

Casr Cas9-CKO Strategy

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Project Overview

Project Name

Casr

Project type

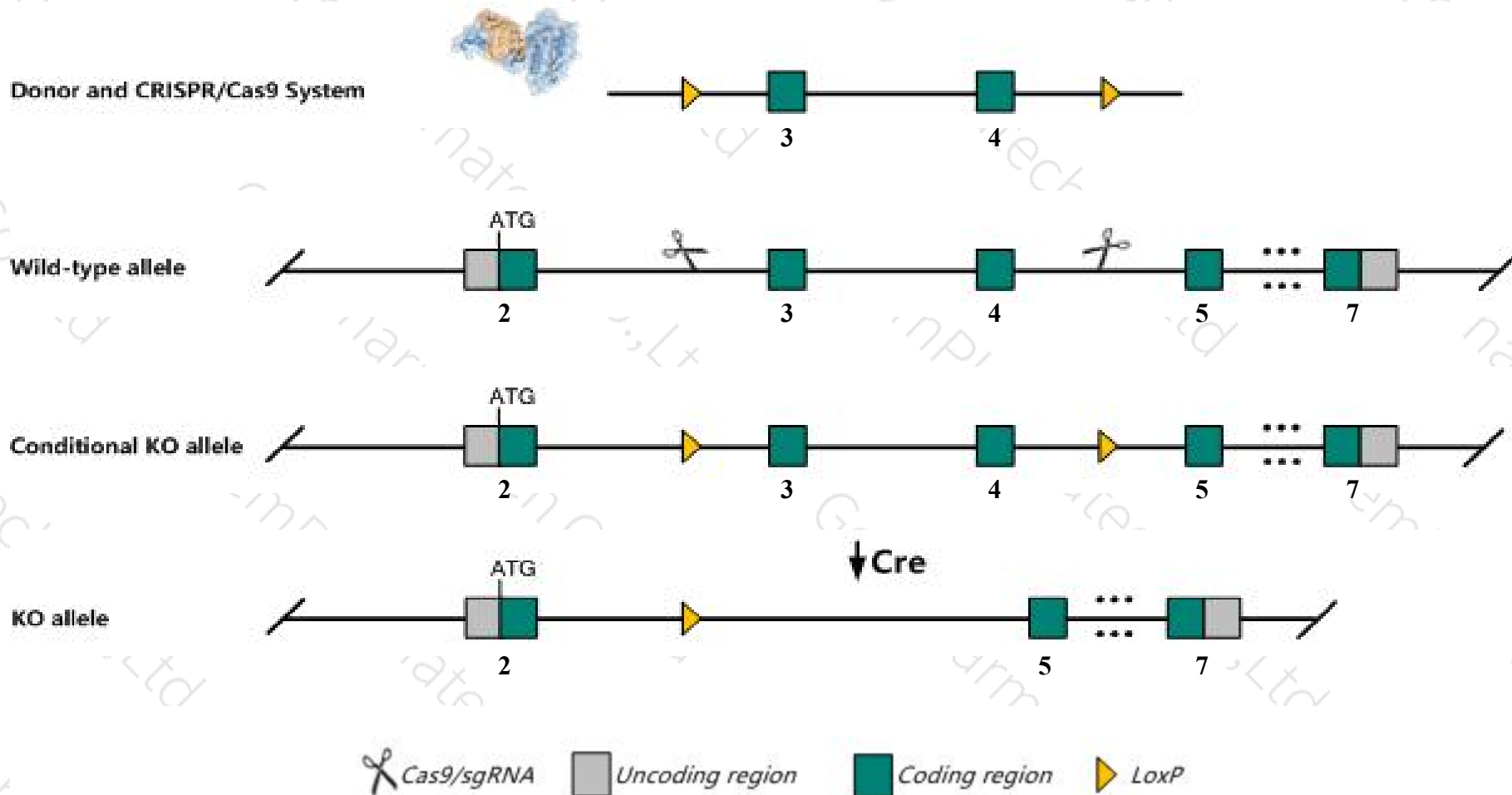
Cas9-CKO

Strain background

C57BL/6J

Conditional Knockout strategy

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



Technical routes

- The *Casr* gene has 4 transcripts. According to the structure of *Casr* gene, exon3-exon4 of *Casr-201* (ENSMUST00000063597.13) transcript is recommended as the knockout region. The region contains 1192bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Casr* 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/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 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.

- According to the existing MGI data, Homozygotes for a targeted null mutation exhibit high levels of serum calcium and parathyroid hormone, parathyroid hyperplasia, bone defects, reduced growth, and early death. Carriers have elevated serum calcium, magnesium, and parathyroid hormone levels.
- The *Casr* gene is located on the Chr16. 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)

Casr calcium-sensing receptor [Mus musculus (house mouse)]

Gene ID: 12374, updated on 31-Jan-2019

Summary



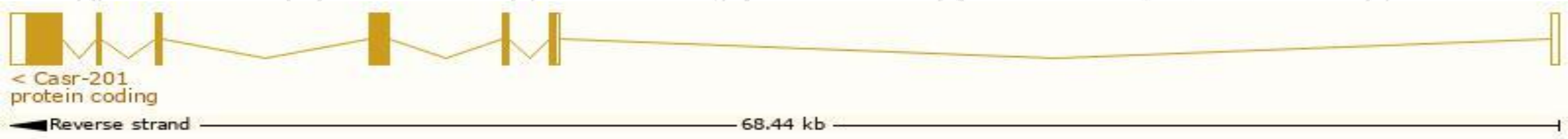
Official Symbol	Casr provided by MGI
Official Full Name	calcium-sensing receptor provided by MGI
Primary source	MGI:MGI:1351351
See related	Ensembl:ENSMUSG000000051980
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	CaR, Gprc2a
Expression	Biased expression in kidney adult (RPKM 6.6) and mammary gland adult (RPKM 0.5) See more
Orthologs	human all

Transcript information (Ensembl)

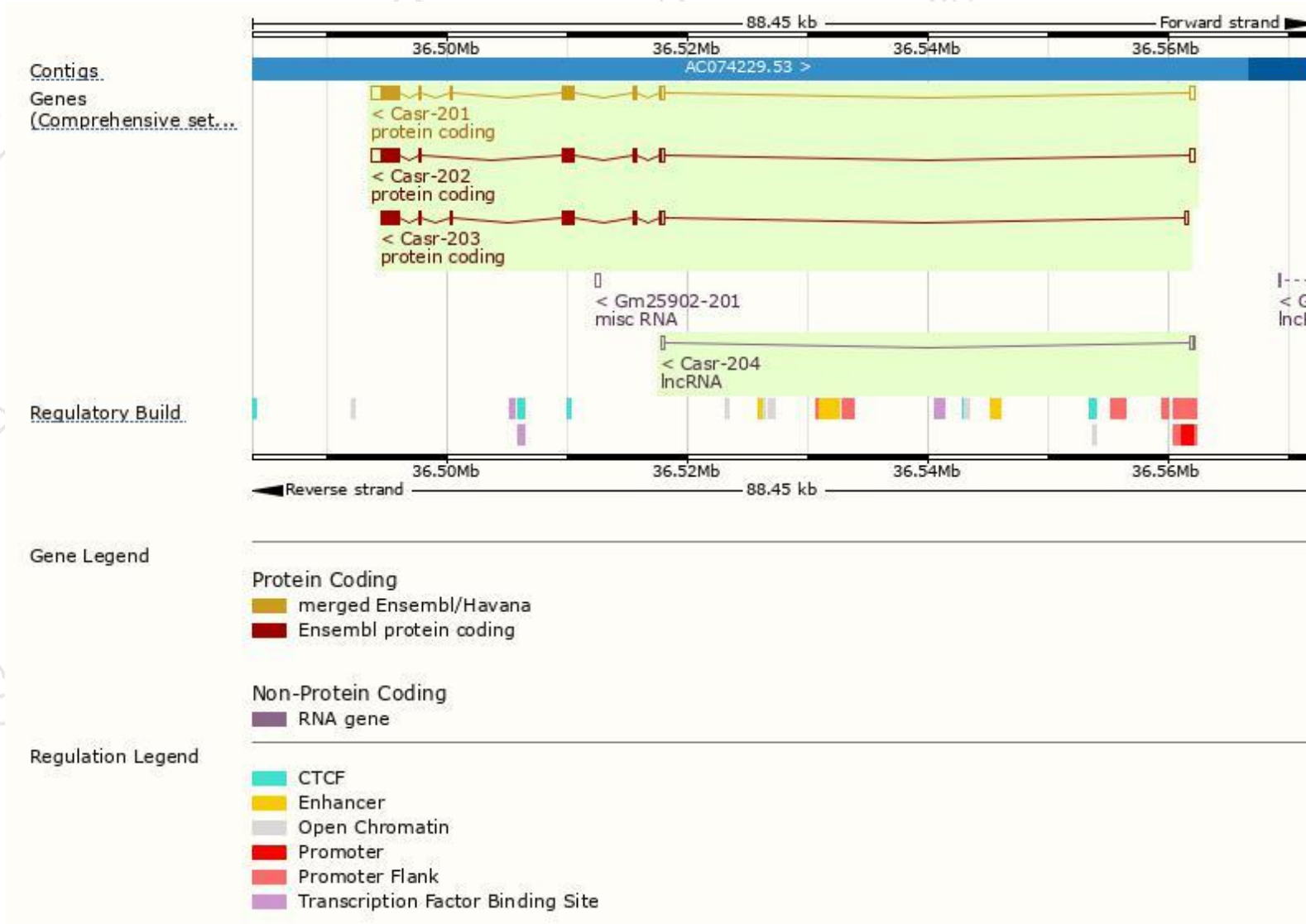
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Casr-201	ENSMUST00000063597.13	4534	1079aa	Protein coding	CCDS28154	Q9QY96	TSL:1 GENCODE basic APPRIS P1
Casr-203	ENSMUST00000172826.1	3659	1079aa	Protein coding	CCDS28154	Q9QY96	TSL:5 GENCODE basic APPRIS P1
Casr-202	ENSMUST00000114847.8	4303	1002aa	Protein coding	-	Q9QY96	TSL:1 GENCODE basic
Casr-204	ENSMUST00000174750.1	400	No protein	lncRNA	-	-	TSL:3

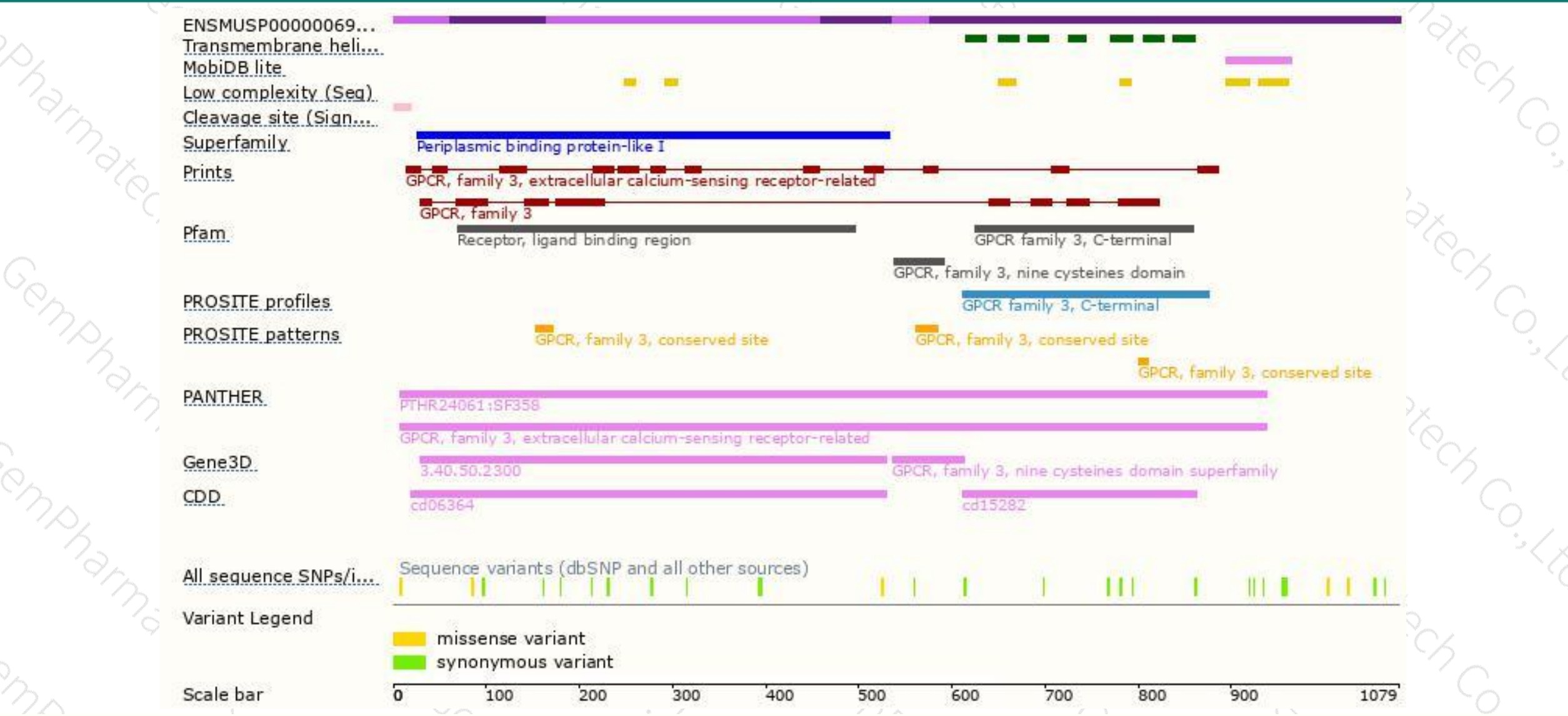
The strategy is based on the design of *Casr-201* transcript,The transcription is shown below



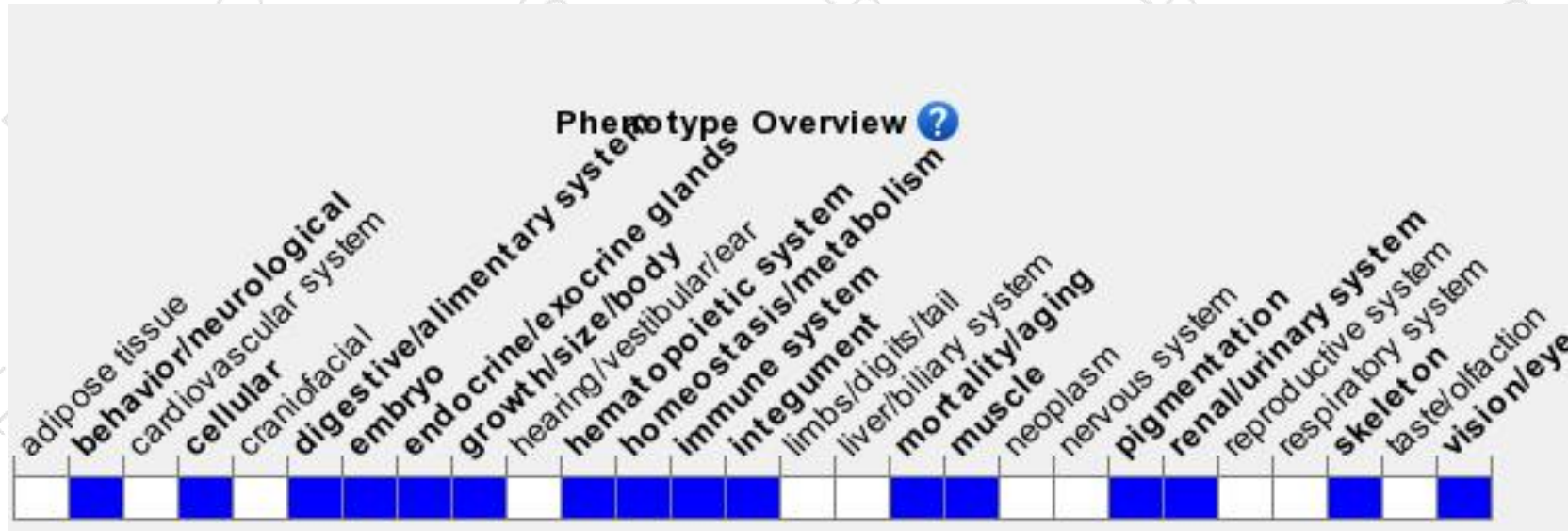
Genomic location distribution



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/>).

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

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