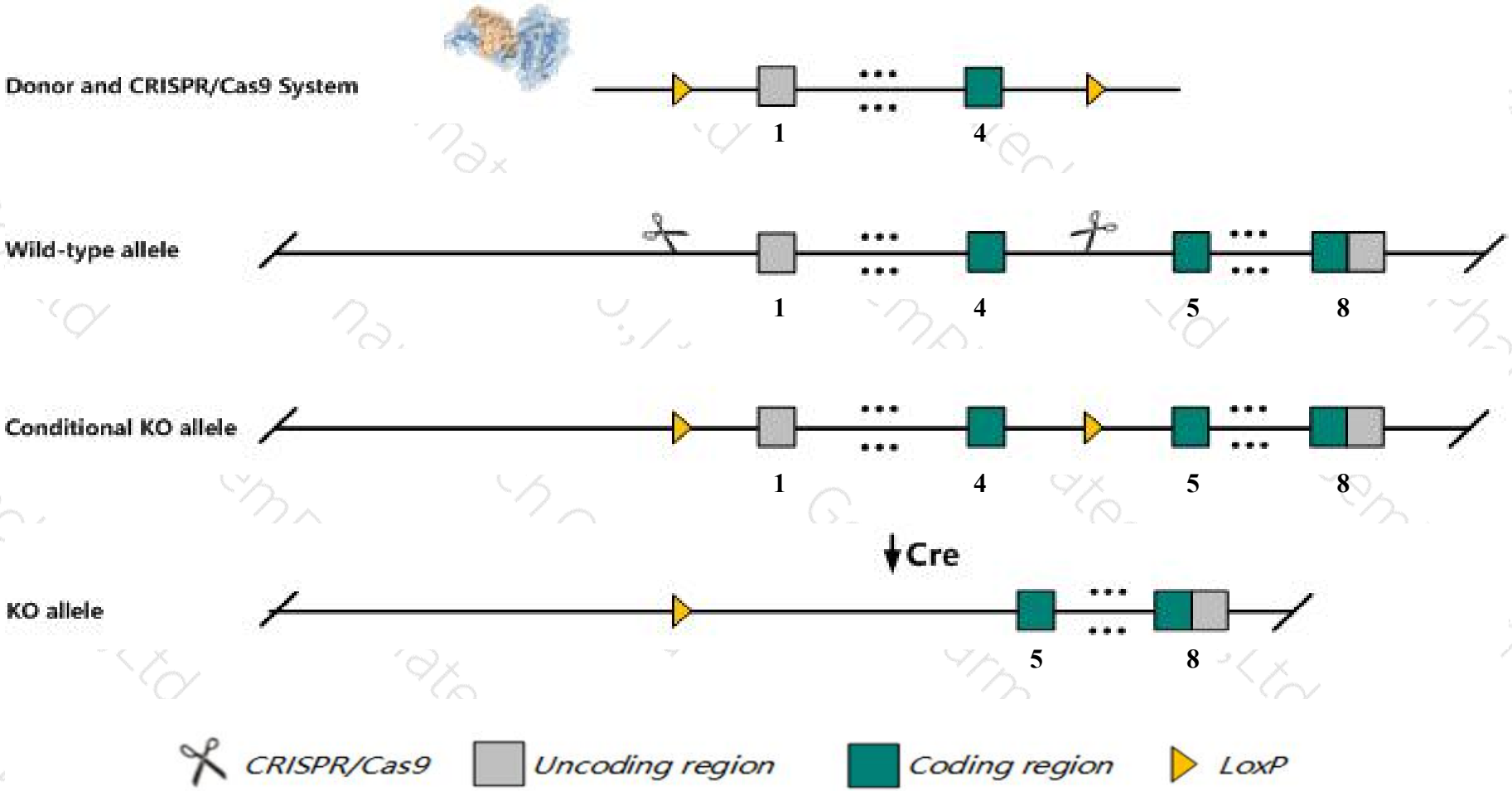
Design Date: 2018-12-26

Project Overview

Project Name	***Ucp2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ucp2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Ucp2* gene has 11 transcripts. According to the structure of *Ucp2* gene, exon1-exon4 of *Ucp2-202*(ENSMUST00000126534.7) transcript is recommended as the knockout region.The region contains start condon ATG. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Ucp2* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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 null mutants have elevated pancreatic islet cell ATP levels and increased glucose-stimulated secretion of insulin. Homozygotes also show reduced mitochondrial proton leak in thymocytes and increased resistance to infection by Toxoplasma gondii.

➢ The *Ucp2* gene is located on the Chr7. 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）

集萃药康 GemPharmatech

Ucp2 uncoupling protein 2 (mitochondrial, proton carrier) [Mus musculus (house mouse)]

Gene ID: 22228, updated on 13-Mar-2020

Summary

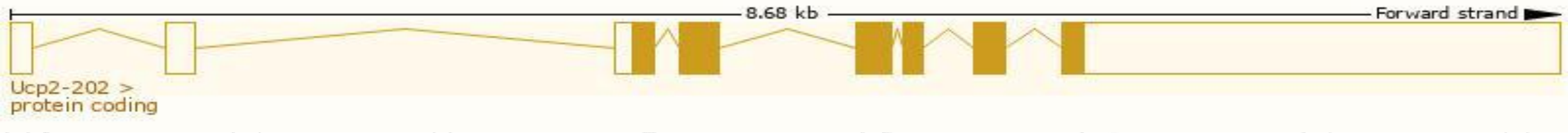
Official Symbol	Ucp2 provided by MGI
Official Full Name	uncoupling protein 2 (mitochondrial, proton carrier) provided byMGI
Primary source	MGI:MGI:109354
See related	Ensembl:ENSMUSG00000033685
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Slc25a8, UCP 2, UCPH
Expression	Broad expression in stomach adult (RPKM 802.8), duodenum adult (RPKM 693.5) and 19 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

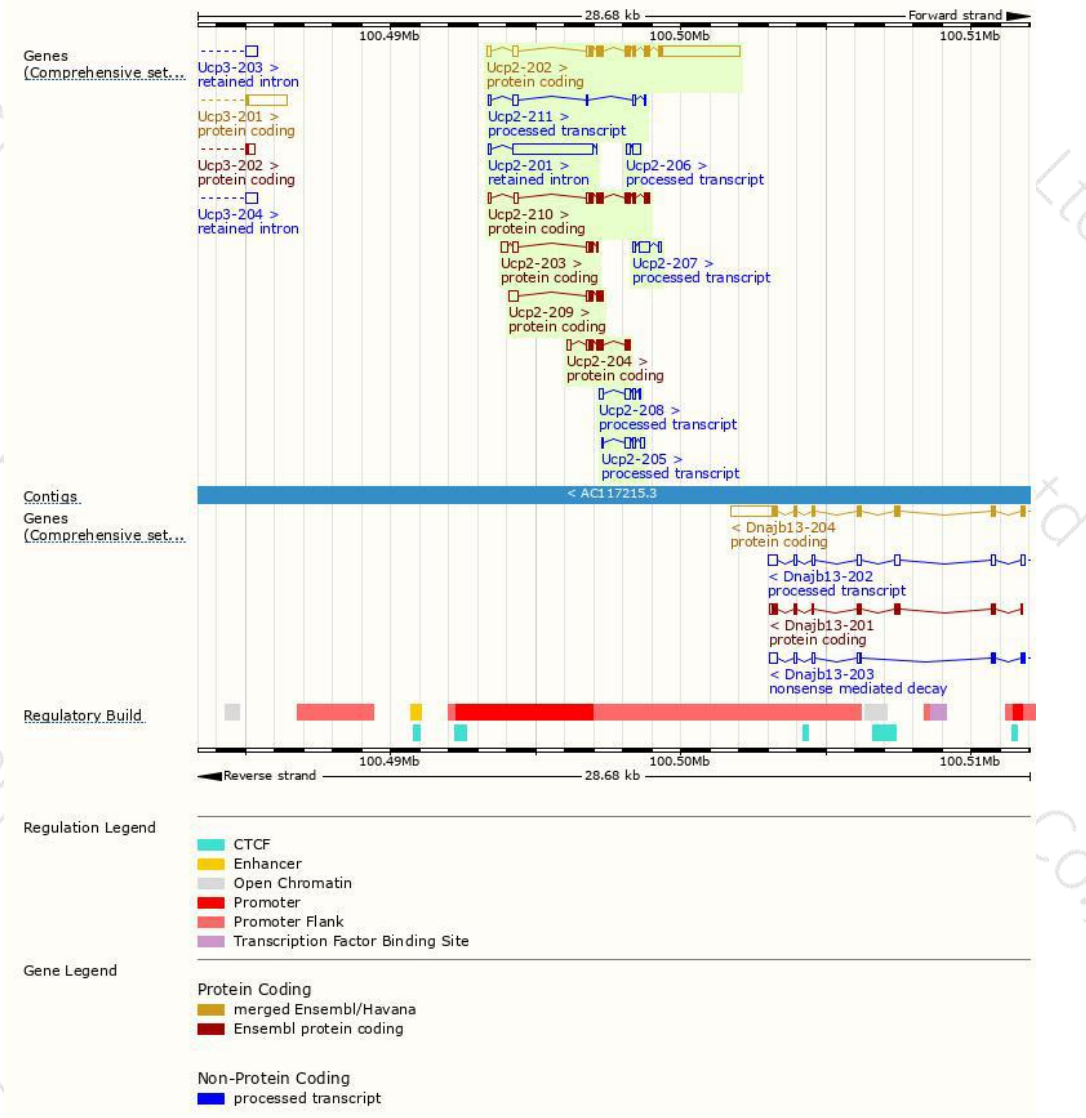
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ucp2-202	ENSMUST00000126534.7	3988	309aa	Protein coding	CCDS21498	P70406 Q549J5	TSL:1 GENCODE basic APPRIS P1
Ucp2-210	ENSMUST00000207748.1	1116	271aa	Protein coding	-	A0A140LHA5	CDS 3' incomplete TSL:5
Ucp2-209	ENSMUST00000153287.7	743	112aa	Protein coding	-	D3YXM5	CDS 3' incomplete TSL:2
Ucp2-204	ENSMUST00000133044.2	742	172aa	Protein coding	-	D3YZG5	CDS 3' incomplete TSL:3
Ucp2-203	ENSMUST00000129324.7	670	65aa	Protein coding	-	D3YZE5	CDS 3' incomplete TSL:5
Ucp2-207	ENSMUST00000149808.1	519	No protein	Processed transcript	-	-	TSL:5
Ucp2-211	ENSMUST00000207890.1	498	No protein	Processed transcript	-	-	TSL:5
Ucp2-205	ENSMUST00000133498.1	478	No protein	Processed transcript	-	-	TSL:2
Ucp2-208	ENSMUST00000151221.7	464	No protein	Processed transcript	-	-	TSL:3
Ucp2-206	ENSMUST00000138673.1	396	No protein	Processed transcript	-	-	TSL:3
Ucp2-201	ENSMUST00000126381.2	2841	No protein	Retained intron	-	-	TSL:5

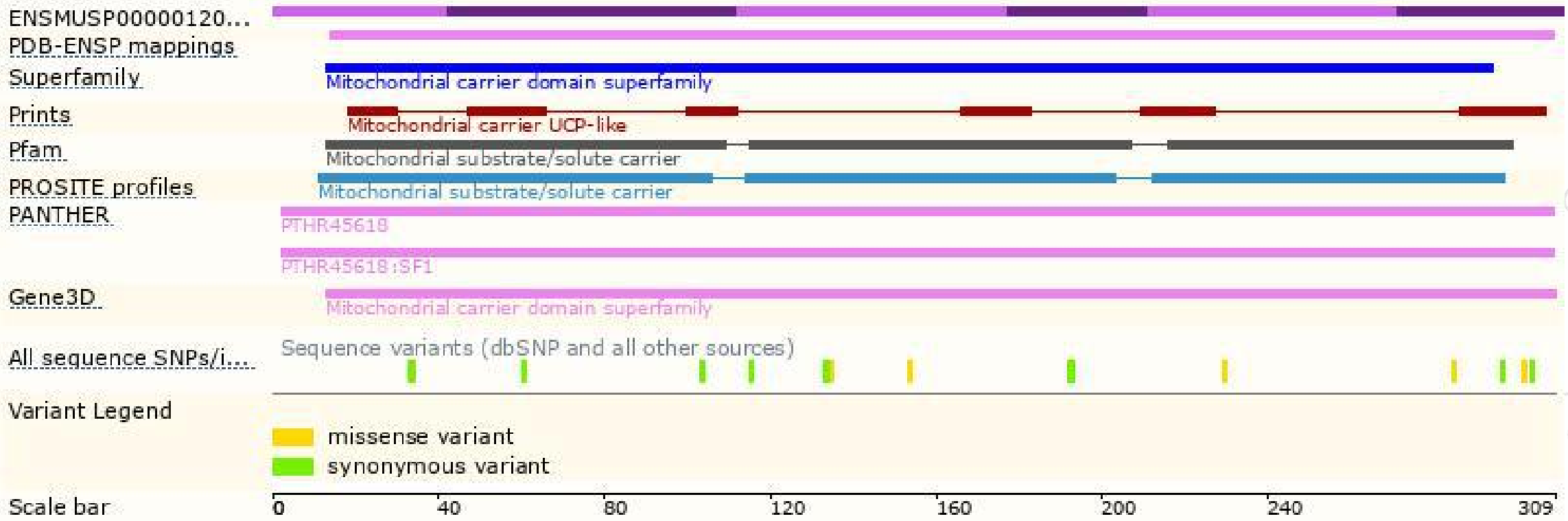
The strategy is based on the design of *Ucp2-202* transcript,the transcription is shown below:



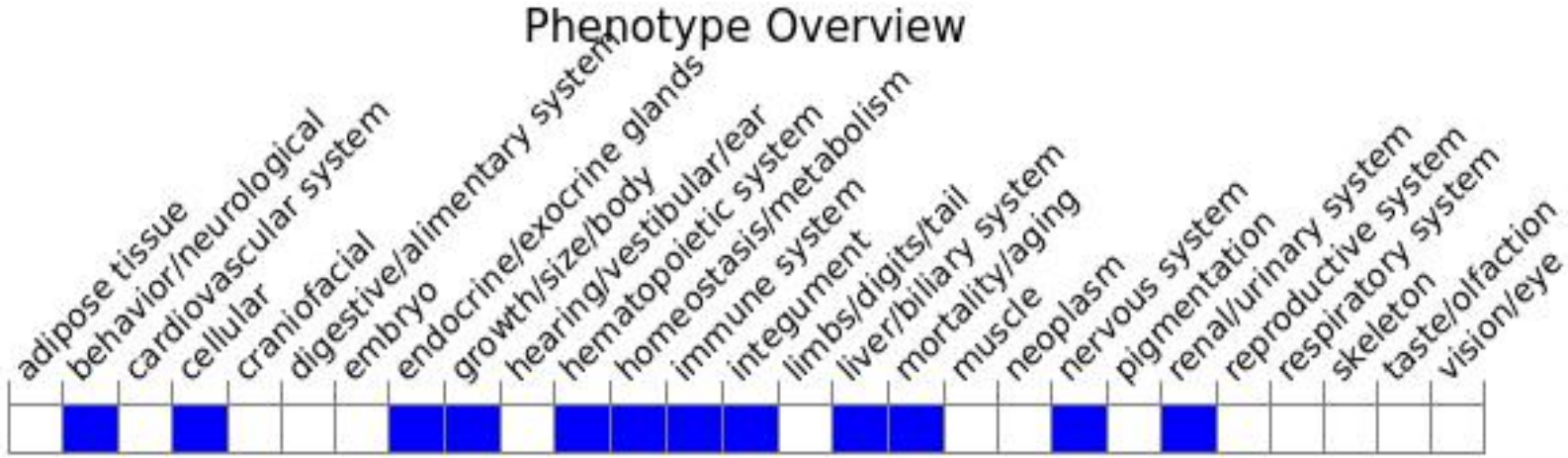
Genomic location distribution



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/).

According to the existing MGI data,homozygous null mutants have elevated pancreatic islet cell ATP levels and increased glucose-stimulated secretion of insulin. Homozygotes also show reduced mitochondrial proton leak in thymocytes and increased resistance to infection by Toxoplasma gondii.

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd