

# *Car2* Cas9-KO Strategy

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Reviewer: Yanhua Shen

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# Project Overview

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**Project Name**

*Car2*

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**Project type**

**Cas9-KO**

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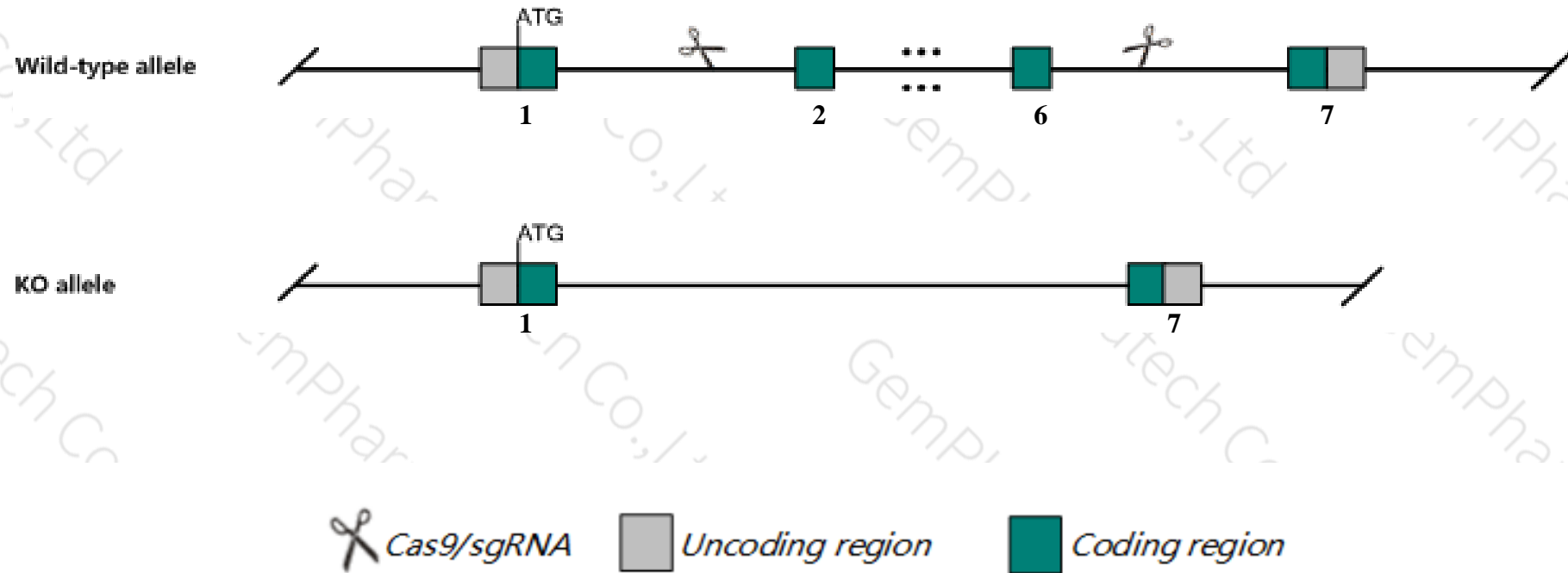
**Strain background**

**C57BL/6JGpt**

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# Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Car2* gene. The schematic diagram is as follows:



- The *Car2* gene has 3 transcripts. According to the structure of *Car2* gene, exon2-exon6 of *Car2-201* (ENSMUST00000029078.8) transcript is recommended as the knockout region. The region contains 629bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Car2* gene. The brief process is as follows: sgRNA was transcribed in vitro. Cas9 and sgRNA 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.

- According to the existing MGI data, Homozygous mutant mice are growth retarded, display renal tubular acidosis, but mutants have not been recovered that display osteopetrosis as found in human CA-II deficiency.
- The *Car2* gene is located on the Chr3. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.



# Gene information (NCBI)

## Car2 carbonic anhydrase 2 [ *Mus musculus* (house mouse) ]

Gene ID: 12349, updated on 24-Oct-2019

### Summary

- Official Symbol** Car2 provided by MGI
- Official Full Name** carbonic anhydrase 2 provided by MGI
- Primary source** [MGI:MGI:88269](#)
- See related** [Ensembl:ENSMUSG00000027562](#)
- 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** Ca2; CAII; Car-2; Ltw-5; Lvtw-5; AI131712
- Expression** Biased expression in liver E14 (RPKM 556.3), liver E14.5 (RPKM 538.2) and 14 other tissues [See more](#)
- Orthologs** [human](#) [all](#)

### Genomic context

**Location:** 3 A1; 3 3.23 cM See Car2 in [Genome Data Viewer](#)

**Exon count:** 7

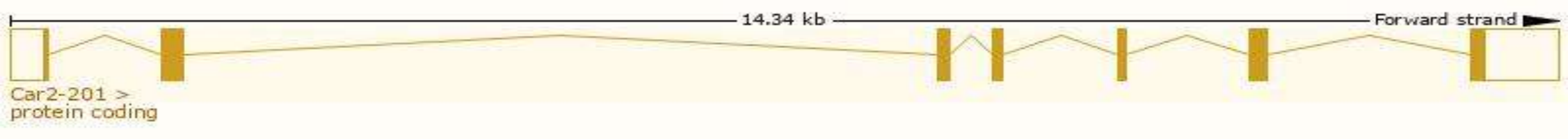
Annotation release	Status	Assembly	Chr	Location
<a href="#">108</a>	current	GRCm38.p6 ( <a href="#">GCF_000001635.26</a> )	3	NC_000069.6 (14886269..14900770)
Build 37.2	previous assembly	MGSCv37 ( <a href="#">GCF_000001635.18</a> )	3	NC_000069.5 (14886426..14900770)

# Transcript information (Ensembl)

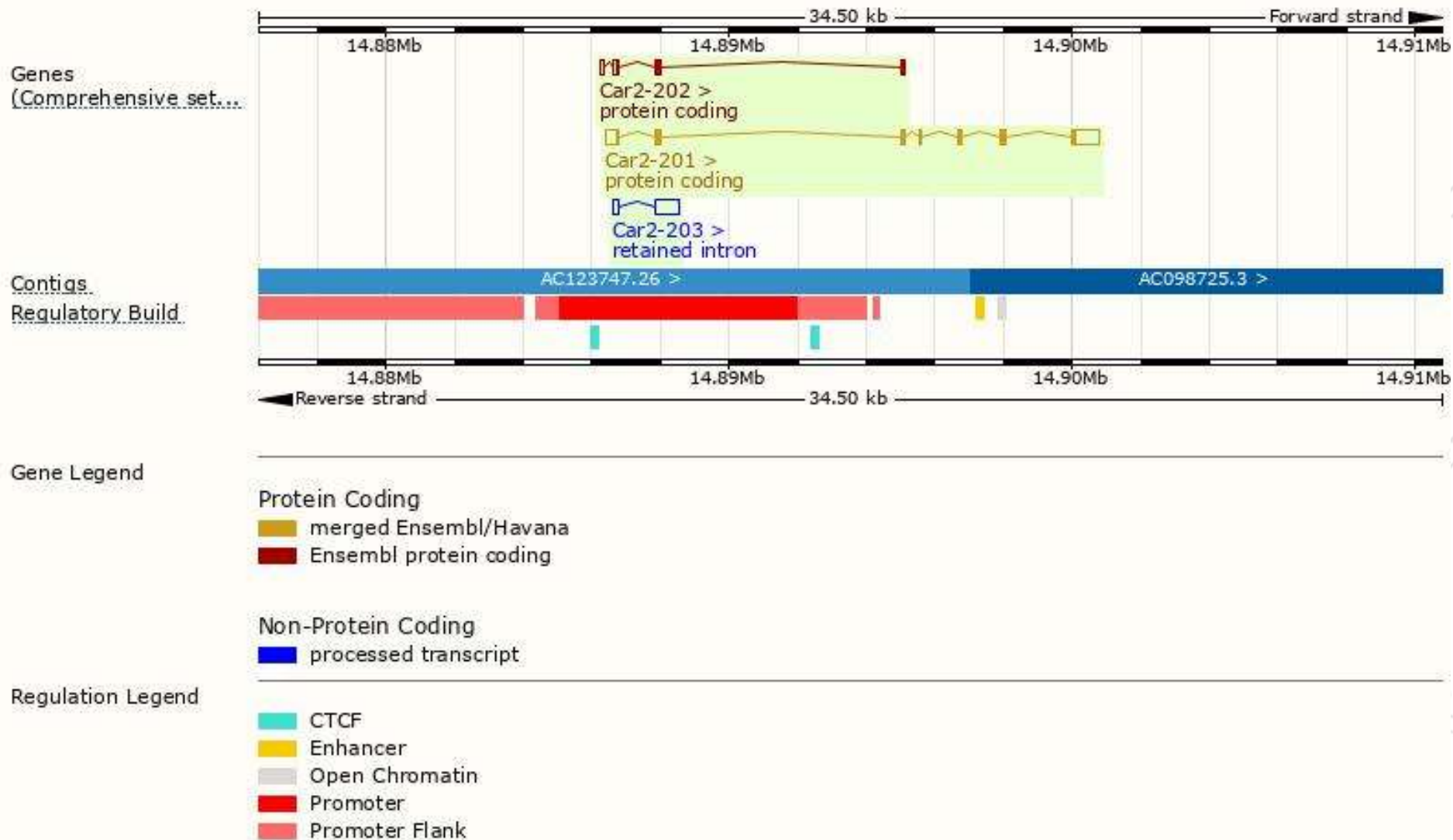
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Car2-201	<a href="#">ENSMUST00000029078.8</a>	1788	<a href="#">260aa</a>	Protein coding	<a href="#">CCDS17251</a>	<a href="#">P00920</a>	TSL:1 GENCODE basic APPRIS P1
Car2-202	<a href="#">ENSMUST00000192609.5</a>	587	<a href="#">115aa</a>	Protein coding	-	<a href="#">A0A0A6YX78</a>	CDS 3' incomplete TSL:3
Car2-203	<a href="#">ENSMUST00000195520.1</a>	881	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Car2-201* transcript,The transcription is shown below



# Genomic location distribution





# Protein domain

ENSMUSP00000029...

Superfamily

Alpha carbonic anhydrase domain superfamily

SMART

Alpha carbonic anhydrase domain

Pfam

Alpha carbonic anhydrase domain

PROSITE profiles

Alpha carbonic anhydrase domain

PROSITE patterns

Carbonic anhydrase, alpha-class, conserved site

PANTHER

PTHR18552:SF120

Carbonic anhydrase, alpha-class



Gene3D

Alpha carbonic anhydrase domain superfamily

All sequence SNPs/i....

Sequence variants (dbSNP and all other sources)

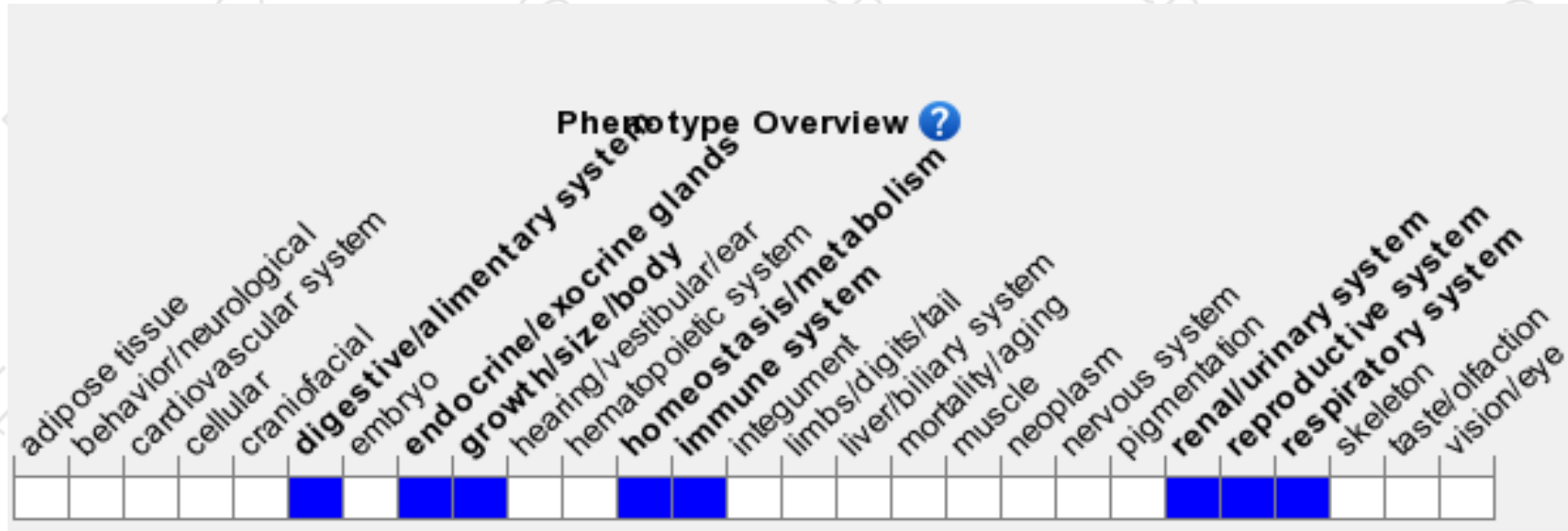
Variant Legend

 missense variant  
 synonymous variant

Scale bar

0 40 80 120 160 200 260

# 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, Homozygous mutant mice are growth retarded, display renal tubular acidosis, but mutants have not been recovered that display osteopetrosis as found in human CA-II deficiency.

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

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