Grin2d Cas9-CKO Strategy

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Reviewer: Ruirui Zhang
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Project Overview

Project Name	***Grin2d***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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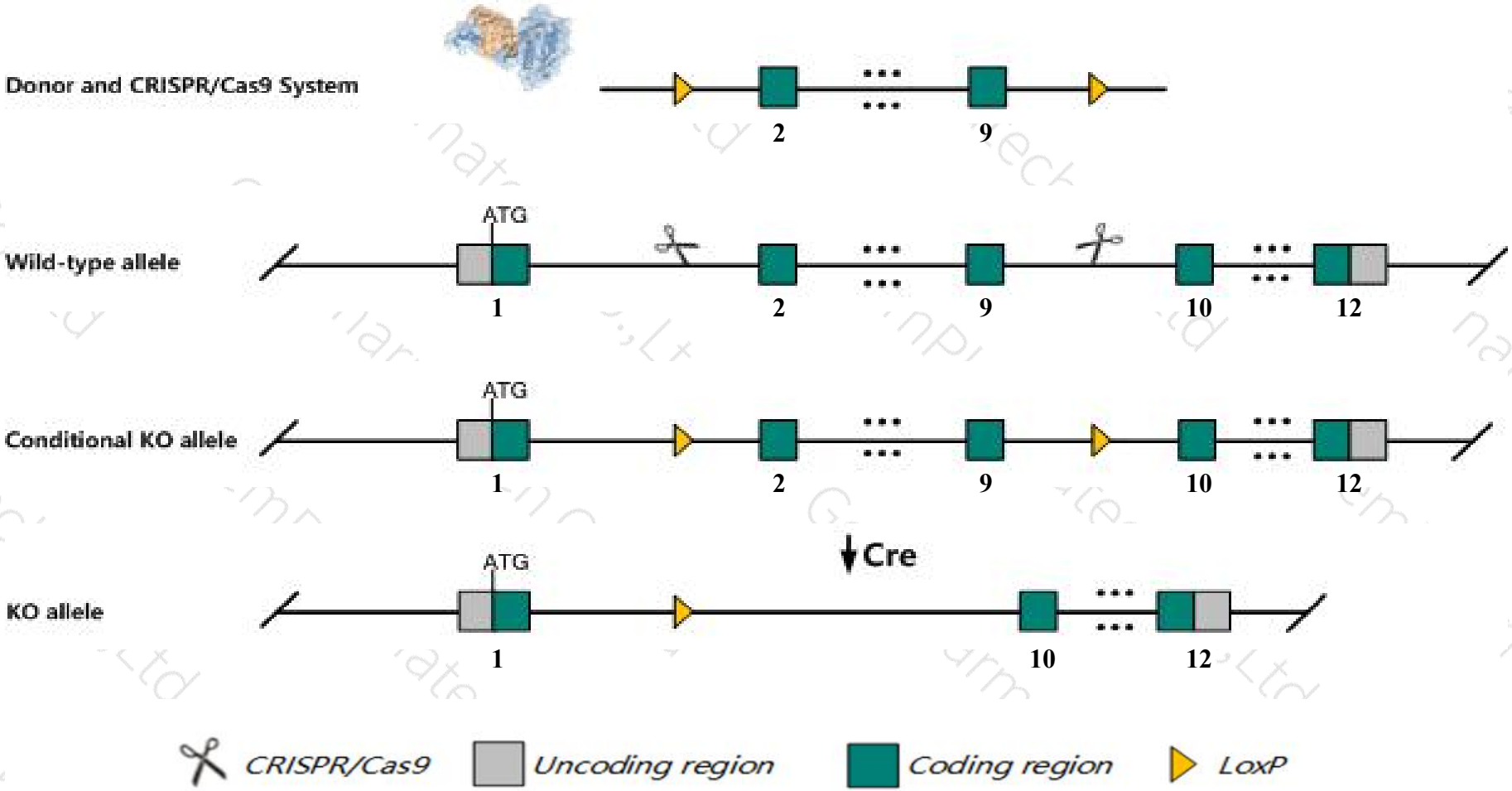
This model will use CRISPR/Cas9 technology to edit the *Grin2d* gene. The schematic diagram is as follows:



Technical routes

- The *Grin2d* gene has 3 transcripts. According to the structure of *Grin2d* gene, exon2-exon9 of *Grin2d-201* (ENSMUST00000002848.9) transcript is recommended as the knockout region.The region contains 1787bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Grin2d* 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/6J 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/6J 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,Homozygotes for a targeted null mutation exhibit reduced spontaneous activity and an elevated auditory brainstem response threshold.

- The *Grin2d* gene is located on the Chr7. 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.

- The *Grin2d-202* transcript has not been affected.

- 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）

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Grin2d glutamate receptor, ionotropic, NMDA2D (epsilon 4) [Mus musculus (house mouse)]

Gene ID: 14814, updated on 19-Mar-2019

Summary

Official Symbol	Grin2d provided by MGI
Official Full Name	glutamate receptor, ionotropic, NMDA2D (epsilon 4) provided by MGI
Primary source	MGI:MGI:95823
See related	Ensembl:ENSMUSG00000002771
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	GluN2D, NMDAR2D, NR2D
Expression	Broad expression in testis adult (RPKM 12.8), CNS E18 (RPKM 5.9) and 19 other tissues See more
Orthologs	human all

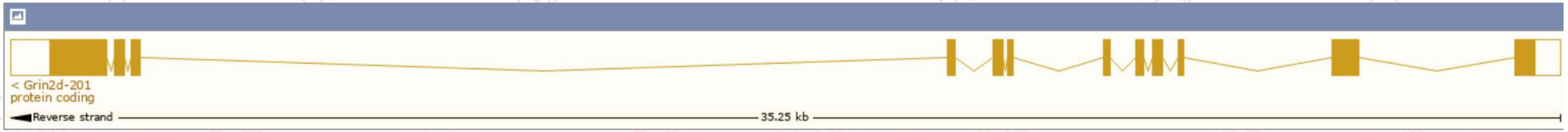
Transcript information（Ensembl）

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

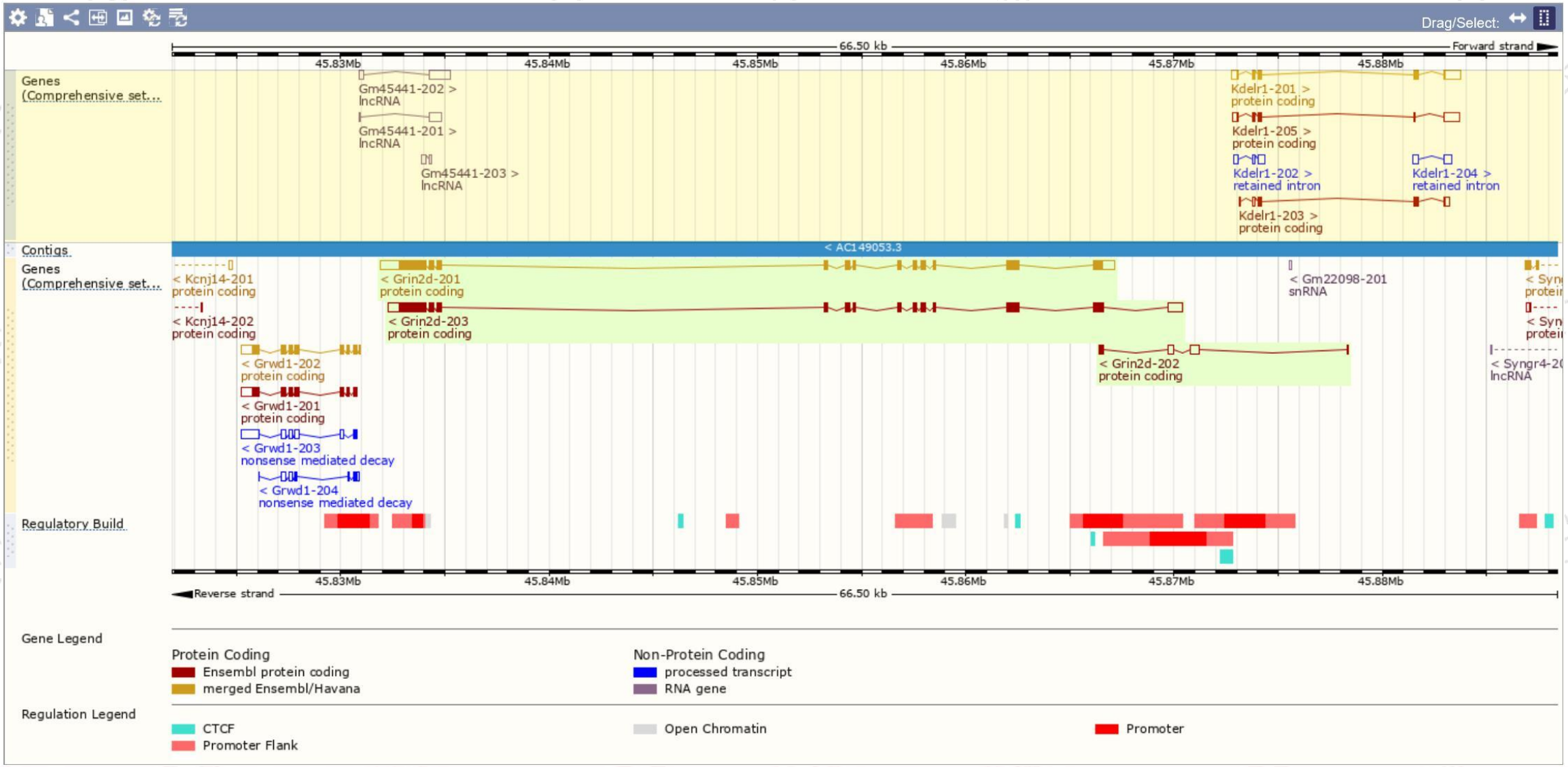
Show/hide columns (1 hidden) Filter

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Grin2d-203	ENSMUST00000211713.1	5244	1323aa	Protein coding	CCDS21267	Q03391	TSL:5 GENCODE basic APPRIS P1
Grin2d-202	ENSMUST00000211250.1	1009	63aa	Protein coding	-	A0A1B0GRF9	CDS 3' incomplete TSL:5
Grin2d-201	ENSMUST00000002848.9	5431	1323aa	Protein coding	CCDS21267	Q03391	TSL:1 GENCODE basic APPRIS P1

The strategy is based on the design of *Grin2d-201* transcript,The transcription is shown below

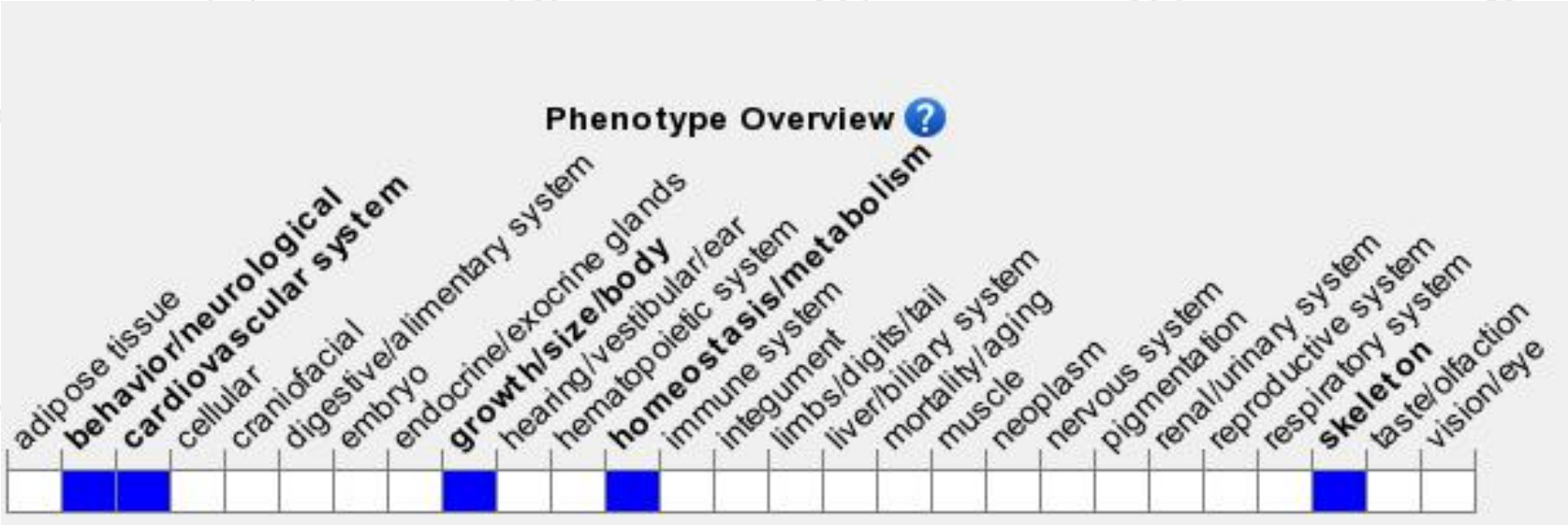


Genomic location distribution



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,Homozygotes for a targeted null mutation exhibit reduced spontaneous activity and an elevated auditory brainstem response threshold.

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

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