

***Gnat2* Cas9-CKO Strategy**

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Reviewer:

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Design Date:

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Project Overview

Project Name

Gnat2

Project type

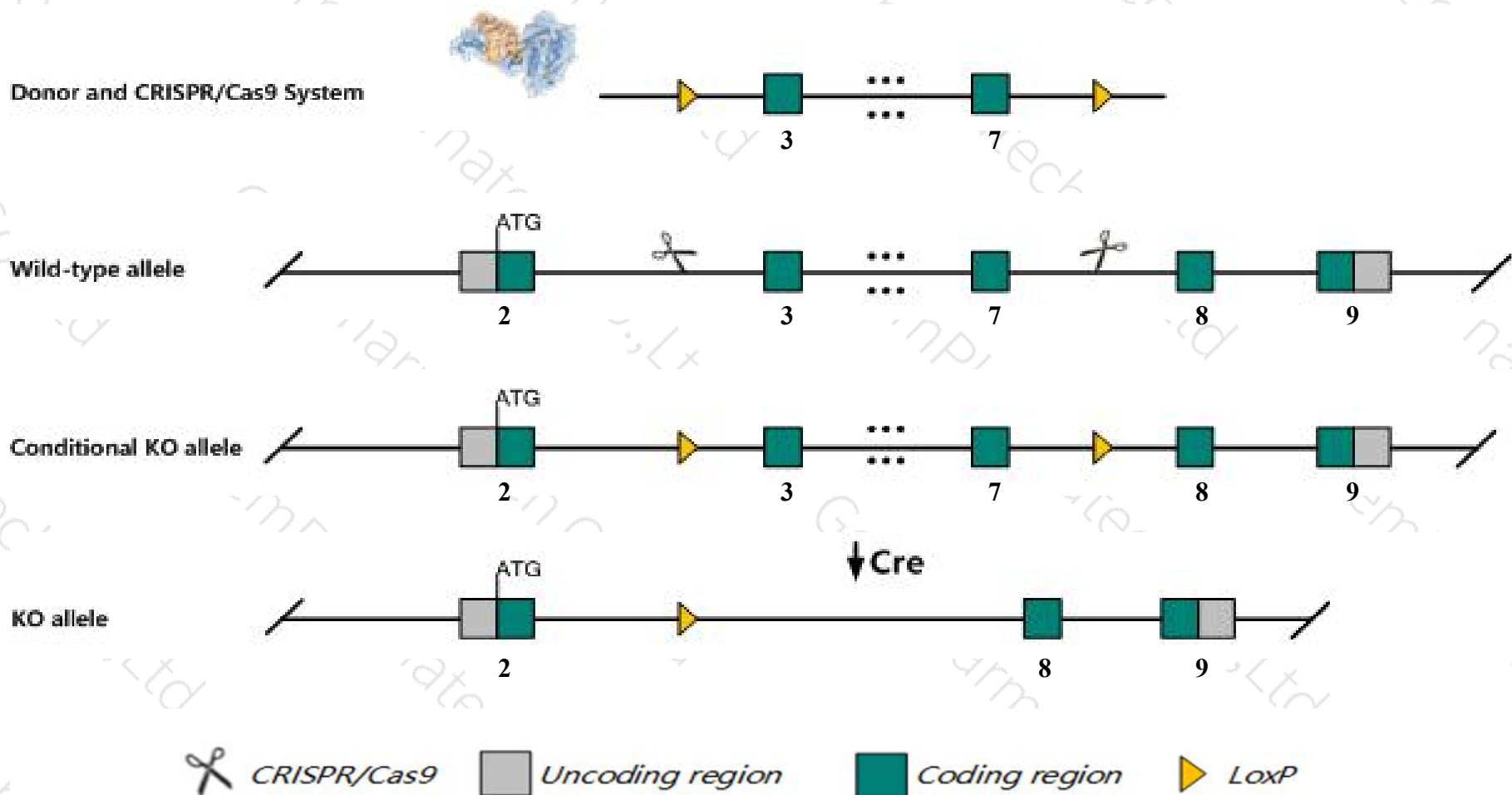
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Gnat2* gene. The schematic diagram is as follows:



Technical routes

- The *Gnat2* gene has 6 transcripts. According to the structure of *Gnat2* gene, exon3-exon7 of *Gnat2*-201 (ENSMUST00000058669.14) transcript is recommended as the knockout region. The region contains 602bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Gnat2* 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.

- According to the existing MGI data, Mutations in this gene result in progressive degeneration of photoreceptors and normal ERG responses.
- The *Gnat2* gene is located on the Chr3. 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)

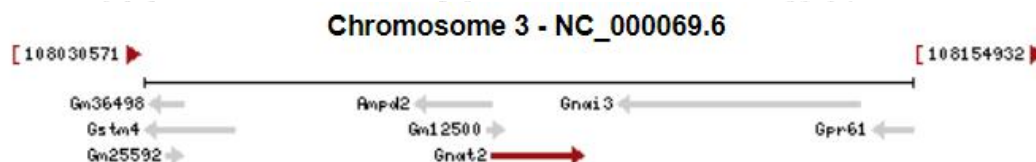
Gnat2 guanine nucleotide binding protein, alpha transducing 2 [*Mus musculus* (house mouse)]

Gene ID: 14686, updated on 26-Nov-2019

Summary



Official Symbol	Gnat2 provided by MGI
Official Full Name	guanine nucleotide binding protein, alpha transducing 2 provided by MGI
Primary source	MGI:MGI:95779
See related	Ensembl:ENSMUSG00000009108
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	Gt-2; Gnat-2; Tcalpha; AW490837
Expression	Biased expression in adrenal adult (RPKM 2.2), genital fat pad adult (RPKM 1.0) and 11 other tissues See more
Orthologs	human all

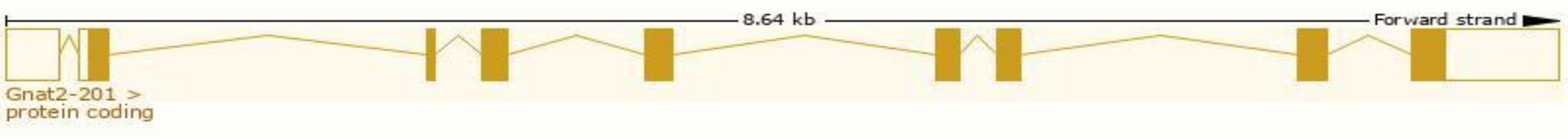


Transcript information (Ensembl)

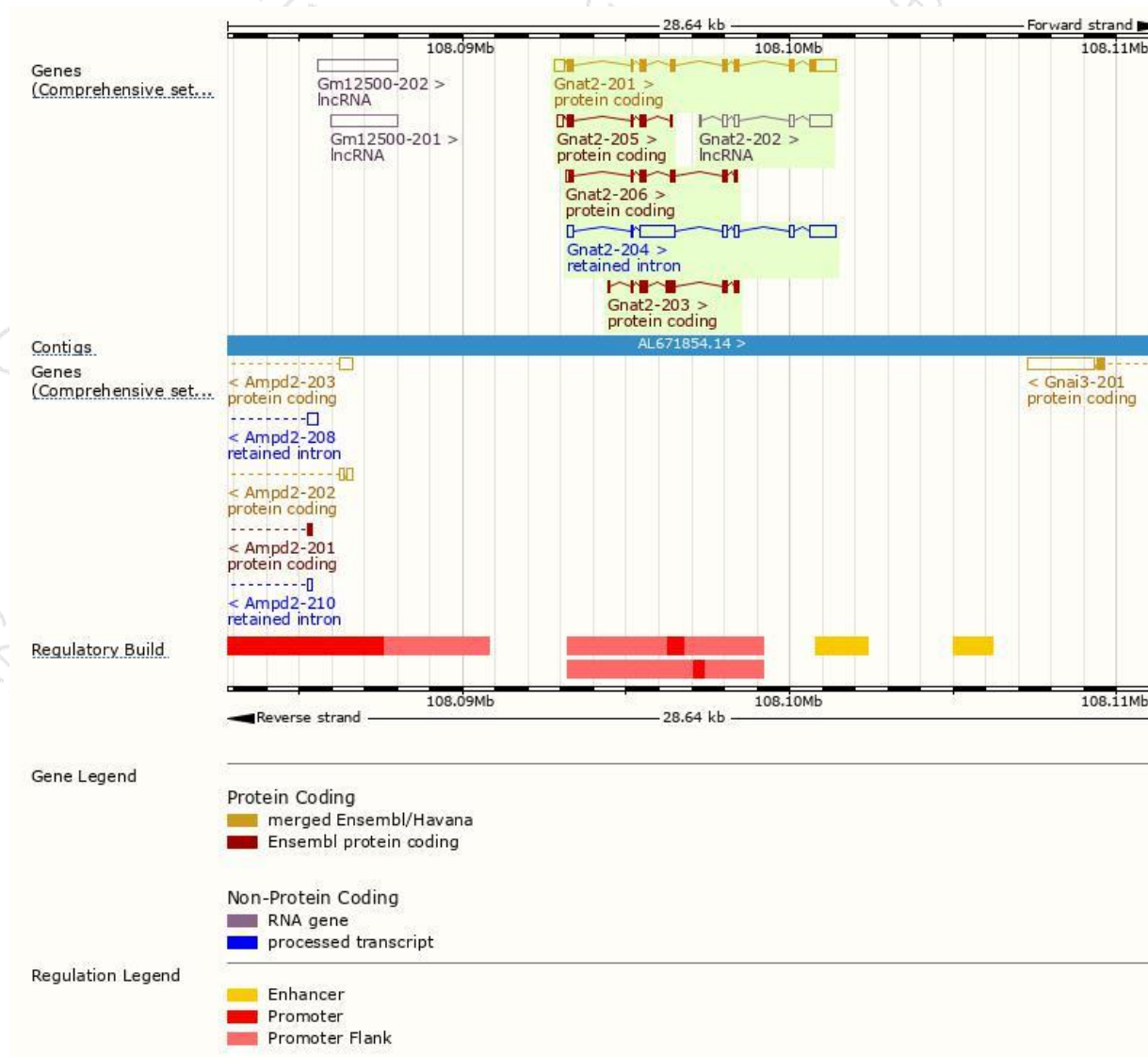
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags
Gnat2-201	ENSMUST00000058669.14	2054	354aa	ENSMUSP00000053818.8	Protein coding	CCDS17750	A2AE33 P50149	TSL:1 GENCODE basic APPRIS P1
Gnat2-203	ENSMUST00000131856.2	842	249aa	ENSMUSP00000121533.2	Protein coding	-	F6QPU5	CDS 3' incomplete TSL:5
Gnat2-206	ENSMUST00000151326.7	808	232aa	ENSMUSP00000120425.1	Protein coding	-	A2AE32	CDS 3' incomplete TSL:2
Gnat2-205	ENSMUST00000145101.7	587	112aa	ENSMUSP00000123125.1	Protein coding	-	A2AE31	CDS 3' incomplete TSL:3
Gnat2-204	ENSMUST00000142568.7	2518	No protein	-	Retained intron	-	-	TSL:2
Gnat2-202	ENSMUST00000130013.1	1152	No protein	-	lncRNA	-	-	TSL:1

The strategy is based on the design of *Gnat2-201* transcript,The transcription is shown below



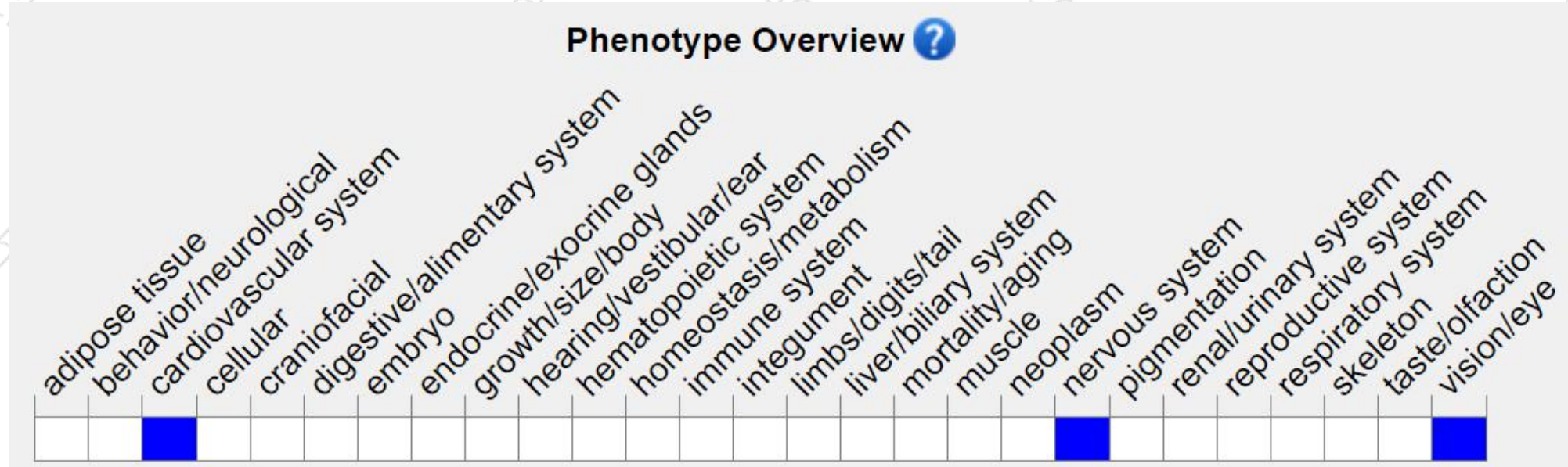
Genomic location distribution



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, Mutations in this gene result in progressive degeneration of photoreceptors and normal ERG responses.

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

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