Cars Cas9-CKO Strategy

Designer: Daohua Xu
Reviewer: Huimin Su

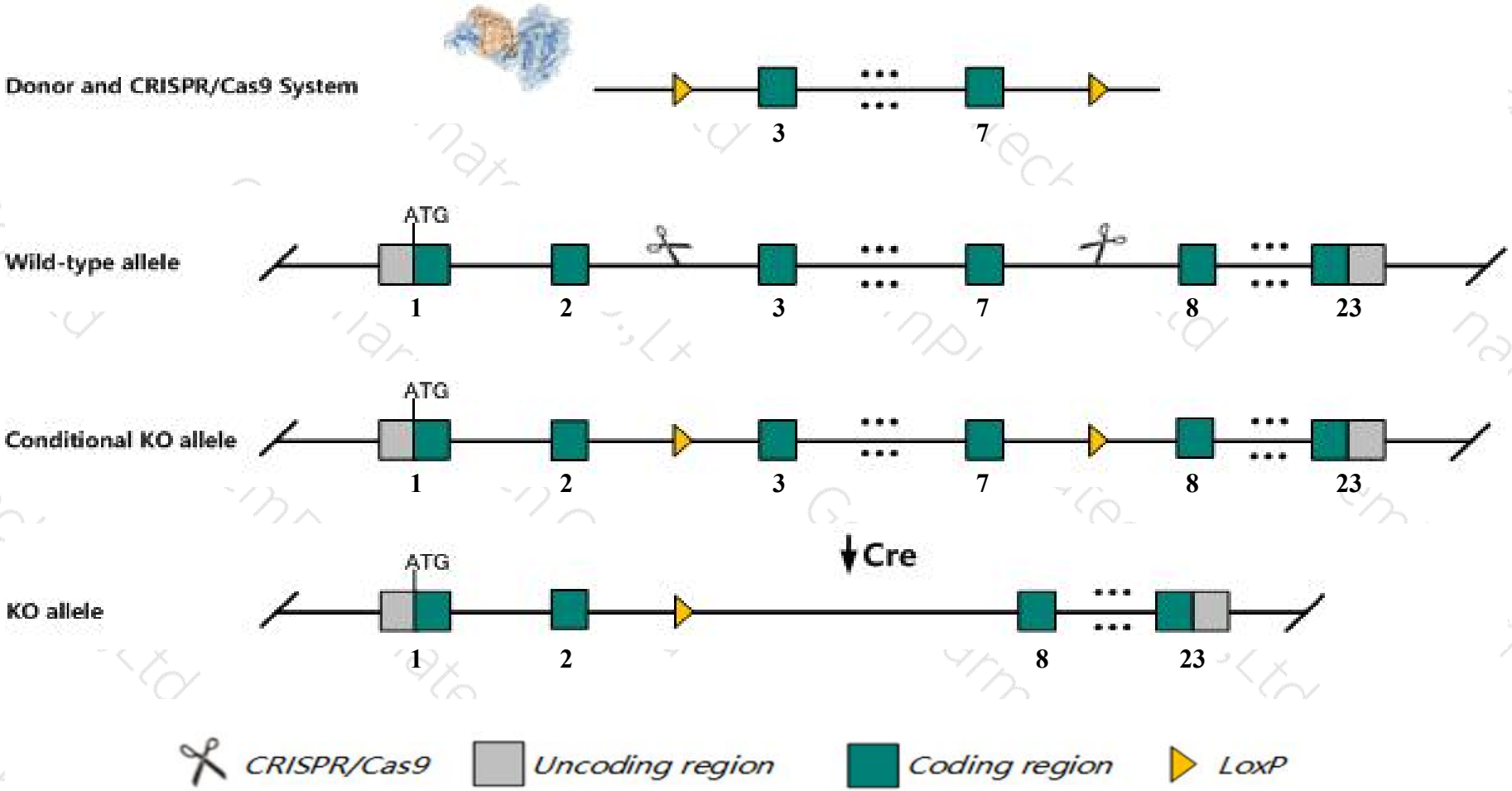
Design Date: 2019-11-25

Project Overview

Project Name	***Cars***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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



Technical routes

- The *Cars* gene has 11 transcripts. According to the structure of *Cars* gene, exon3-exon7 of *Cars-201* (ENSMUST00000010899.13) transcript is recommended as the knockout region.The region contains 527bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Cars* 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

➢ The *Cars* gene is located on the Chr7. 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）

集萃药康 GemPharmatech

Cars cysteinyl-tRNA synthetase [Mus musculus (house mouse)]

Gene ID: 27267, updated on 31-Jan-2019

Summary

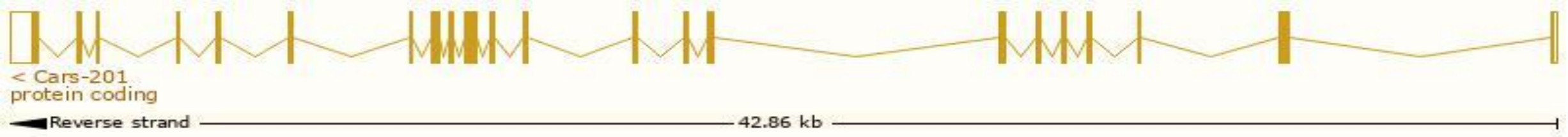
Official Symbol	Cars provided by MGI
Official Full Name	cysteinyl-tRNA synthetase provided by MGI
Primary source	MGI:MGI:1351477
See related	Ensembl:ENSMUSG00000010755
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	CA3
Expression	Ubiquitous expression in CNS E11.5 (RPKM 11.8), liver E14 (RPKM 11.5) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

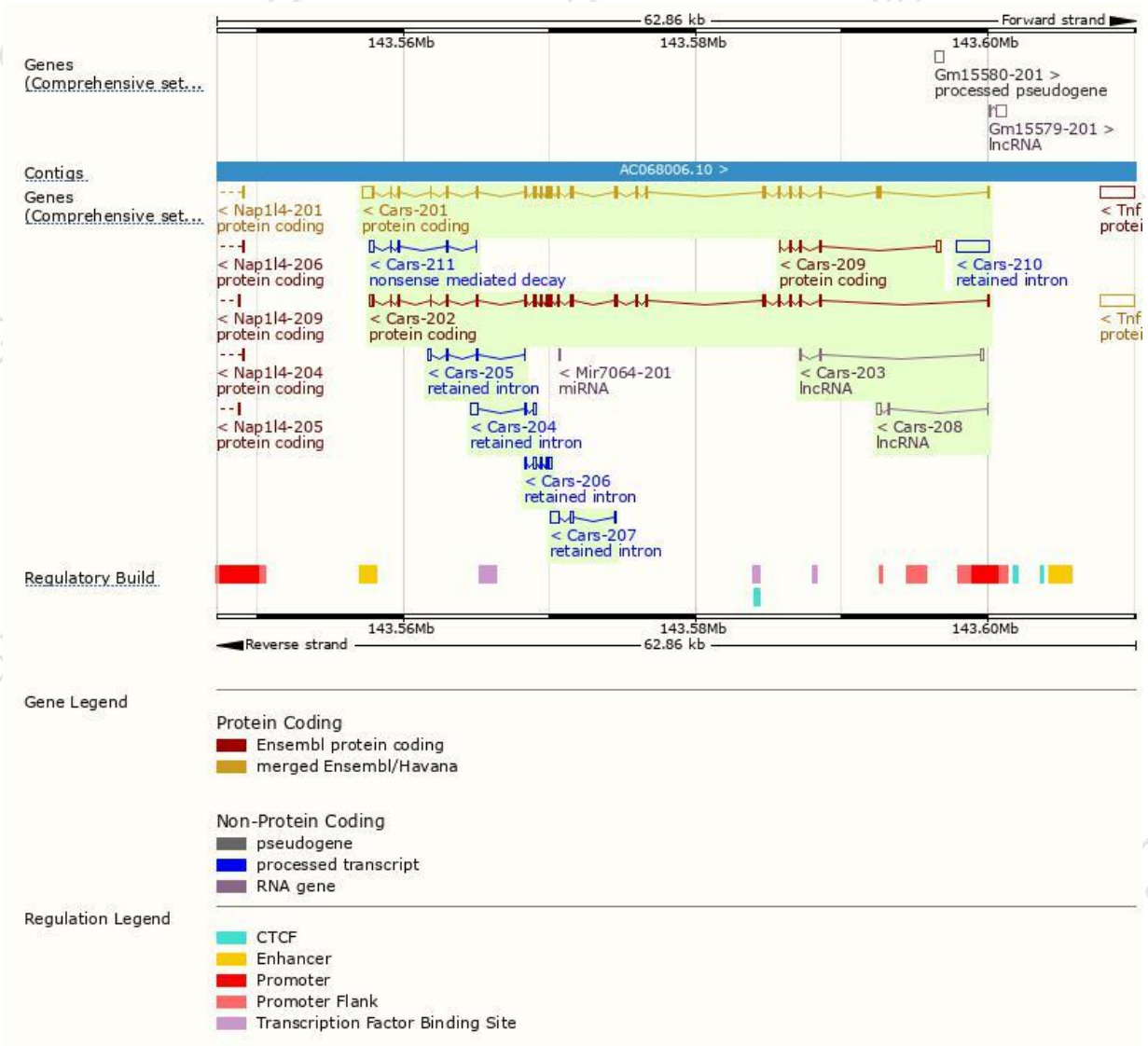
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cars-201	ENSMUST00000010899.13	3222	831aa	Protein coding	CCDS40198	Q9ER72	TSL:1 GENCODE basic APPRIS P3
Cars-202	ENSMUST00000105909.3	2462	748aa	Protein coding	CCDS57597	Q3U716 Q9ER72	TSL:1 GENCODE basic APPRIS ALT1
Cars-209	ENSMUST00000154022.7	539	53aa	Protein coding	-	A0A140LIB6	CDS 3' incomplete TSL:3
Cars-211	ENSMUST00000208575.1	558	39aa	Nonsense mediated decay	-	A0A140LII7	CDS 5' incomplete TSL:3
Cars-210	ENSMUST00000207168.1	2213	No protein	Retained intron	-	-	TSL:NA
Cars-207	ENSMUST00000146904.1	833	No protein	Retained intron	-	-	TSL:3
Cars-204	ENSMUST00000134753.7	731	No protein	Retained intron	-	-	TSL:5
Cars-206	ENSMUST00000146462.2	592	No protein	Retained intron	-	-	TSL:5
Cars-205	ENSMUST00000135032.1	384	No protein	Retained intron	-	-	TSL:2
Cars-208	ENSMUST00000151574.1	430	No protein	lncRNA	-	-	TSL:3
Cars-203	ENSMUST00000134128.1	307	No protein	lncRNA	-	-	TSL:3

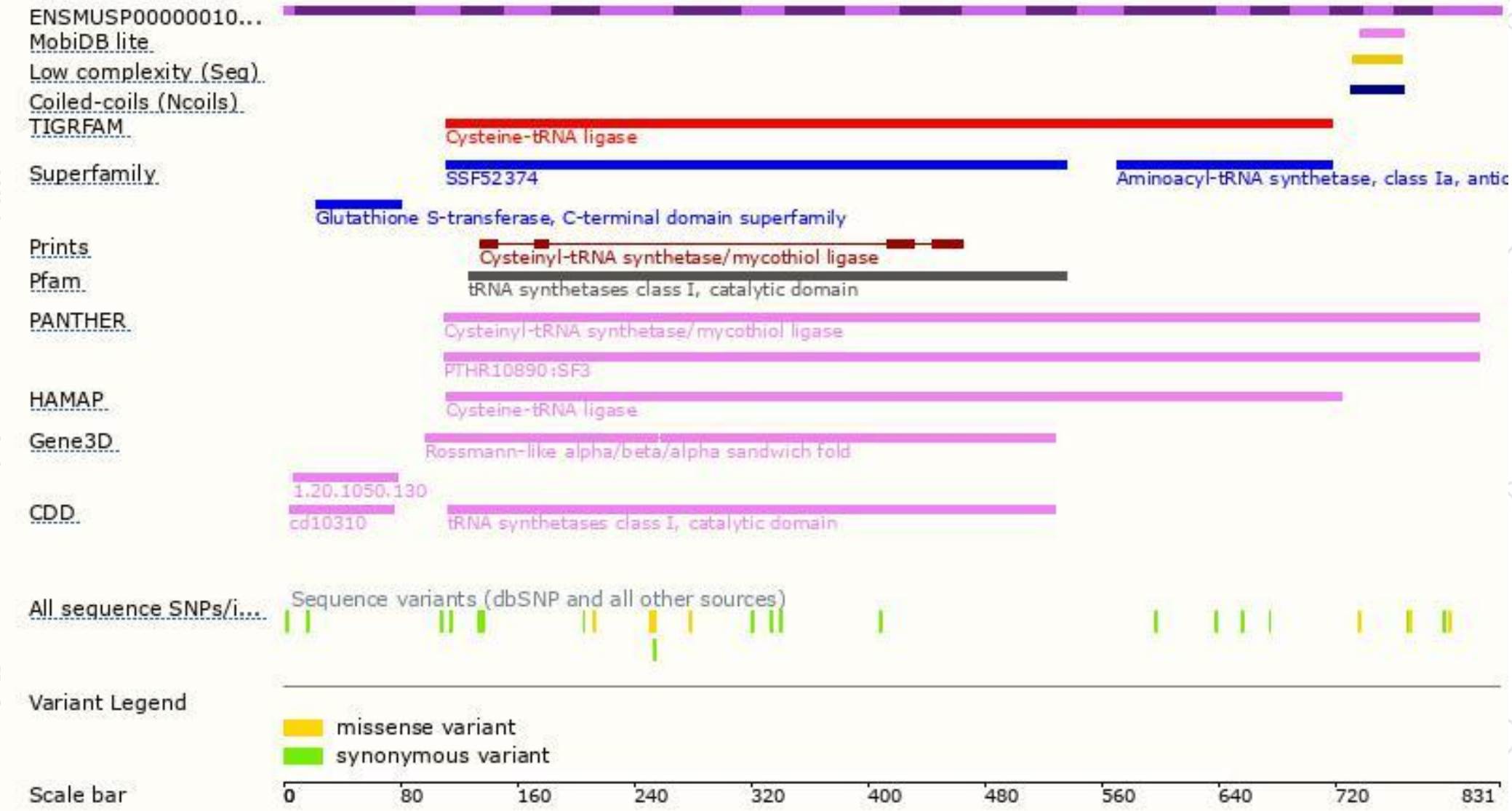
The strategy is based on the design of *Cars-201* transcript,The transcription is shown below



Genomic location distribution



Protein domain



集萃药康
GemPharmatech

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd