

Car9 Cas9-CKO Strategy

Designer:

Daohua Xu

Reviewer :

Huimin Su

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Project Overview



Project Name

Car9

Project type

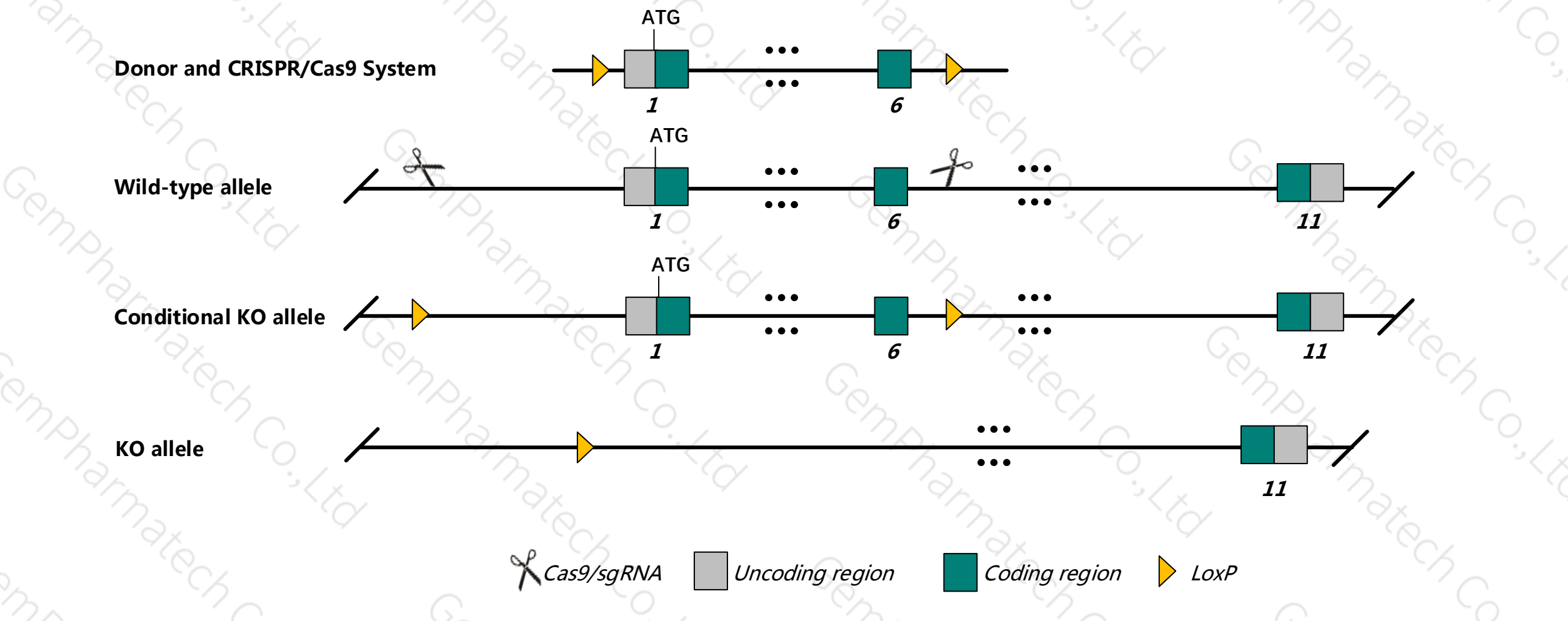
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Car9* gene. The schematic diagram is as follows:



- The *Car9* gene has 7 transcripts. According to the structure of *Car9* gene, exon1-exon6 of *Car9*-201 (ENSMUST00000030183.9) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Car9* 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/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 was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues or cell types.

- According to the existing MGI data , Mice homozygous for a targeted mutation are viable and fertile but develop hyperplasia of the glandular gastric epithelium with numerous cysts. Mice homozygous for a different mutation show an increased mean percentage of mature B cells in bone marrow.
- The KO region contains the *Gm12454* gene. Knockout the region will affect the function of *Gm12454* gene.
- The *Car9* gene is located on the Chr4. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)

Car9 carbonic anhydrase 9 [*Mus musculus* (house mouse)]

Gene ID: 230099, updated on 17-Dec-2019

Summary

Official Symbol	Car9 provided by MGI
Official Full Name	carbonic anhydrase 9 provided by MGI
Primary source	MGI:MGI:2447188
See related	Ensembl:ENSMUSG00000028463
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	Ca9; CAIX; MN/CA9
Expression	Biased expression in stomach adult (RPKM 45.1), duodenum adult (RPKM 29.1) and 6 other tissues See more
Orthologs	human all

Genomic context

Location: 4; 4 A5
Exon count: 12

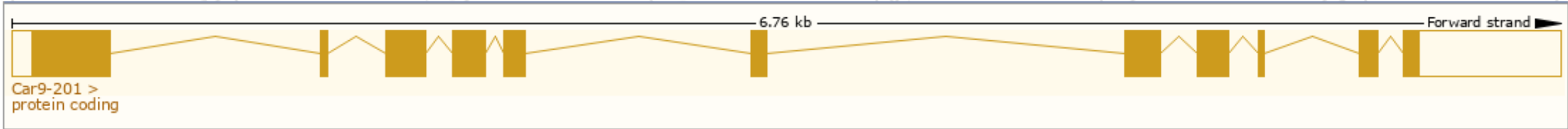
See Car9 in [Genome Data Viewer](#)

Transcript information (Ensembl)

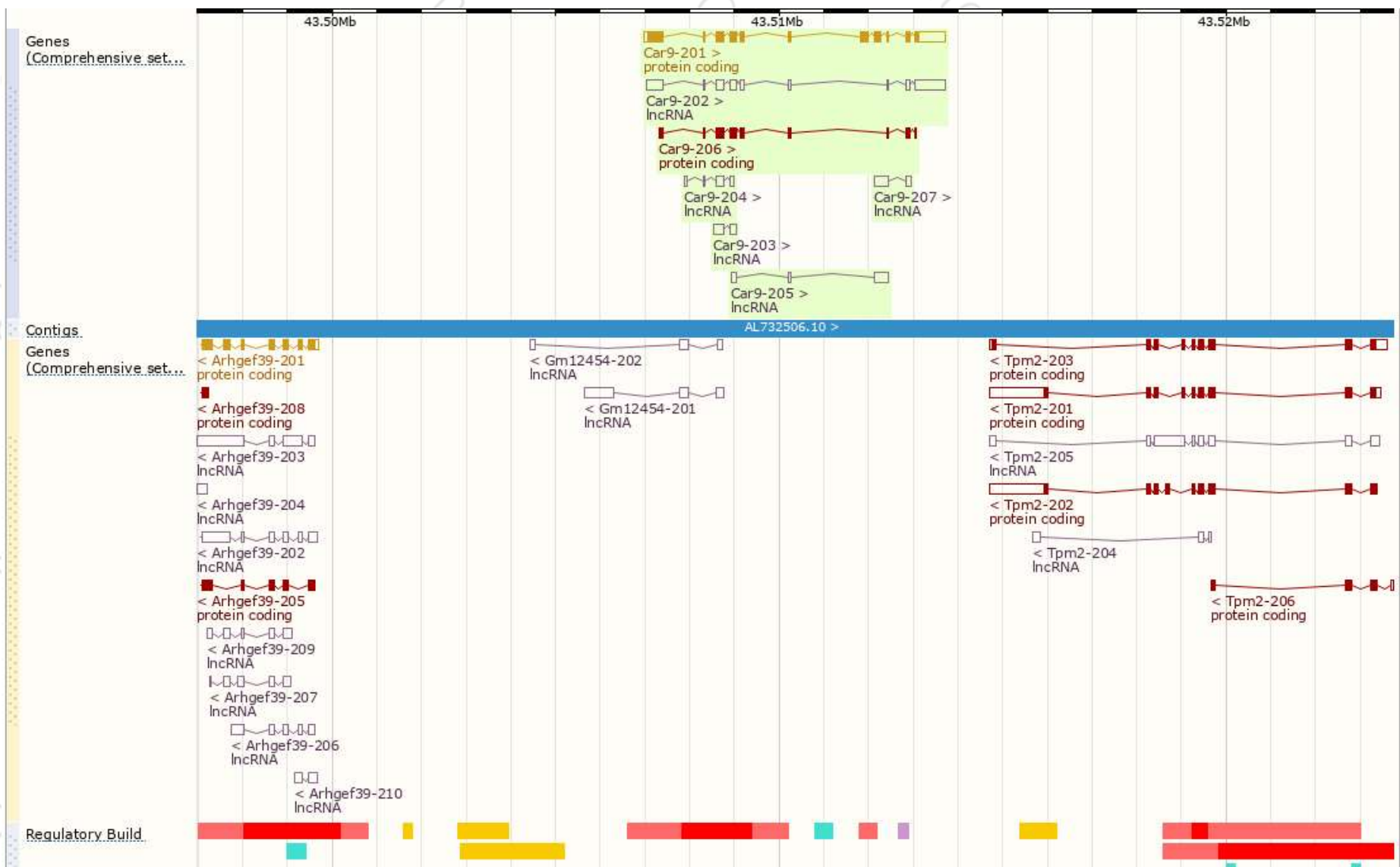
The gene has 7 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Car9-201	ENSMUST00000030183.9	2023	437aa	<div><div></div>Protein coding</div>	CCDS18099	Q3UUZ9 Q8VHB5	TSL:1 GENCODE basic APPRIS P1
Car9-206	ENSMUST00000138073.1	712	237aa	<div><div></div>Protein coding</div>	-	F6XXU0	CDS 5' and 3' incomplete TSL:1
Car9-202	ENSMUST00000124114.7	1667	No protein	<div><div></div>lncRNA</div>	-	-	TSL:5
Car9-205	ENSMUST00000129996.1	480	No protein	<div><div></div>lncRNA</div>	-	-	TSL:5
Car9-207	ENSMUST00000154251.1	398	No protein	<div><div></div>lncRNA</div>	-	-	TSL:5
Car9-203	ENSMUST00000126750.1	374	No protein	<div><div></div>lncRNA</div>	-	-	TSL:3
Car9-204	ENSMUST00000128232.1	334	No protein	<div><div></div>lncRNA</div>	-	-	TSL:3

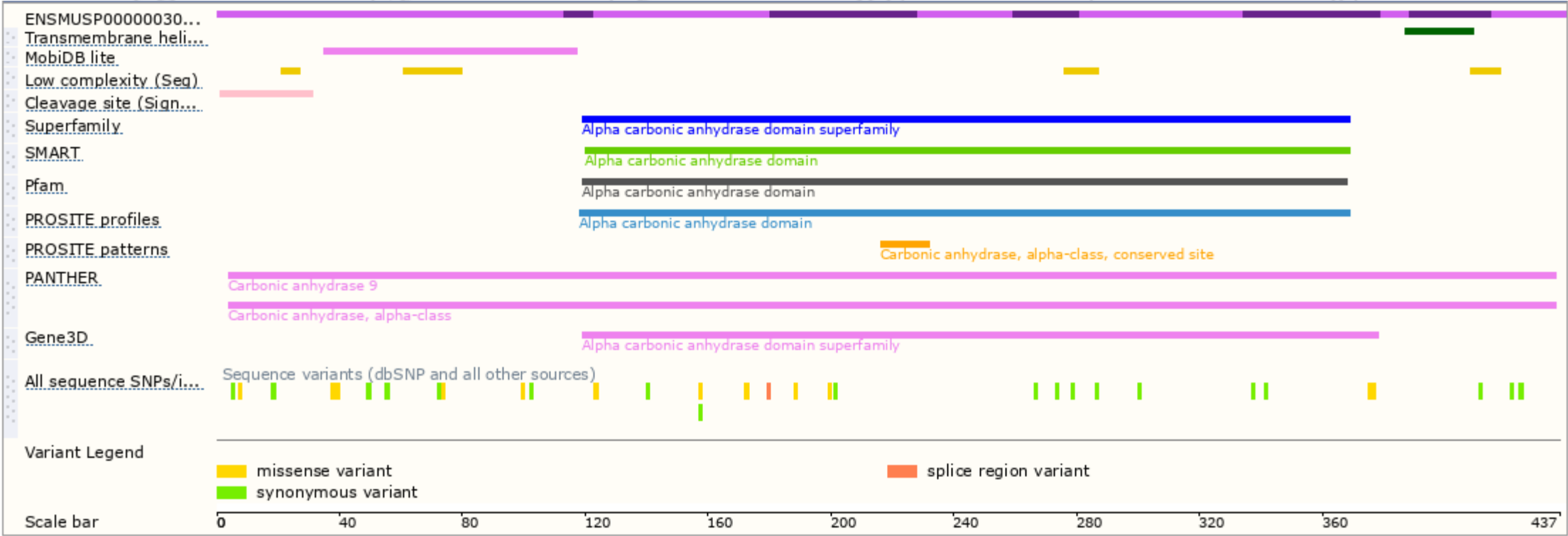
The strategy is based on the design of *Car9-201* transcript, The transcription is shown below



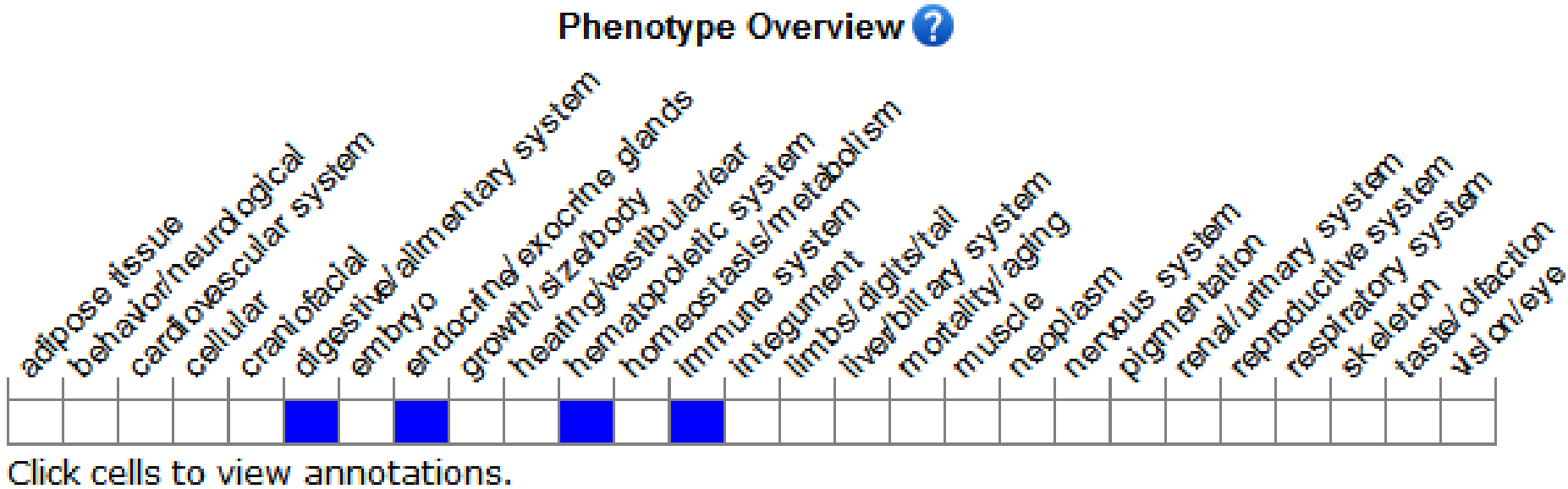
Genomic location distribution



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/>).

According to the existing MGI data, Mice homozygous for a targeted mutation are viable and fertile but develop hyperplasia of the glandular gastric epithelium with numerous cysts. Mice homozygous for a different mutation show an increased mean percentage of mature B cells in bone marrow.

If you have any questions, you are welcome to inquire.
Tel: 025-5864 1534



集萃药康生物科技
GemPharmatech Co.,Ltd

