

# Rbp2 Cas9-CKO Strategy

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Reviewer: Huimin Su

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## **Project Overview**



**Project Name** 

Rbp2

**Project type** 

Cas9-CKO

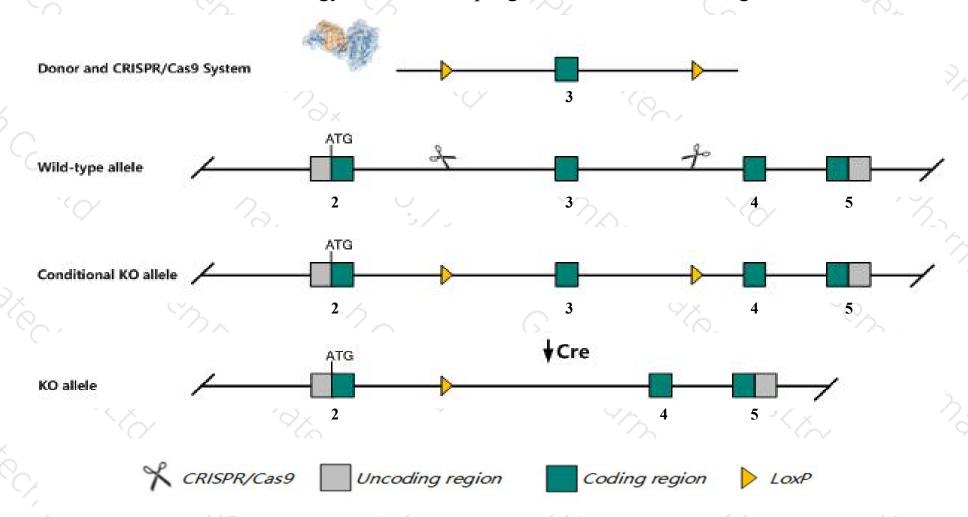
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Rbp2* gene. The schematic diagram is as follows:



### Technical routes



- ➤ The *Rbp2* gene has 4 transcripts. According to the structure of *Rbp2* gene, exon3 of *Rbp2-204*(ENSMUST00000189446.6) transcript is recommended as the knockout region. The region contains 179bp coding sequence.

  Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Rbp2* 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.

### **Notice**



- ➤ According to the existing MGI data, saturable vitamin a (retinol) uptake is impaired in homozygous mutant mice. serum retinol levels are unaffected when with normal dietary intake, however, pups of homozygous dams fed a marginal retinol diet show increased neonatal lethality due to inadequate retinal transport to the fetus.
- > The *Rbp2* 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.
- 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)



#### Rbp2 retinol binding protein 2, cellular [Mus musculus (house mouse)]

Gene ID: 19660, updated on 13-Mar-2020

#### Summary

☆ ?

Official Symbol Rbp2 provided by MGI

Official Full Name retinol binding protein 2, cellular provided by MGI

Primary source MGI:MGI:97877

See related Ensembl: ENSMUSG00000032454

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 Crbp-2, Crbpll, Rbp-2

Expression Biased expression in liver E18 (RPKM 216.6), small intestine adult (RPKM 147.9) and 3 other tissuesSee more

Orthologs <u>human all</u>

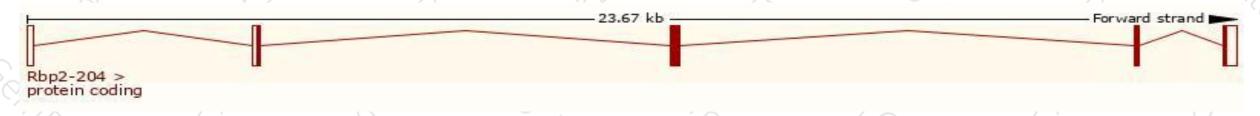
## Transcript information (Ensembl)



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

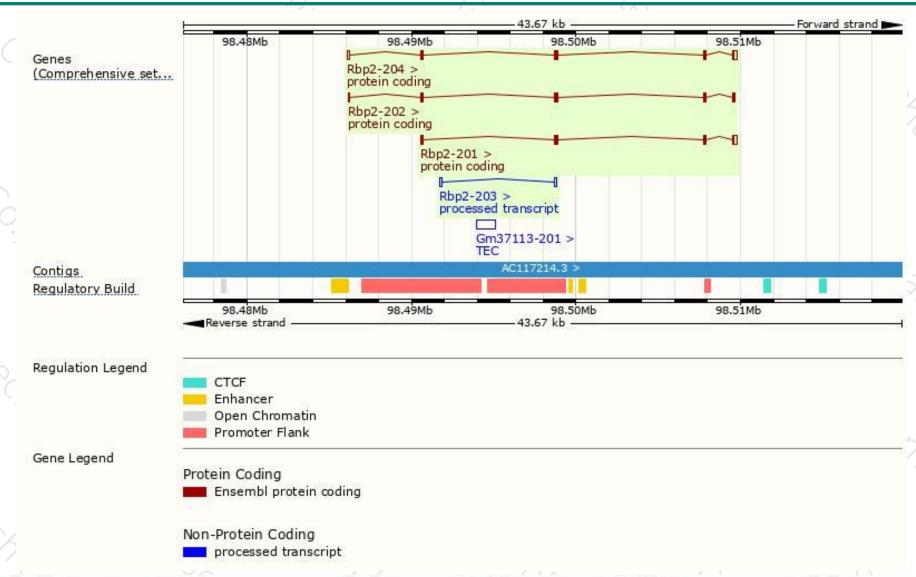
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rbp2-204	ENSMUST00000189446.6	803	<u>134aa</u>	Protein coding	CCDS23425	Q059R7 Q08652	TSL:3 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Rbp2-201	ENSMUST00000035029.2	672	<u>134aa</u>	Protein coding	CCDS23425	Q059R7 Q08652	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Rbp2-202	ENSMUST00000187905.6	611	<u>134aa</u>	Protein coding	CCDS23425	Q059R7 Q08652	TSL:3 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Rbp2-203	ENSMUST00000188779.1	291	No protein	Processed transcript	25	1/29	TSL:1

The strategy is based on the design of *Rbp2-204* transcript, the transcription is shown below:



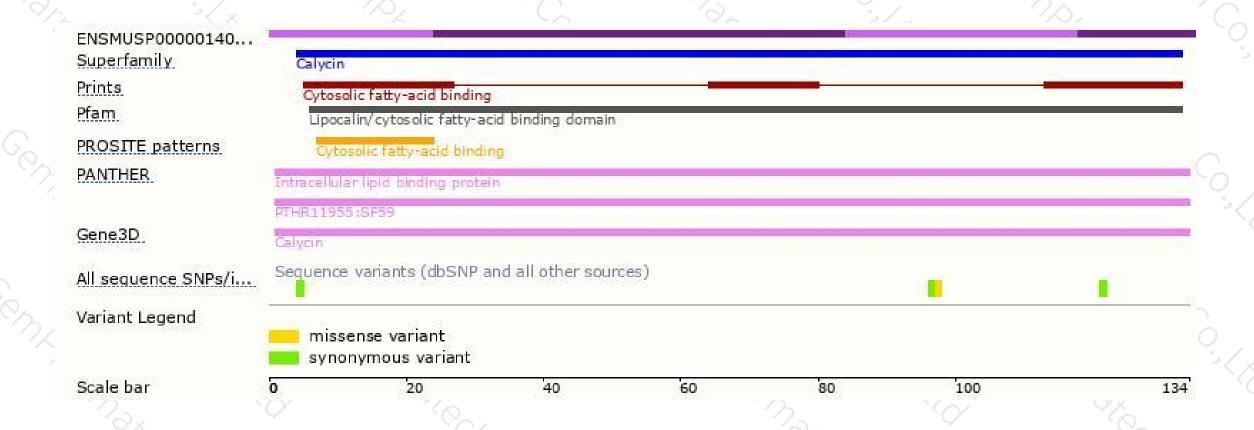
### Genomic location distribution





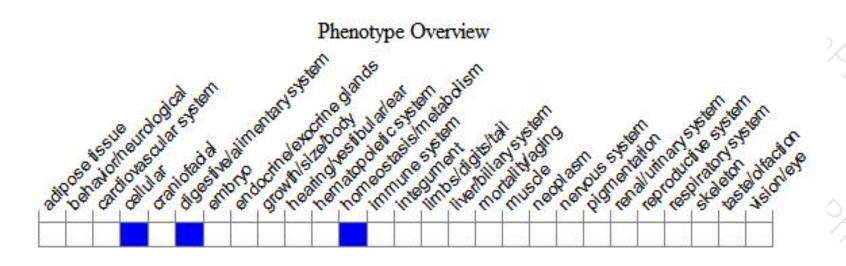
### Protein domain





## 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, saturable vitamin A (retinol) uptake is impaired in homozygous mutant mice. Serum retinol levels are unaffected when with normal dietary intake, however, pups of homozygous dams fed a marginal retinol diet show increased neonatal lethality due to inadequate retinal transport to the fetus.



If you have any questions, you are welcome to inquire. Tel: 400-9660890





