

# ***Car5b Cas9-CKO Strategy***

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# Project Overview

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**Project Name**

*Car5b*

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**Project type**

Cas9-CKO

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**Animal background**

C57BL/6JGpt

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# Conditional Knockout strategy

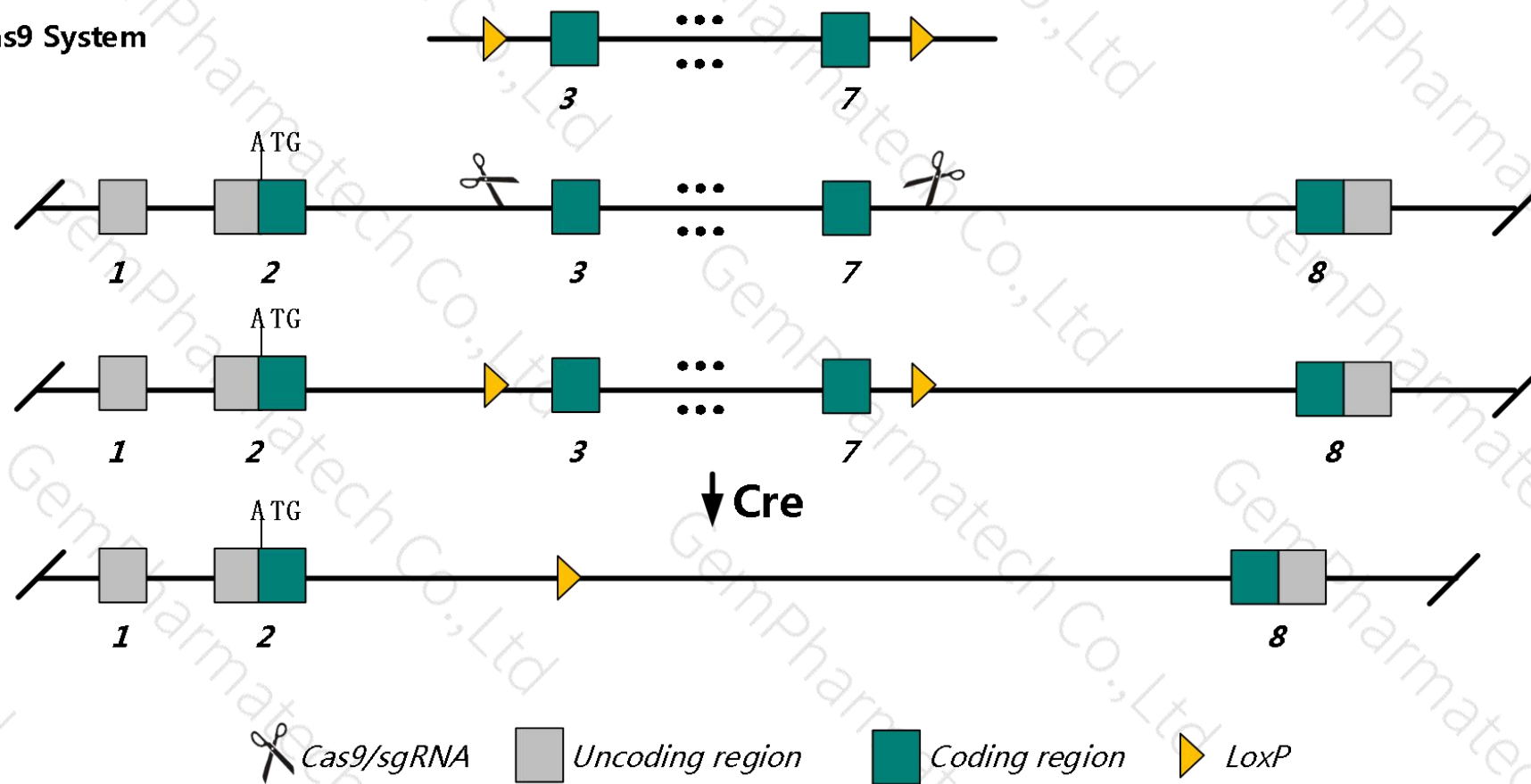
This model will use CRISPR/Cas9 technology to edit the Car5b gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System

Wild-type allele

Conditional KO allele

KO allele



- The *Car5b* gene has 2 transcripts, According to the structure of *Car5b* gene, exon3-7 of *Car5b* -201 transcript is recommended as the knockout region. The region contains the 632bp coding sequence. Knock out the region, result in destruction of protein.
- This project uses CRISPR/Cas9 technology to modify *Car5b* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed, Cas9, gRNA and donor were microinjected into fertilized eggs of C57BL/6JGpt mice and homologous recombination was carried out to obtain F0 mice. A stable and hereditary F1 generation mouse model was obtained by mating F0 generation mice with C57BL/6JGpt mice which were confirmed positive by PCR-sequencing.
- 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 that are either homozygous or hemizygous for a knock-out allele exhibit normal survival and show no detectable differences in blood ammonia or fasting glucose levels relative to control littermates.
- The *Car5b* gene is located in the ChrX. If the knockout mice are mixed with other mice, two target genes are avoided on the same chromosome as possible, otherwise the offspring of mice with double gene positive and homozygous gene knockout can not be obtained.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of gene transcription and translation processes, all risks cannot be predicted under existing information.



# Gene information ( NCBI )

## Car5b carbonic anhydrase 5b, mitochondrial [ *Mus musculus* (house mouse) ]

Gene ID: 56078, updated on 31-Jan-2019

### Summary

Official Symbol	Car5b provided by <a href="#">MGI</a>
Official Full Name	carbonic anhydrase 5b, mitochondrial provided by <a href="#">MGI</a>
Primary source	<a href="#">MGI:MGI:1926249</a>
See related	<a href="#">Ensembl:ENSMUSG00000031373</a>
Gene type	protein coding
RefSeq status	VALIDATED
Organism	<a href="#">Mus musculus</a>
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	CAVB; Ca5b; CarVb; 7330410H16Rik; D730005F19Rik
Expression	Biased expression in subcutaneous fat pad adult (RPKM 31.5), genital fat pad adult (RPKM 23.6) and 7 other tissues <a href="#">See more</a>
Orthologs	<a href="#">human</a> <a href="#">all</a>

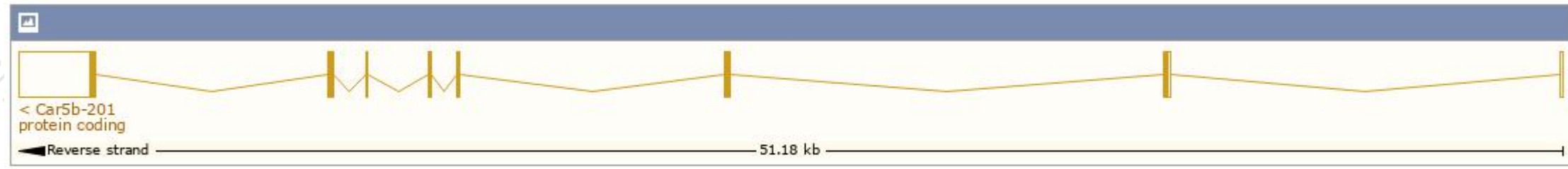
# Transcript information (Ensembl)



The gene has 2 transcripts, and all transcripts are shown below :

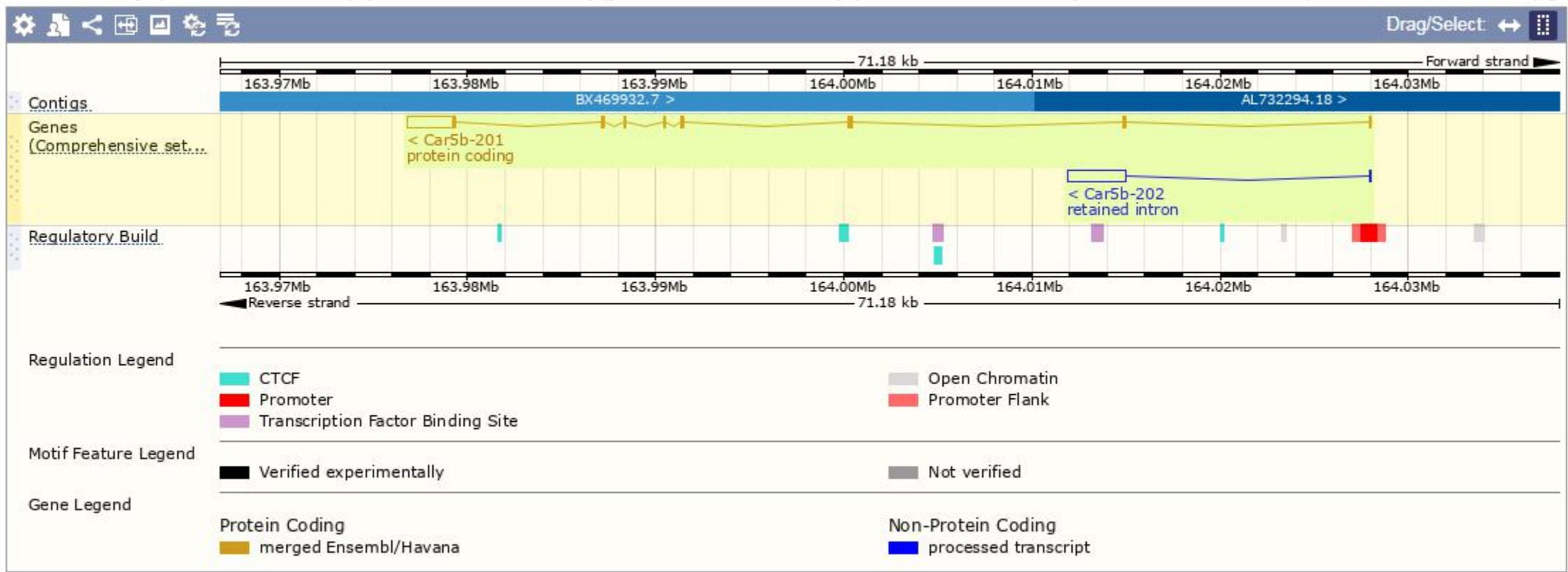
Show/hide columns (1 hidden) <span>Filter</span>								
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags
Car5b-201	<a href="#">ENSMUST00000033739.4</a>	3436	<a href="#">317aa</a>	Protein coding	<a href="#">CCDS30515</a>	<a href="#">Q9QZA0</a>	<a href="#">NM_181315</a> <a href="#">NP_851832</a>	TSL:1 Gencode basic APPRIS P1
Car5b-202	<a href="#">ENSMUST00000126650.1</a>	3157	No protein	Retained intron	-	-	-	TSL:2

The strategy is based on the design of *Car5b* -201 transcript,The transcription is shown below



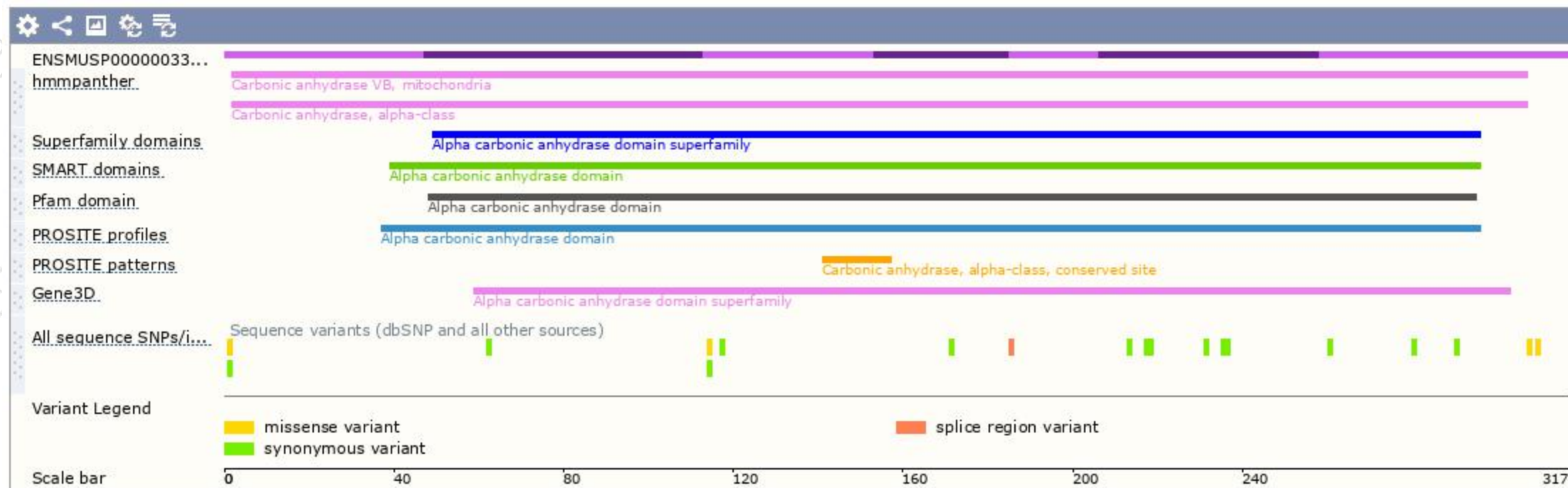
Statistics Exons: 8, Coding exons: 7, Transcript length: 3,436 bps, Translation length: 317 residues

# Genomic location distribution





# Protein domain



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

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