

***Gramd2* Cas9-CKO Strategy**

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Project Overview

Project Name

Gramd2

Project type

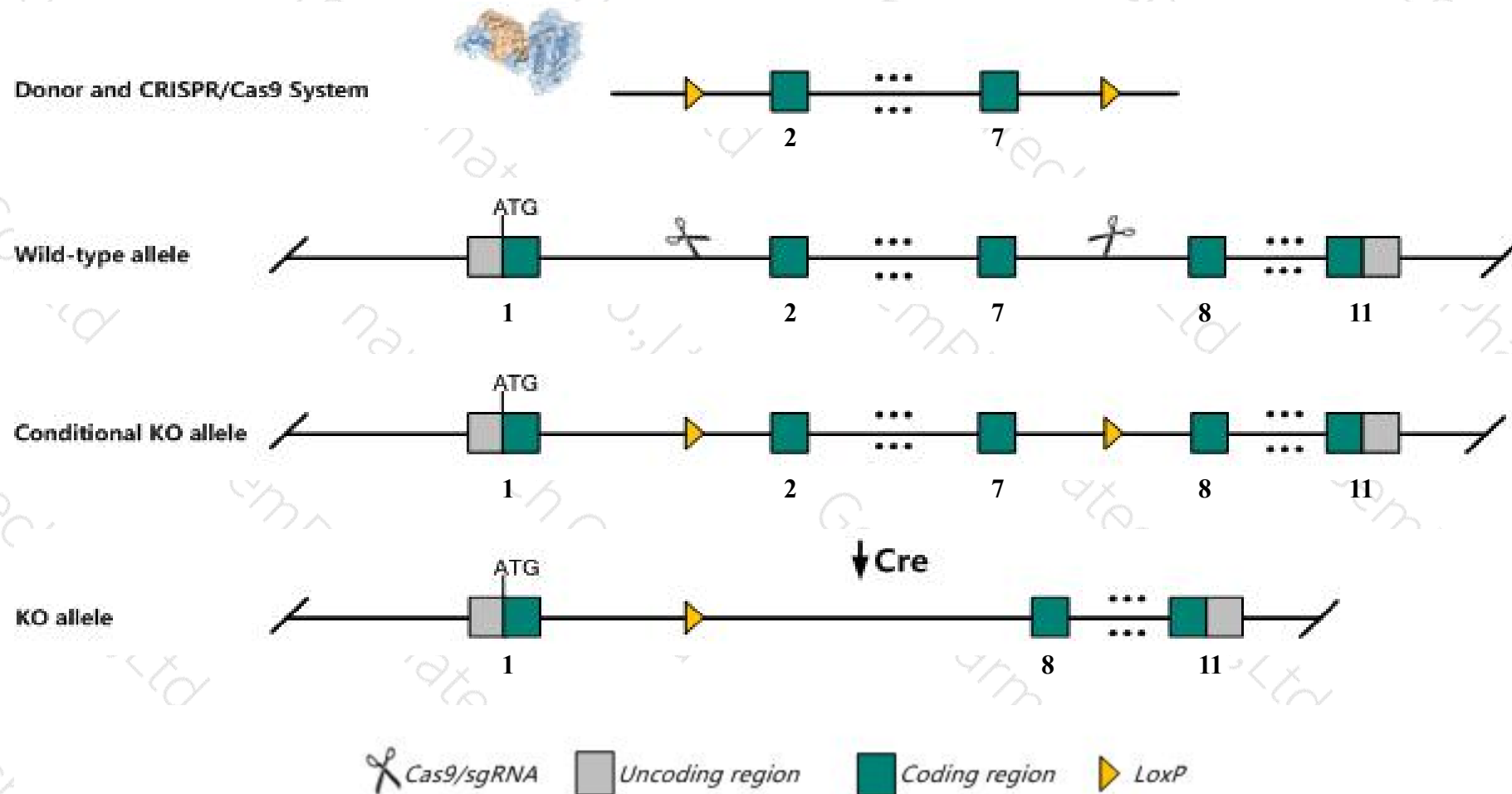
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Gramd2* gene. The schematic diagram is as follows:



- The *Gramd2* gene has 6 transcripts. According to the structure of *Gramd2* gene, exon2-exon7 of *Gramd2-201*(ENSMUST00000098661.9) transcript is recommended as the knockout region. The region contains 472bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Gramd2* 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.

Notice

- The *Gramd2* gene is located on the Chr9. 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 *Gramd2*-206 may not be affected.
- The effect on transcript *Gramd2*-204 is unknown.
- 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)

Gramd2 GRAM domain containing 2 [Mus musculus (house mouse)]

Gene ID: 546134, updated on 13-Mar-2020

Summary



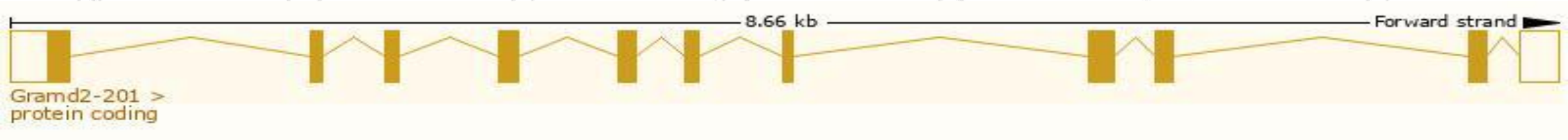
Official Symbol	Gramd2 provided by MGI
Official Full Name	GRAM domain containing 2 provided by MGI
Primary source	MGI:MGI:3528937
See related	Ensembl:ENSMUSG00000074259
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	BC064463, Gramd2a
Expression	Biased expression in lung adult (RPKM 18.5), genital fat pad adult (RPKM 5.3) and 11 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

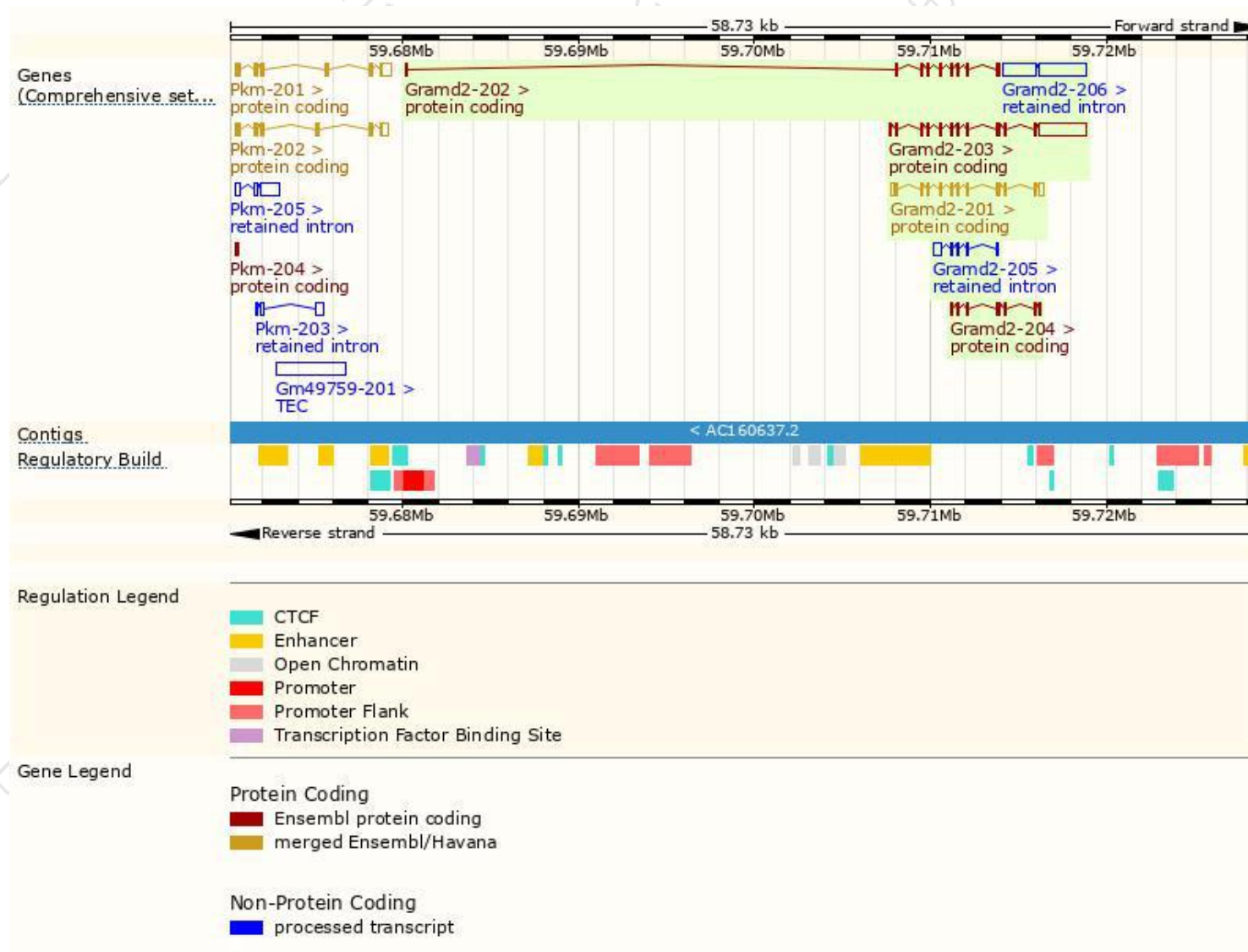
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Gramd2-201	ENSMUST00000098661.9	1368	312aa	Protein coding	CCDS52818	Q3V3G7	TSL:1 GENCODE basic APPRIS P2
Gramd2-203	ENSMUST00000128944.7	3705	320aa	Protein coding	-	Q3V3G7	TSL:1 GENCODE basic APPRIS ALT2
Gramd2-202	ENSMUST00000123914.7	805	245aa	Protein coding	-	D3YVC0	CDS 3' incomplete TSL:5
Gramd2-204	ENSMUST00000129129.1	706	200aa	Protein coding	-	F6W980	CDS 5' incomplete TSL:5
Gramd2-206	ENSMUST00000143224.1	4579	No protein	Retained intron	-	-	TSL:1
Gramd2-205	ENSMUST00000133822.1	729	No protein	Retained intron	-	-	TSL:3

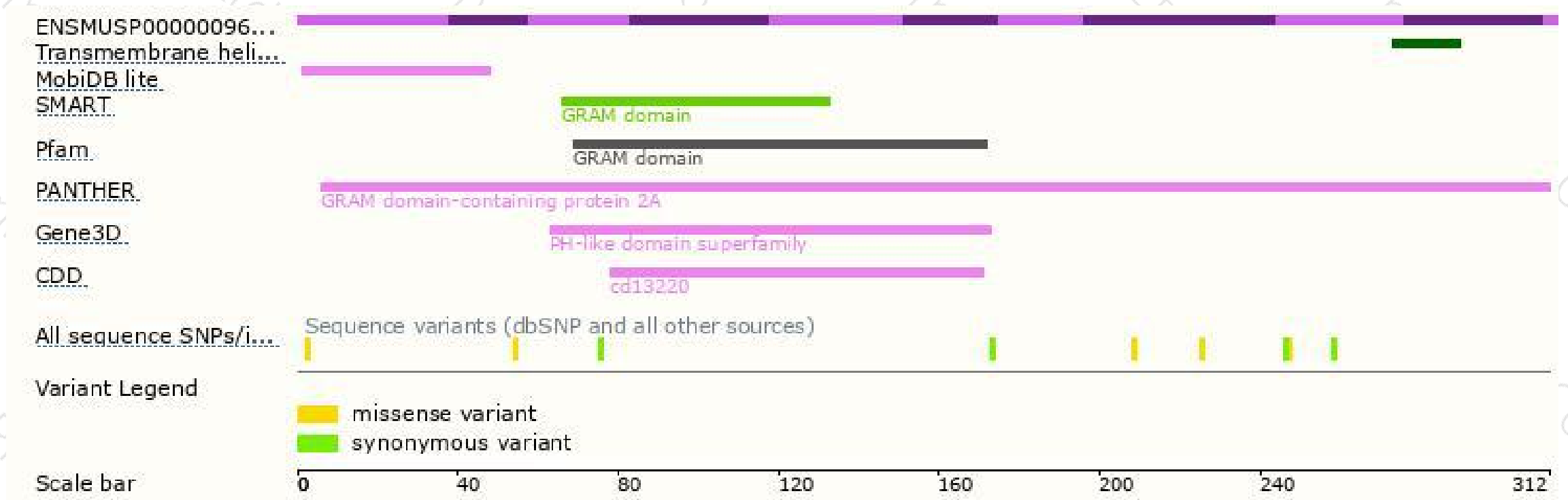
The strategy is based on the design of *Gramd2-201* transcript,the transcription is shown below:



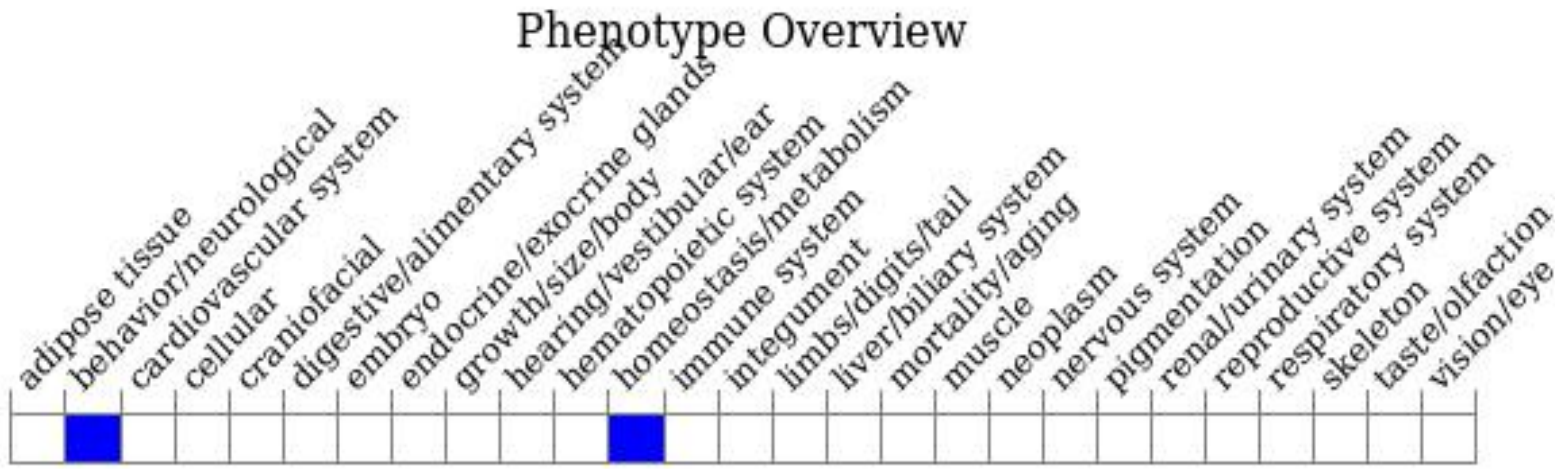
Genomic location distribution



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/>).

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

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