

# Gpr50 Cas9-CKO Strategy

Designer: Xueting Zhang

Design Date: 2019-8-5

## **Project Overview**



**Project Name** 

Gpr50

**Project type** 

Cas9-CKO

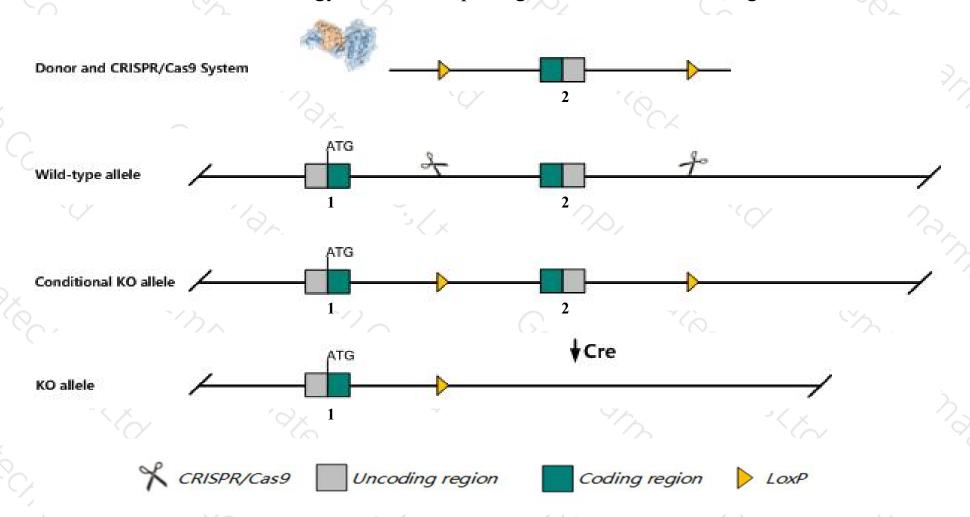
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Gpr50* gene. The schematic diagram is as follows:



### Technical routes



- The *Gpr50* gene has 3 transcripts. According to the structure of *Gpr50* gene, exon2 of *Gpr50-201*(ENSMUST00000070449.5) transcript is recommended as the knockout region. The region contains 1565bp coding sequence.

  Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Gpr50* 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.
- 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, Mice homozygous for a targeted disruption of this gene display alterations in energy balance and metabolism.
- > The *Gpr50* gene is located on the ChrX. 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)



#### Gpr50 G-protein-coupled receptor 50 [Mus musculus (house mouse)]

Gene ID: 14765, updated on 3-Feb-2019

#### Summary

**↑** ?

Official Symbol Gpr50 provided by MGI

Official Full Name G-protein-coupled receptor 50 provided by MGI

Primary source MGI:MGI:1333877

See related Ensembl:ENSMUSG00000056380

Gene type protein coding
RefSeq status REVIEWED

Organism Mus musculus

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;

Muroidea; Muridae; Murinae; Mus; Mus

Also known as H9

Summary This gene encodes a multipass membrane protein that is thought to act as a G protein-coupled receptor. Activity of this protein may be

important in neurotransmitter and glucocorticoid signalling. Mutation of this gene causes a decreased ability to maintain a constant body temperature, resulting in torpor, as well as an increased metabolic rate. Alternative splicing results in multiple transcript variants. [provided

by RefSeq, May 2015]

Expression Biased expression in placenta adult (RPKM 5.5), whole brain E14.5 (RPKM 1.0) and 7 other tissues See more

Orthologs <u>human</u> all

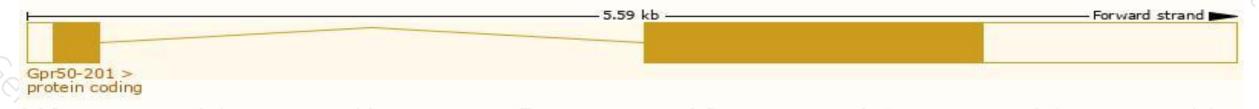
## Transcript information (Ensembl)



The gene has 3 transcripts, all transcripts are shown below:

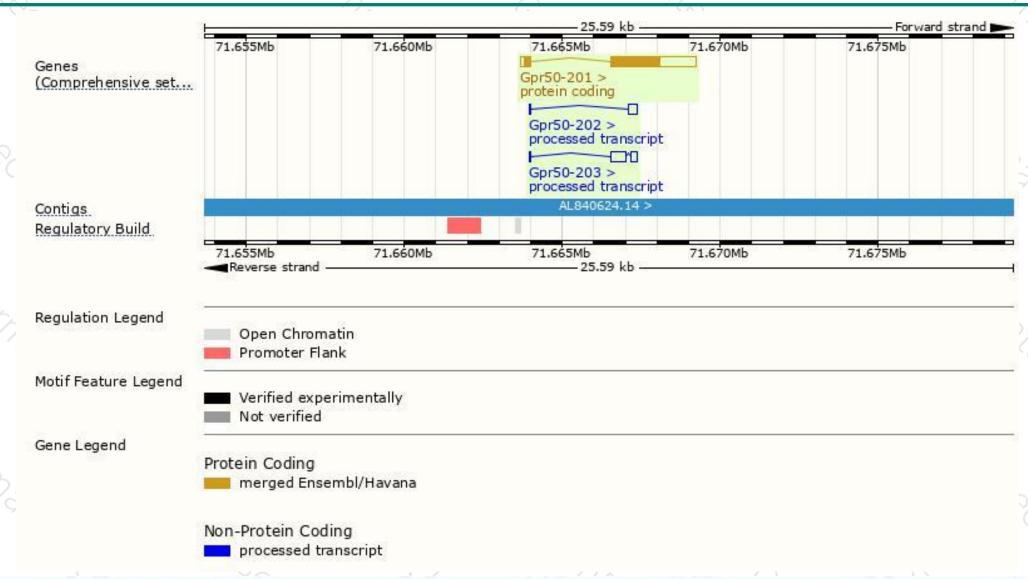
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Gpr50-201	ENSMUST00000070449.5	3069	<u>591aa</u>	Protein coding	CCDS30181	<u>088495</u>	TSL:1 GENCODE basic APPRIS P1
Gpr50-203	ENSMUST00000144712.6	754	No protein	Processed transcript	-		TSL:5
Gpr50-202	ENSMUST00000131554.2	350	No protein	Processed transcript	20	2	TSL:5

The strategy is based on the design of *Gpr50-201* transcript, The transcription is shown below



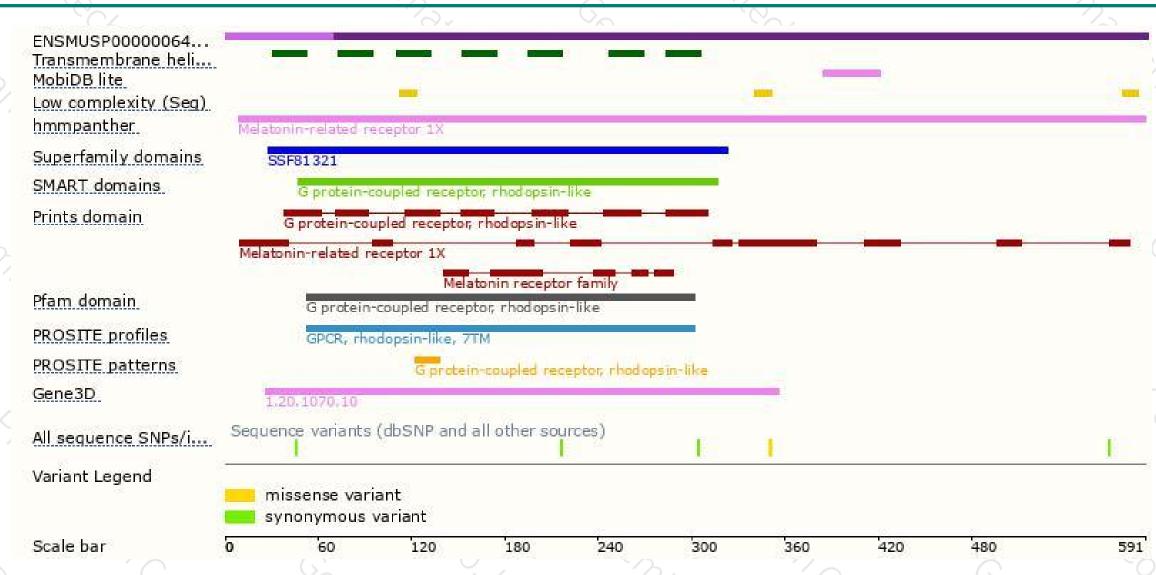
### Genomic location distribution





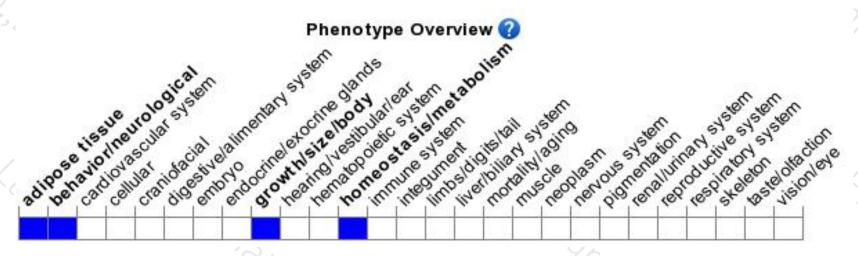
### Protein domain





## Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

According to the existing MGI data, Mice homozygous for a targeted disruption of this gene display alterations in energy balance and metabolism.



If you have any questions, you are welcome to inquire. Tel: 400-9660890





