

Ngb Cas9-CKO Strategy

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Project Overview

Project Name

Ngb

Project type

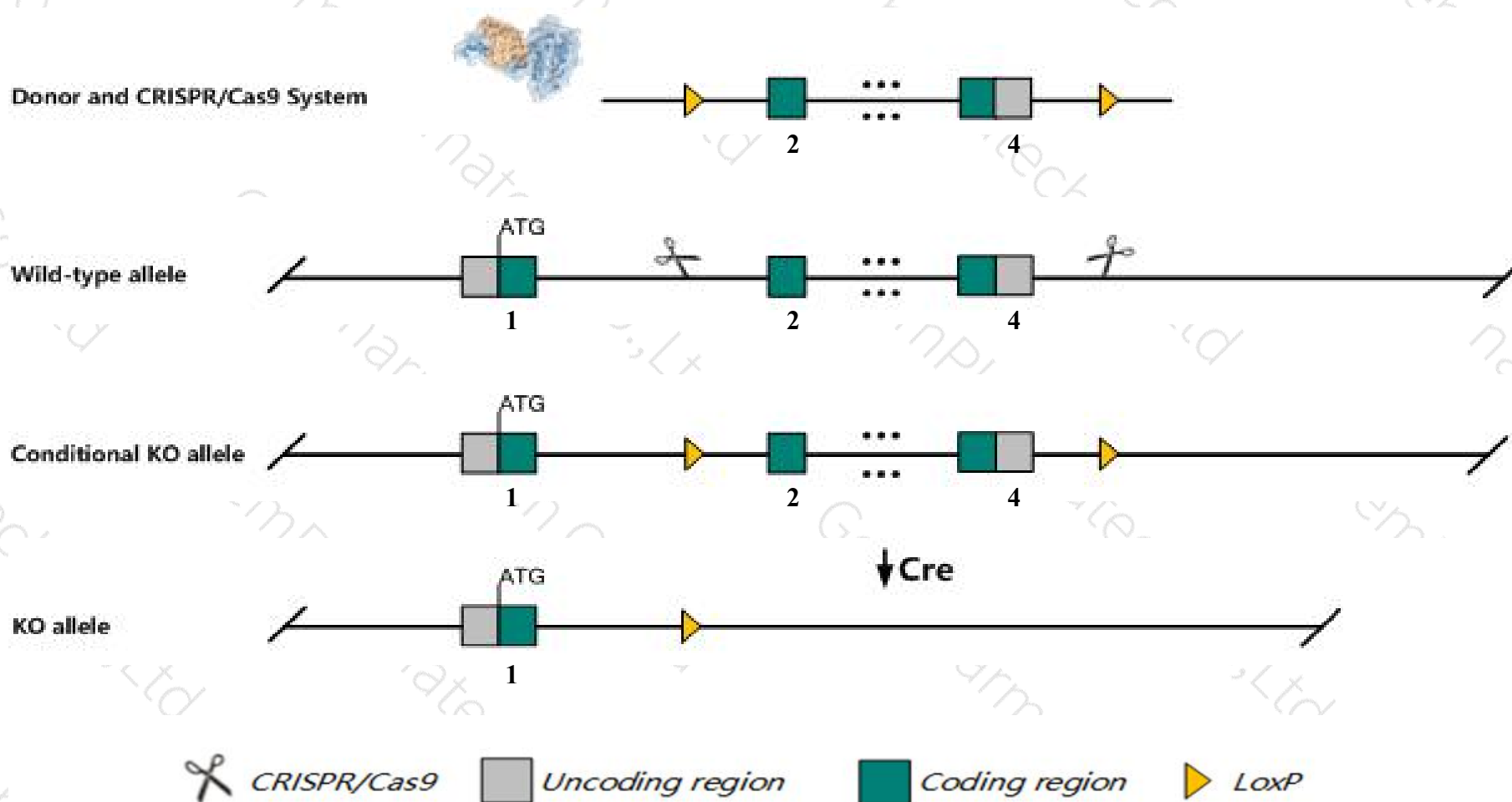
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



Technical routes

- The *Ngb* gene has 3 transcripts. According to the structure of *Ngb* gene, exon2-exon4 of *Ngb-201* (ENSMUST00000021420.13) transcript is recommended as the knockout region. The region contains most of coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Ngb* 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.

- According to the existing MGI data, Mice homozygous for a knock-out allele exhibit a significantly larger phase delay of circadian rhythm upon light stimulation at early night.
- The *Ngb* 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)

Ngb neuroglobin [*Mus musculus* (house mouse)]

Gene ID: 64242, updated on 3-Sep-2019

Summary

Official Symbol	Ngb provided by MGI
Official Full Name	neuroglobin provided by MGI
Primary source	MGI:MGI:2151886
See related	Ensembl:ENSMUSG00000021032
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
Expression	Biased expression in bladder adult (RPKM 22.2), stomach adult (RPKM 7.1) and 6 other tissues See more
Orthologs	human all

Genomic context

Location: 12; 12 D2

See Ngb in [Genome Data Viewer](#)

Exon count: 4

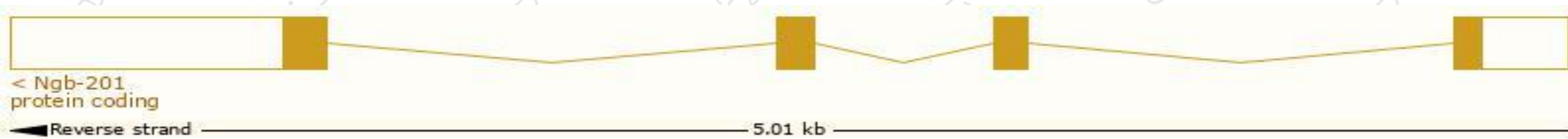
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	12	NC_000078.6 (87097531..87102539, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	12	NC_000078.5 (88438481..88443489, complement)

Transcript information (Ensembl)

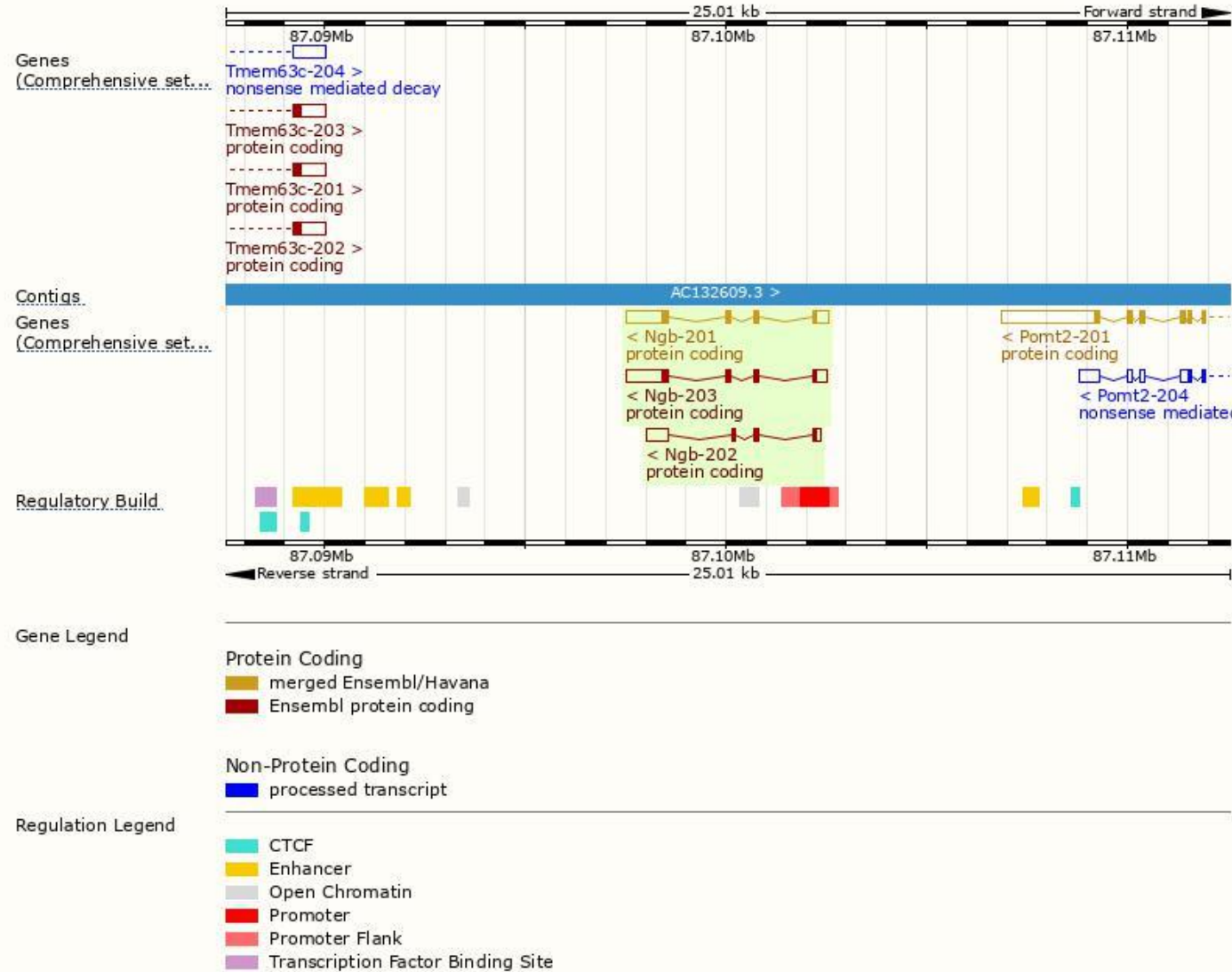
The gene has 3 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ngb-201	ENSMUST00000021420.13	1616	151aa	Protein coding	CCDS36501	Q5ZPR7 Q9ER97	TSL:1 GENCODE basic APPRIS P3
Ngb-203	ENSMUST00000110177.8	1611	155aa	Protein coding	CCDS79141	Q3USR6	TSL:1 GENCODE basic APPRIS ALT 1
Ngb-202	ENSMUST00000110176.1	908	92aa	Protein coding	-	D3YW07	TSL:3 GENCODE basic

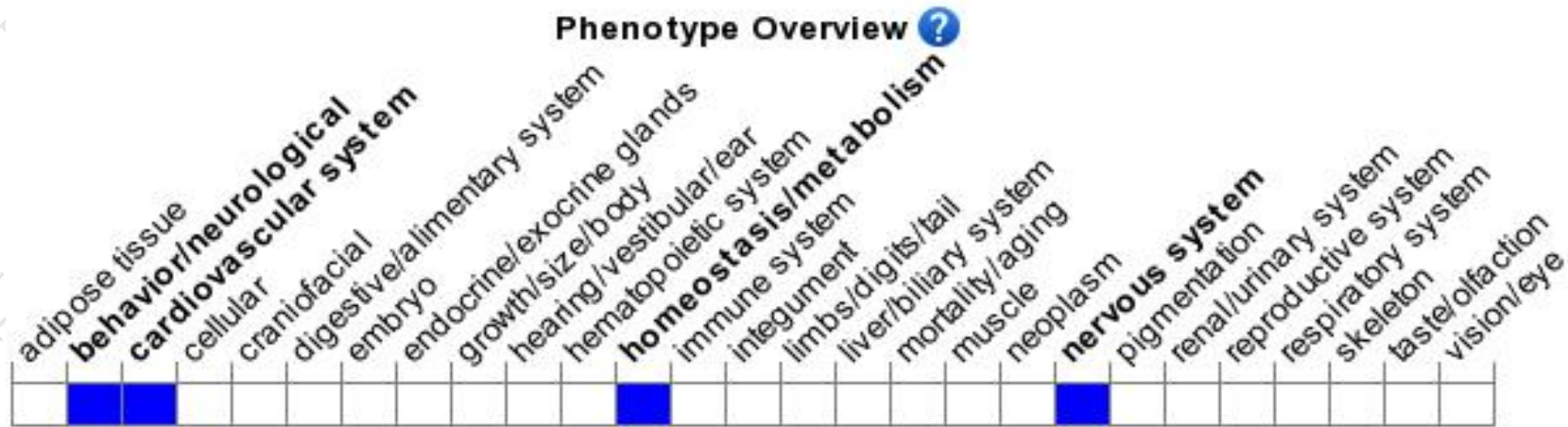
The strategy is based on the design of *Ngb-201* transcript, The transcription is shown below



Genomic location distribution



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 knock-out allele exhibit a significantly larger phase delay of circadian rhythm upon light stimulation at early night.

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

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