

***Gramd4* Cas9-CKO Strategy**

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Project Overview

Project Name

Gramd4

Project type

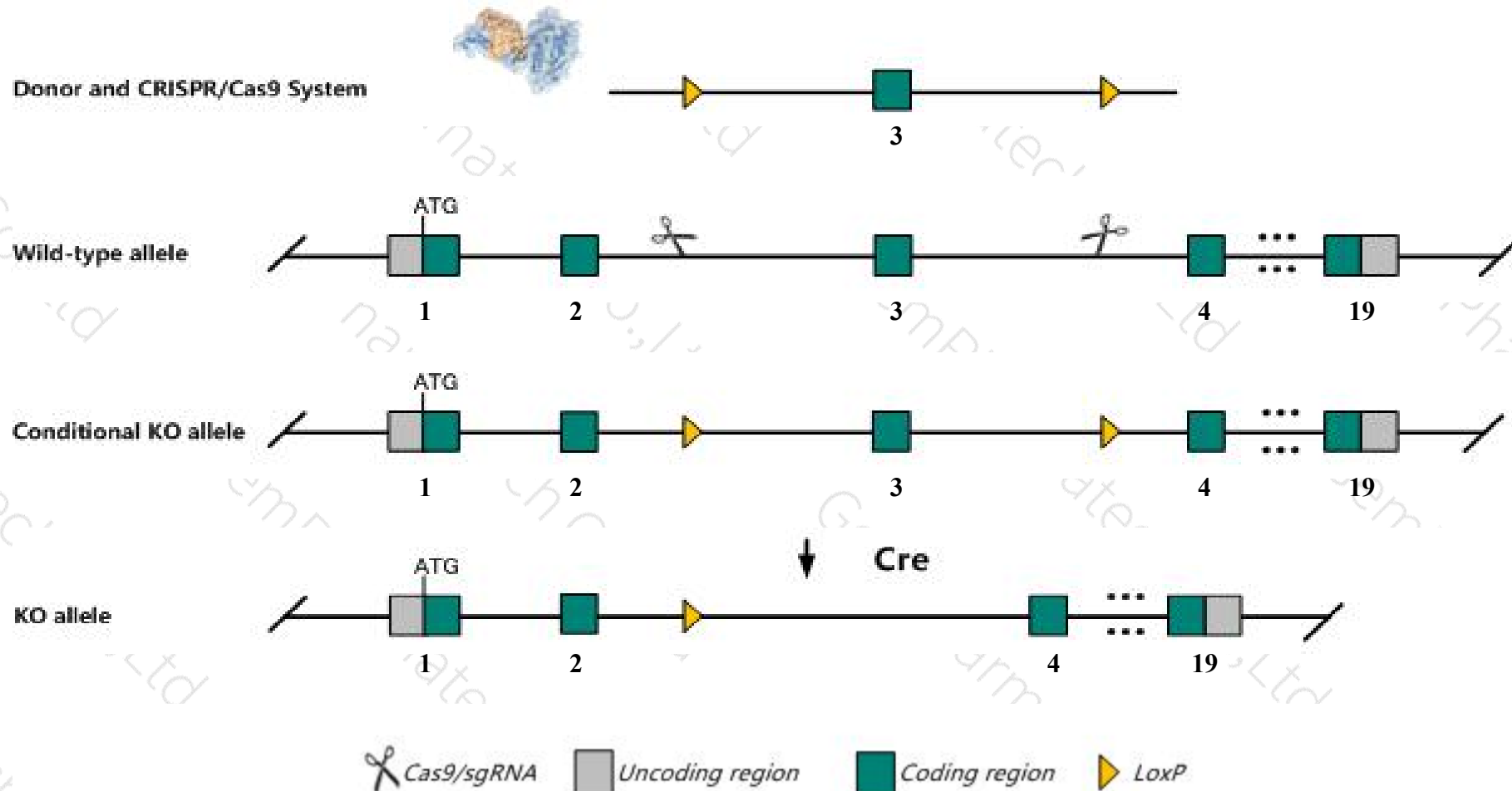
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Gramd4* gene. The schematic diagram is as follows:



- The *Gramd4* gene has 5 transcripts. According to the structure of *Gramd4* gene, exon3 of *Gramd4*-201(ENSMUST00000088931.9) transcript is recommended as the knockout region. The region contains 121bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Gramd4* 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.

- The *Gramd4* gene is located on the Chr15. 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.
- Transcript *Gramd4*-205 may not be 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)

Gramd4 GRAM domain containing 4 [Mus musculus (house mouse)]

Gene ID: 223752, updated on 13-Mar-2020

Summary



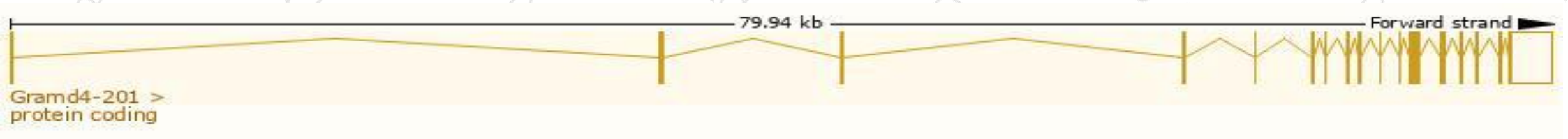
| | |
|---------------------------|---|
| Official Symbol | Gramd4 provided by MGI |
| Official Full Name | GRAM domain containing 4 provided by MGI |
| Primary source | MGI:MGI:2676308 |
| See related | Ensembl:ENSMUSG00000035900 |
| Gene type | protein coding |
| RefSeq status | VALIDATED |
| Organism | Mus musculus |
| Lineage | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| Also known as | 9930016O13 |
| Expression | Ubiquitous expression in whole brain E14.5 (RPKM 10.5), CNS E18 (RPKM 10.3) and 27 other tissues See more |
| Orthologs | human all |

Transcript information (Ensembl)

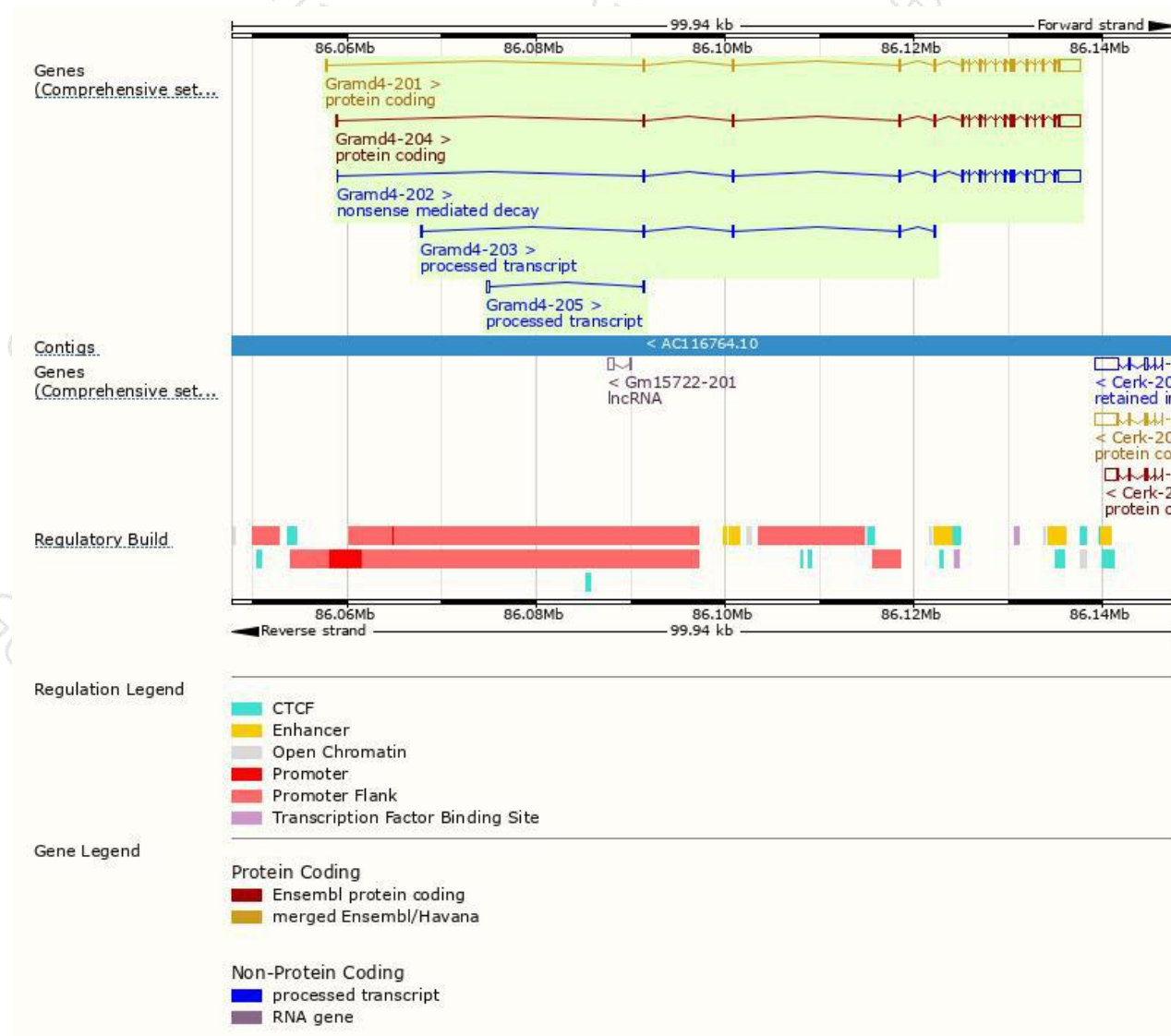
The gene has 5 transcripts,all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|------------|--------------------------------------|------|-----------------------|-------------------------|---------------------------|------------------------|-------------------------------|
| Gramd4-204 | ENSMUST00000138134.7 | 4292 | 627aa | Protein coding | CCDS56998 | D3YUE7 | TSL:1 GENCODE basic |
| Gramd4-201 | ENSMUST00000088931.9 | 4115 | 633aa | Protein coding | CCDS27727 | Q8CB44 | TSL:1 GENCODE basic APPRIS P1 |
| Gramd4-202 | ENSMUST00000123349.1 | 4861 | 323aa | Nonsense mediated decay | - | D6RET7 | TSL:2 |
| Gramd4-203 | ENSMUST00000123474.1 | 597 | No protein | Processed transcript | - | - | TSL:5 |
| Gramd4-205 | ENSMUST00000147286.1 | 374 | No protein | Processed transcript | - | - | TSL:2 |

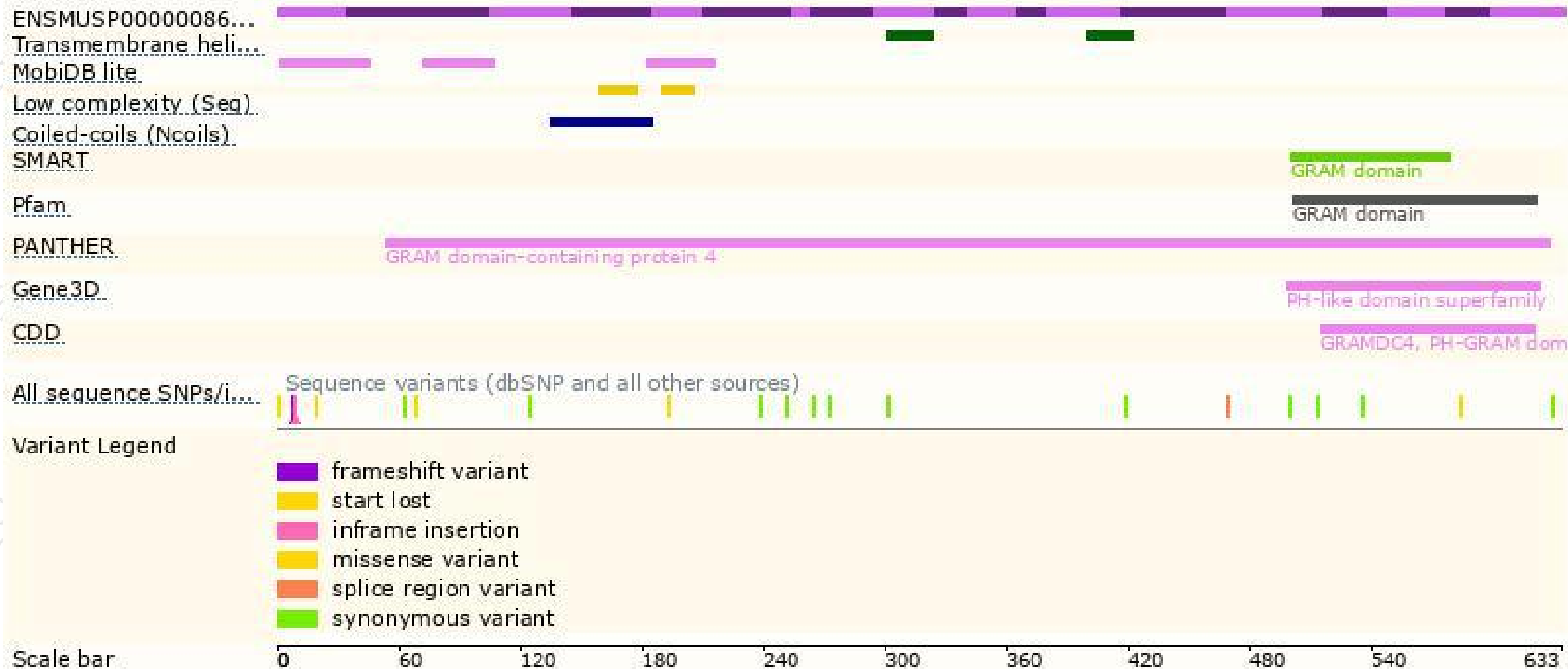
The strategy is based on the design of *Gramd4-201* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

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