

Gpr65 Cas9-CKO Strategy

Designer: Xueting Zhang

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Project Overview

Project Name

Gpr65

Project type

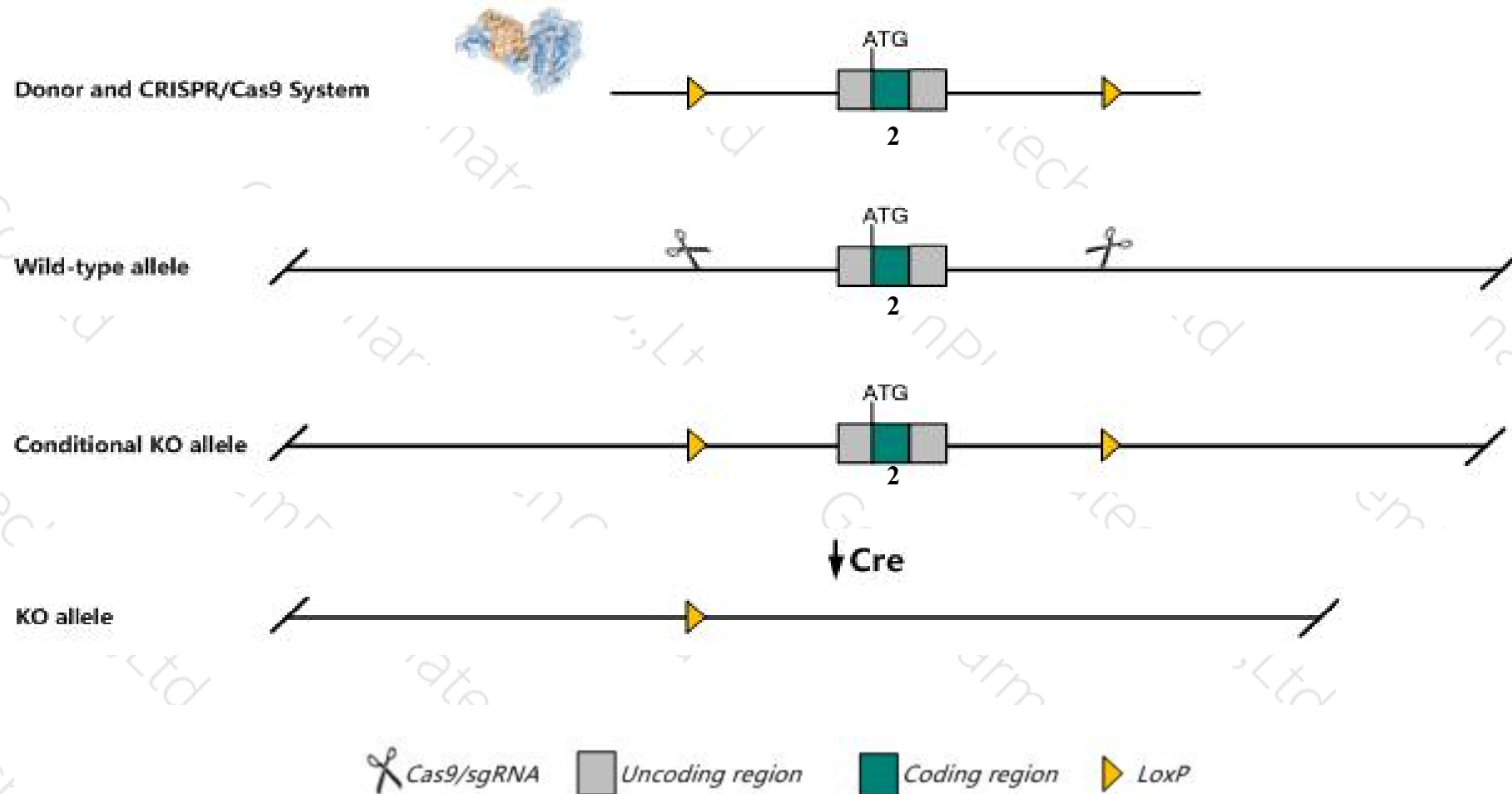
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Gpr65* gene. The schematic diagram is as follows:



- The *Gpr65* gene has 1 transcript. According to the structure of *Gpr65* gene, exon2 of *Gpr65-201* (ENSMUST00000075072.5) transcript is recommended as the knockout region. The region contains all of the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Gpr65* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed. Cas9, sgRNA 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 was 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.

- According to the existing MGI data, Homozygous mutant mice have thymocytes and splenocytes that are insensitive to pH-dependent cAMP production.
- *Gm47566* gene will be deleted together in this strategy.
- The floxed region is near to the N-terminal of *Gm47567* gene, this strategy may influence the regulatory function of the N-terminal of *Gm47567* gene.
- The *Gpr65* gene is located on the Chr12. 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)

Gpr65 G-protein coupled receptor 65 [Mus musculus (house mouse)]

Gene ID: 14744, updated on 16-Mar-2019

Summary



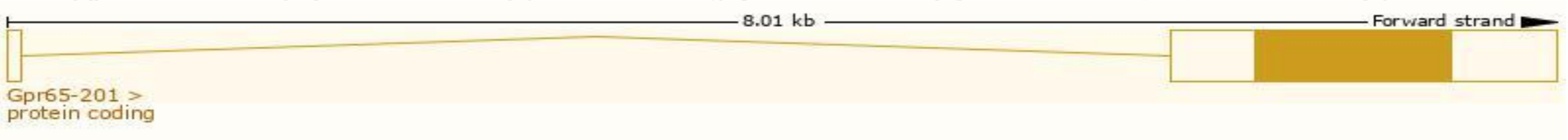
Official Symbol	Gpr65 provided by MGI
Official Full Name	G-protein coupled receptor 65 provided by MGI
Primary source	MGI:MGI:108031
See related	Ensembl:ENSMUSG000000021886
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Dig1, Gpcr25, TDAG8
Expression	Broad expression in thymus adult (RPKM 4.0), spleen adult (RPKM 4.0) and 21 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

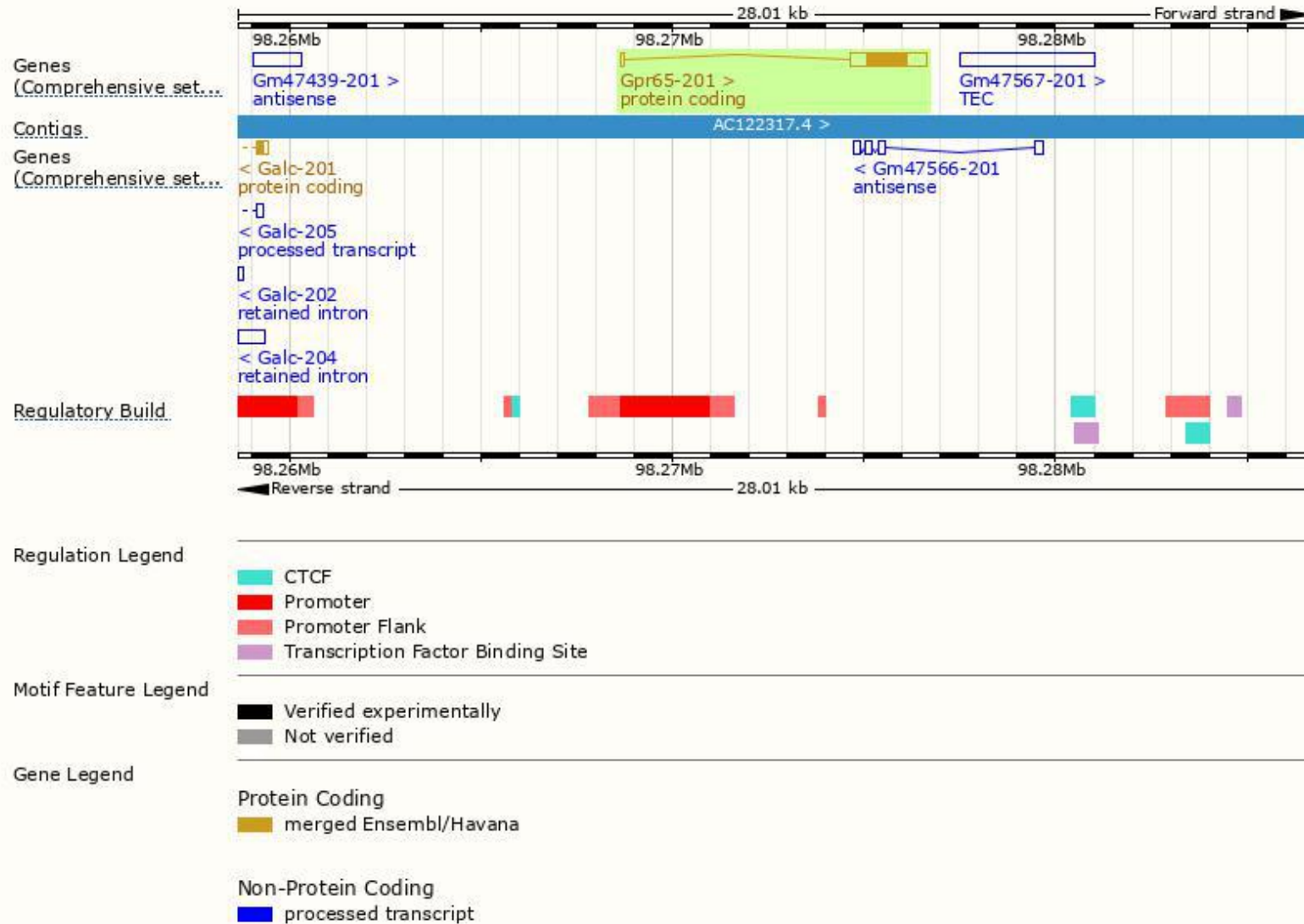
The gene has 1 transcript, and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Gpr65-201	ENSMUST00000075072.5	2069	337aa	Protein coding	CCDS26096	A0A0R4J0Y2	TSL:1 GENCODE basic APPRIS P1

The strategy is based on the design of *Gpr65-201* transcript, The transcription is shown below



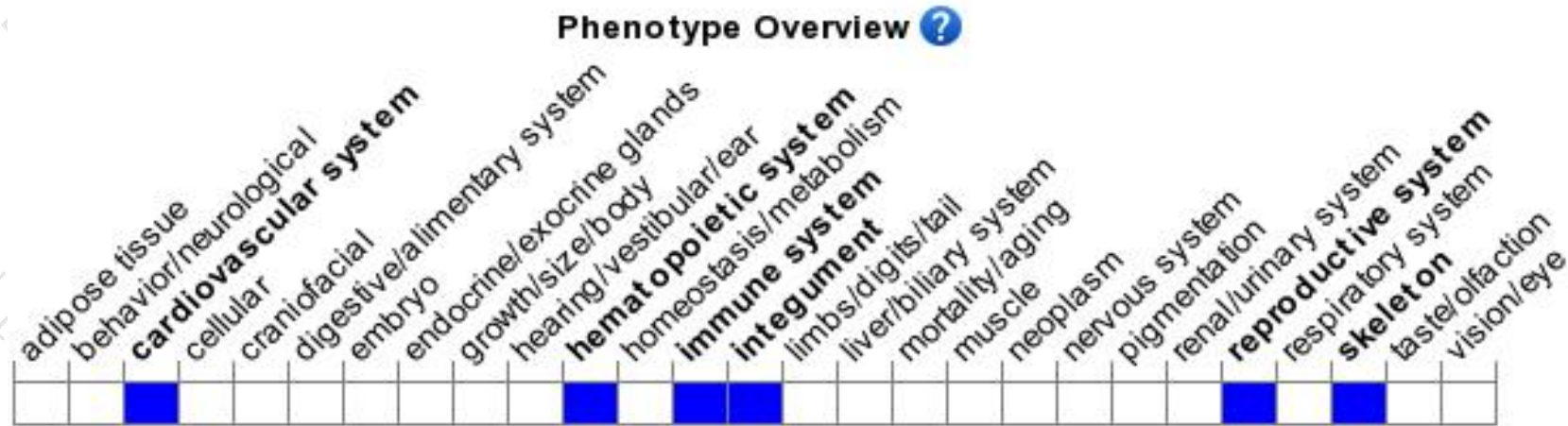
Genomic location distribution



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, Homozygous mutant mice have thymocytes and splenocytes that are insensitive to pH-dependent cAMP production.

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

Tel: 025-5864 1534

