

***Ramp2* Cas9-CKO Strategy**

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Project Overview

Project Name

Ramp2

Project type

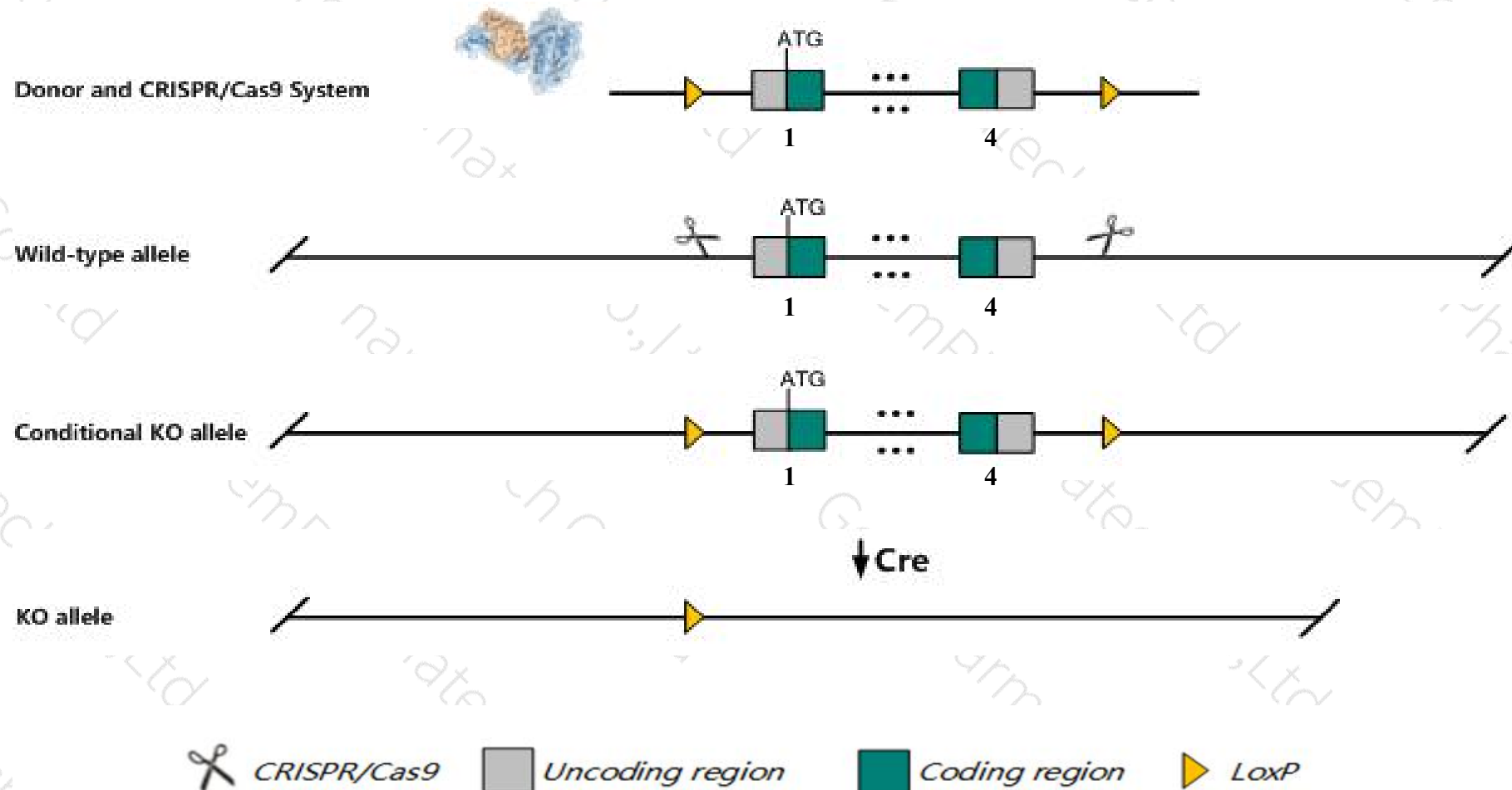
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ramp2* gene. The schematic diagram is as follows:



- The *Ramp2* gene has 8 transcripts. According to the structure of *Ramp2* gene, exon1-exon4 of *Ramp2*-204 (ENSMUST00000129680.7) transcript is recommended as the knockout region. The region contains all of the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Ramp2* 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, mice homozygous for a null allele exhibit embryonic lethality. mice heterozygous for the null allele exhibit decreased litter size beyond the loss of homozygous embryos.
- The floxed region is near to the N-terminal of *Vps25* gene, this strategy may influence the regulatory function of the N-terminal of *Vps25* gene.
- The *Ramp2* 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)

Ramp2 receptor (calcitonin) activity modifying protein 2 [Mus musculus (house mouse)]

Gene ID: 54409, updated on 13-Mar-2020

Summary

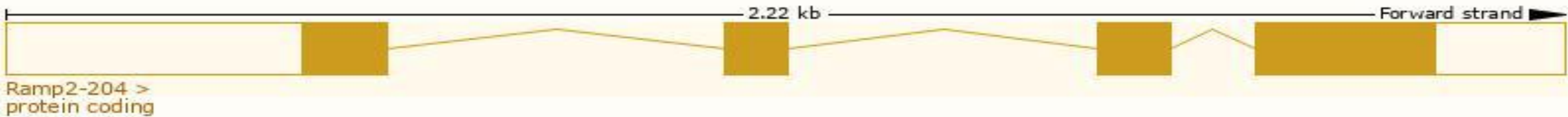
Official Symbol	Ramp2 provided by MGI
Official Full Name	receptor (calcitonin) activity modifying protein 2 provided by MGI
Primary source	MGI:MGI:1859650
See related	Ensembl:ENSMUSG00000001240
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Biased expression in lung adult (RPKM 313.3), subcutaneous fat pad adult (RPKM 94.5) and 14 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

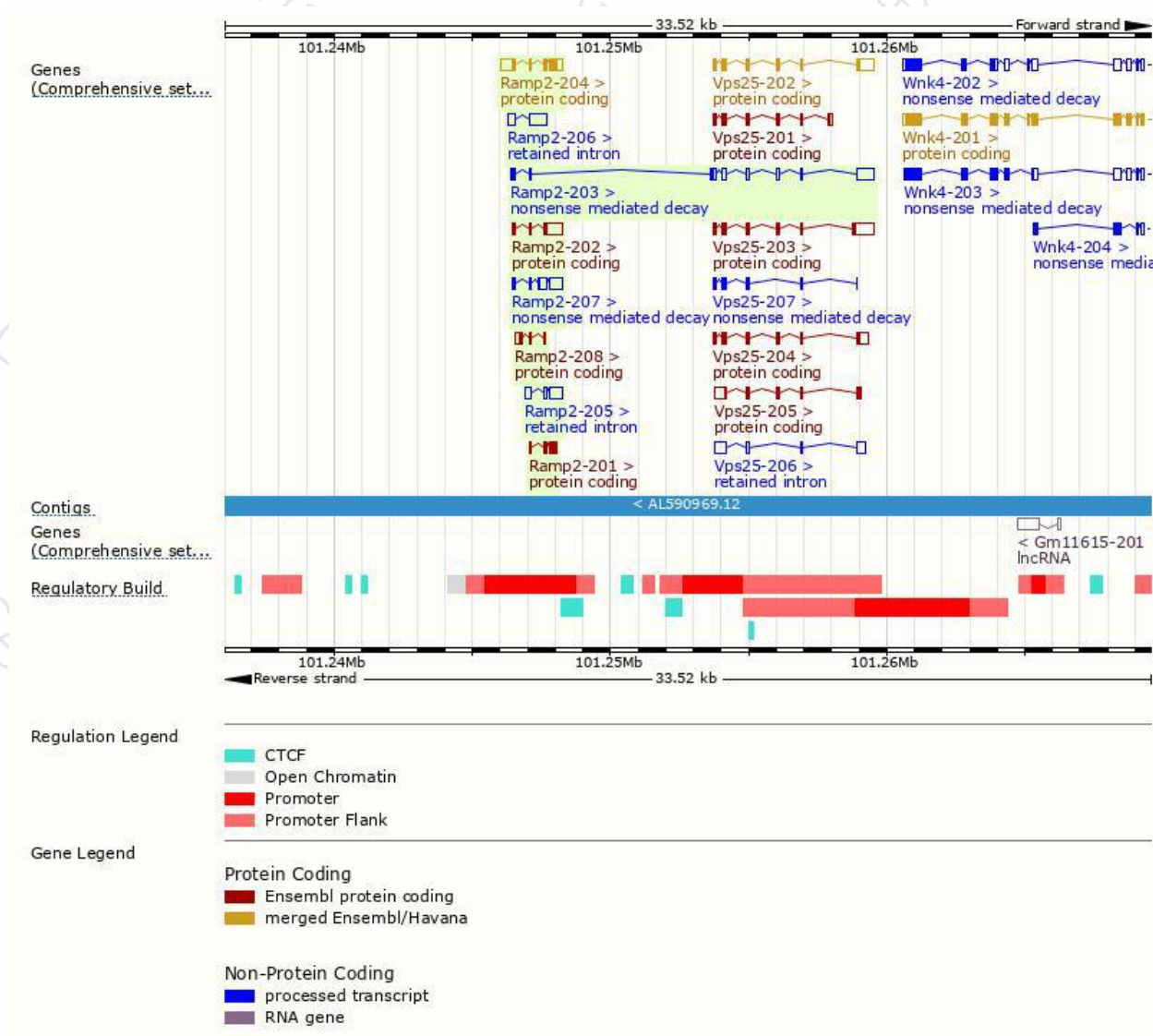
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ramp2-204	ENSMUST00000129680.7	1178	189aa	Protein coding	CCDS25457	Q9WUP0	TSL:1 GENCODE basic APPRIS P2
Ramp2-202	ENSMUST00000122006.7	899	105aa	Protein coding	-	A8XY18	TSL:2 GENCODE basic
Ramp2-201	ENSMUST00000107282.3	424	141aa	Protein coding	-	F6YPZ1	CDS 5' and 3' incomplete TSL:3 APPRIS ALT2
Ramp2-208	ENSMUST00000151830.1	379	81aa	Protein coding	-	A2A4K2	CDS 3' incomplete TSL:3
Ramp2-203	ENSMUST00000128260.8	1478	103aa	Nonsense mediated decay	-	E9Q0S5	TSL:2
Ramp2-207	ENSMUST00000149585.7	957	75aa	Nonsense mediated decay	-	D6RHO4	TSL:3
Ramp2-206	ENSMUST00000149006.1	891	No protein	Retained intron	-	-	TSL:2
Ramp2-205	ENSMUST00000138229.1	755	No protein	Retained intron	-	-	TSL:2

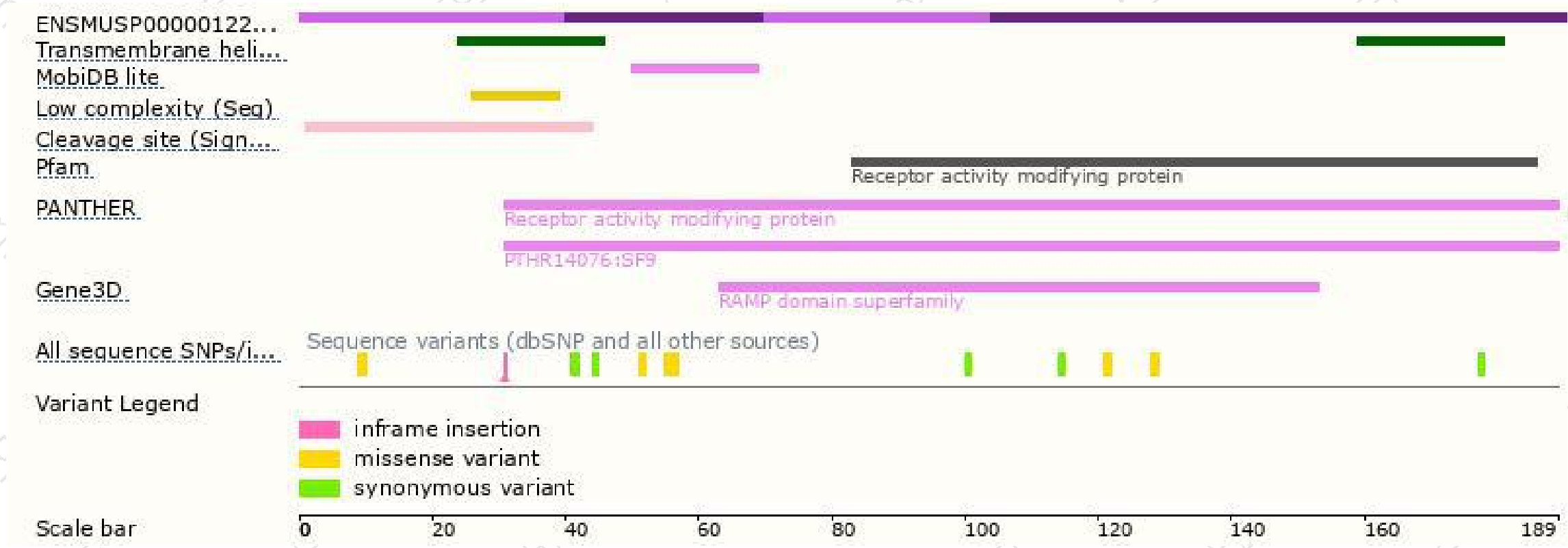
The strategy is based on the design of *Ramp2-204* transcript,the transcription is shown below:



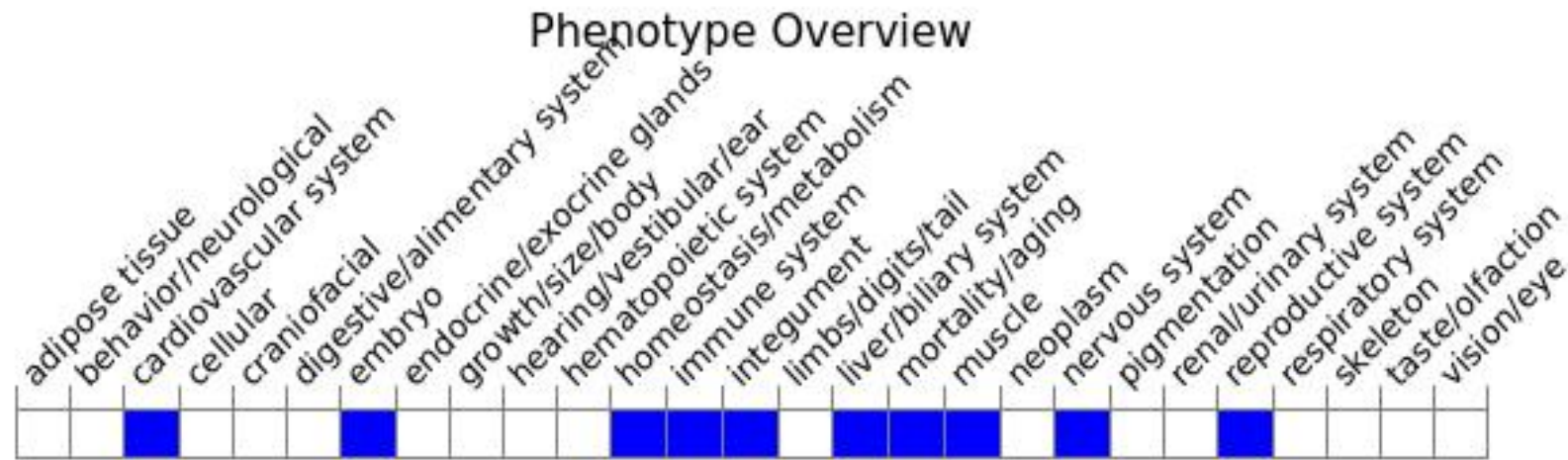
Genomic location distribution



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, mice homozygous for a null allele exhibit embryonic lethality. Mice heterozygous for the null allele exhibit decreased litter size beyond the loss of homozygous embryos.

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

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