

***Grm6* Cas9-CKO Strategy**

Designer:

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Design Date:

2019-7-25

Project Overview

Project Name

Grm6

Project type

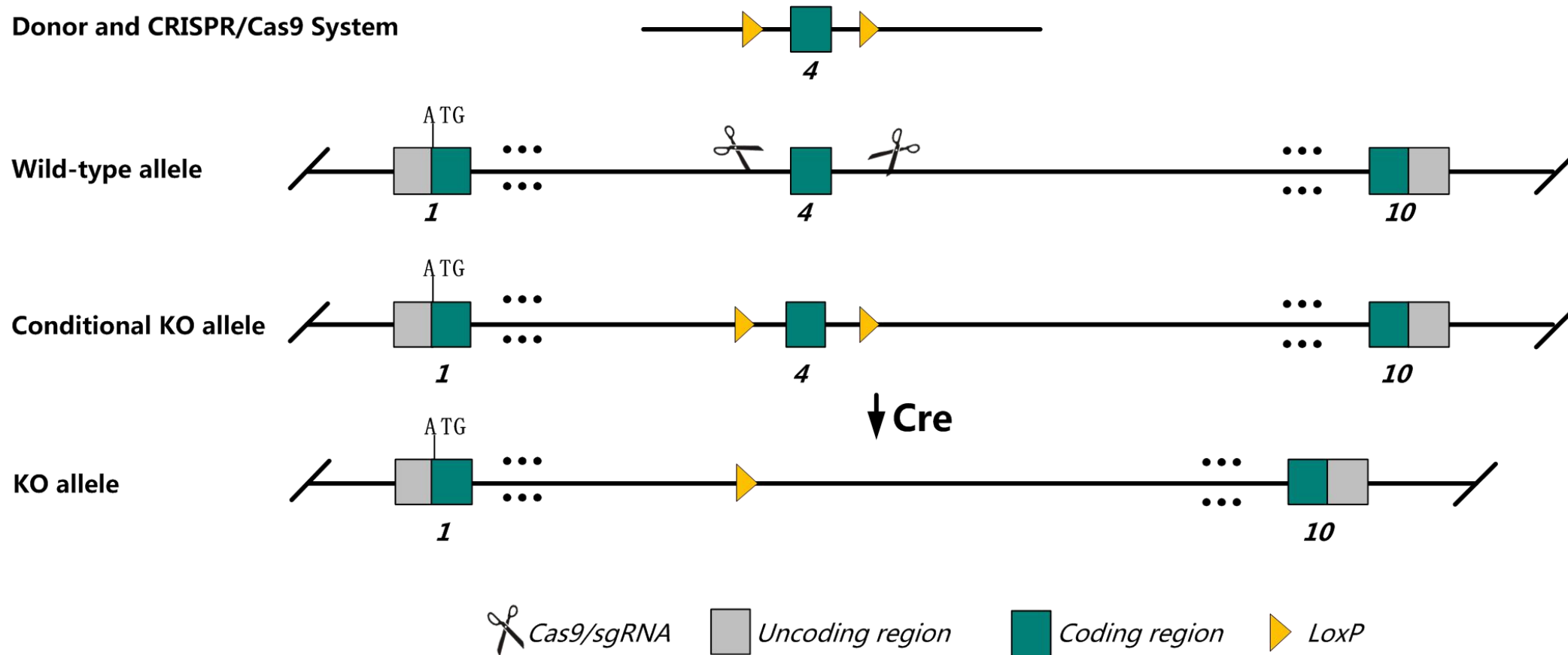
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Grm6* gene. The schematic diagram is as follows:



- The *Grm6* gene has 4 transcripts. According to the structure of *Grm6* gene, exon4 of *Grm6-201* (ENSMUST00000000631.7) transcript is recommended as the knockout region. The region contains 155bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Grm6* 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, Homozygous null mice show loss of ON responses without significant alteration of OFF responses in visual transmission or changes in visual behavioral responses. ENU-induced mutant mice have an ERG that lacks the rod b-wave and scotopic threshold response, while the cone ERG is of large amplitude.
- The *Grm6* gene is located on the Chr11. 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)

Grm6 glutamate receptor, metabotropic 6 [Mus musculus (house mouse)]

Gene ID: 108072, updated on 26-Feb-2019

Summary



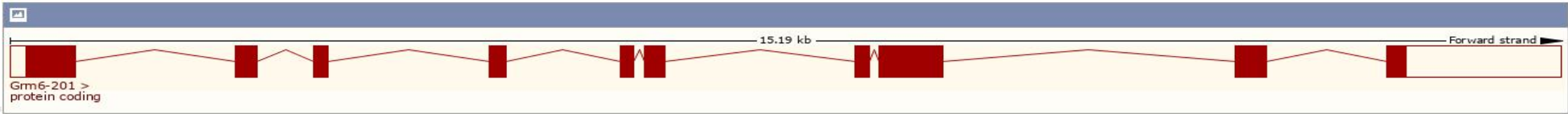
Official Symbol	Grm6 provided by MGI
Official Full Name	glutamate receptor, metabotropic 6 provided by MGI
Primary source	MGI:MGI:1351343
See related	Ensembl:ENSMUSG00000000617
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	BC021919, Gm3, Gprc1f, mGluR6, nerg1, nob2, nob3, nob4
Expression	Low expression observed in reference dataset See more
Orthologs	human all

Transcript information (Ensembl)

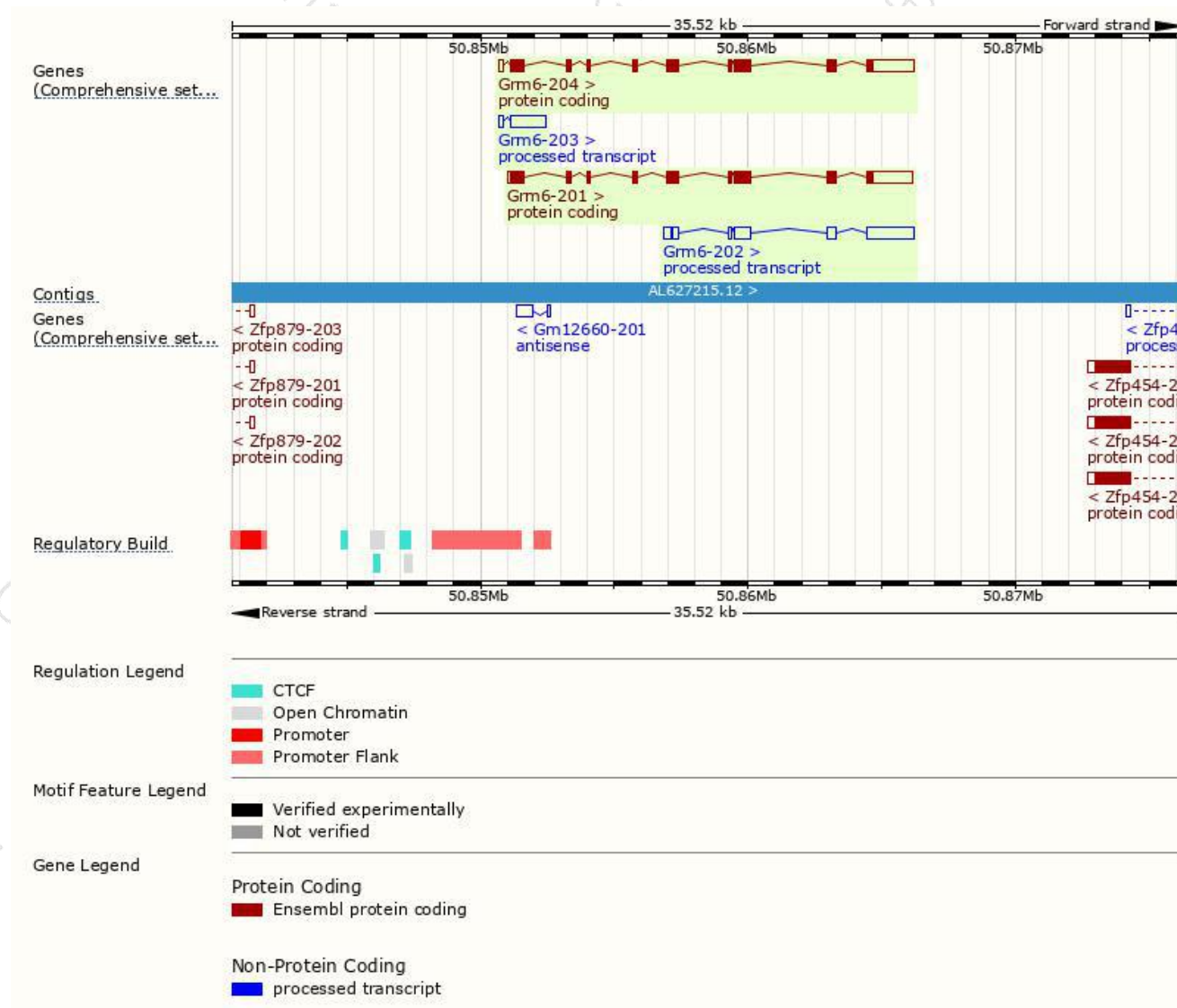
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Grm6-204	ENSMUST00000171427.7	4330	871aa	Protein coding	CCDS48785	Q5NCH9	TSL:5 GENCODE basic APPRIS P1
Grm6-201	ENSMUST00000000631.7	4291	871aa	Protein coding	CCDS48785	Q5NCH9	TSL:5 GENCODE basic APPRIS P1
Grm6-202	ENSMUST00000126890.1	3305	No protein	Processed transcript	-	-	TSL:5
Grm6-203	ENSMUST00000156890.7	1463	No protein	Processed transcript	-	-	TSL:1

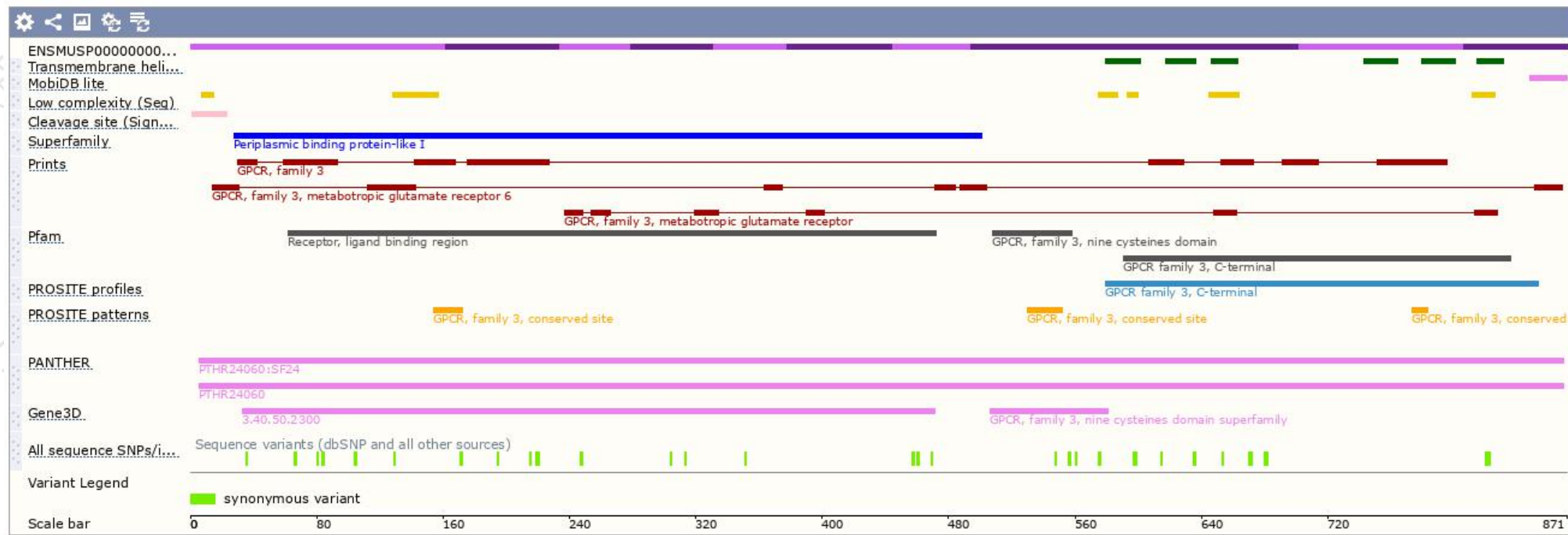
The strategy is based on the design of *Grm6-201* transcript,The transcription is shown below



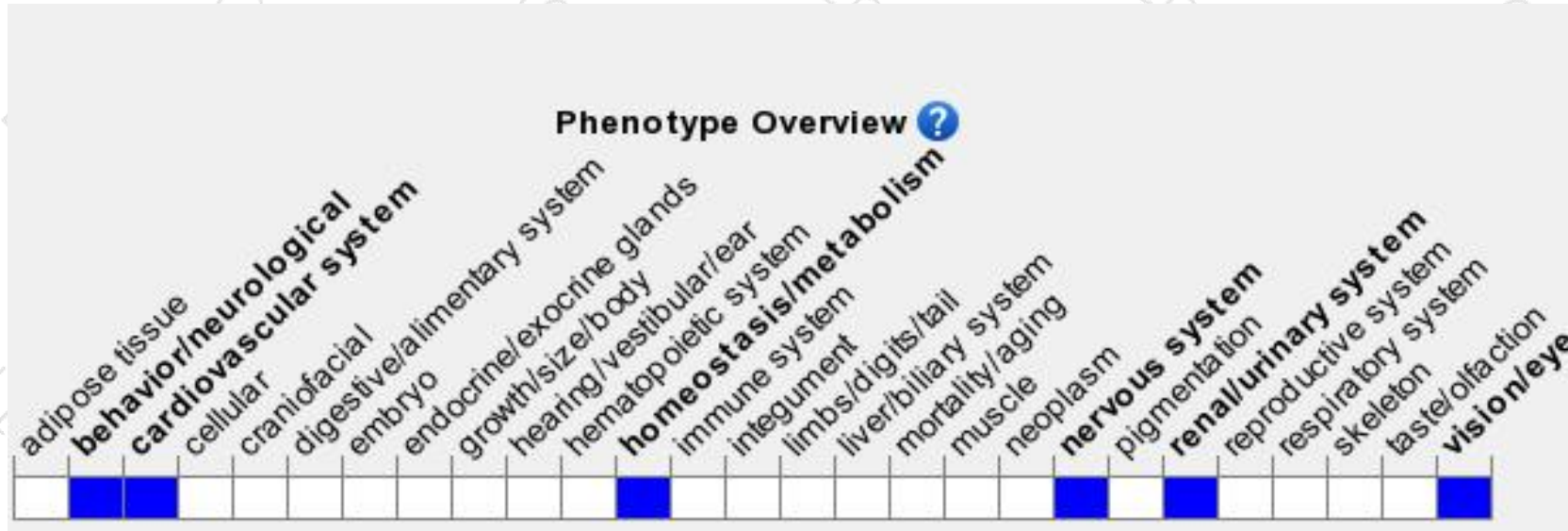
Genomic location distribution



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 null mice show loss of ON responses without significant alteration of OFF responses in visual transmission or changes in visual behavioral responses. ENU-induced mutant mice have an ERG that lacks the rod b-wave and scotopic threshold response, while the cone ERG is of large amplitude.

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

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