Car2 Cas9-CKO Strategy

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Project Overview

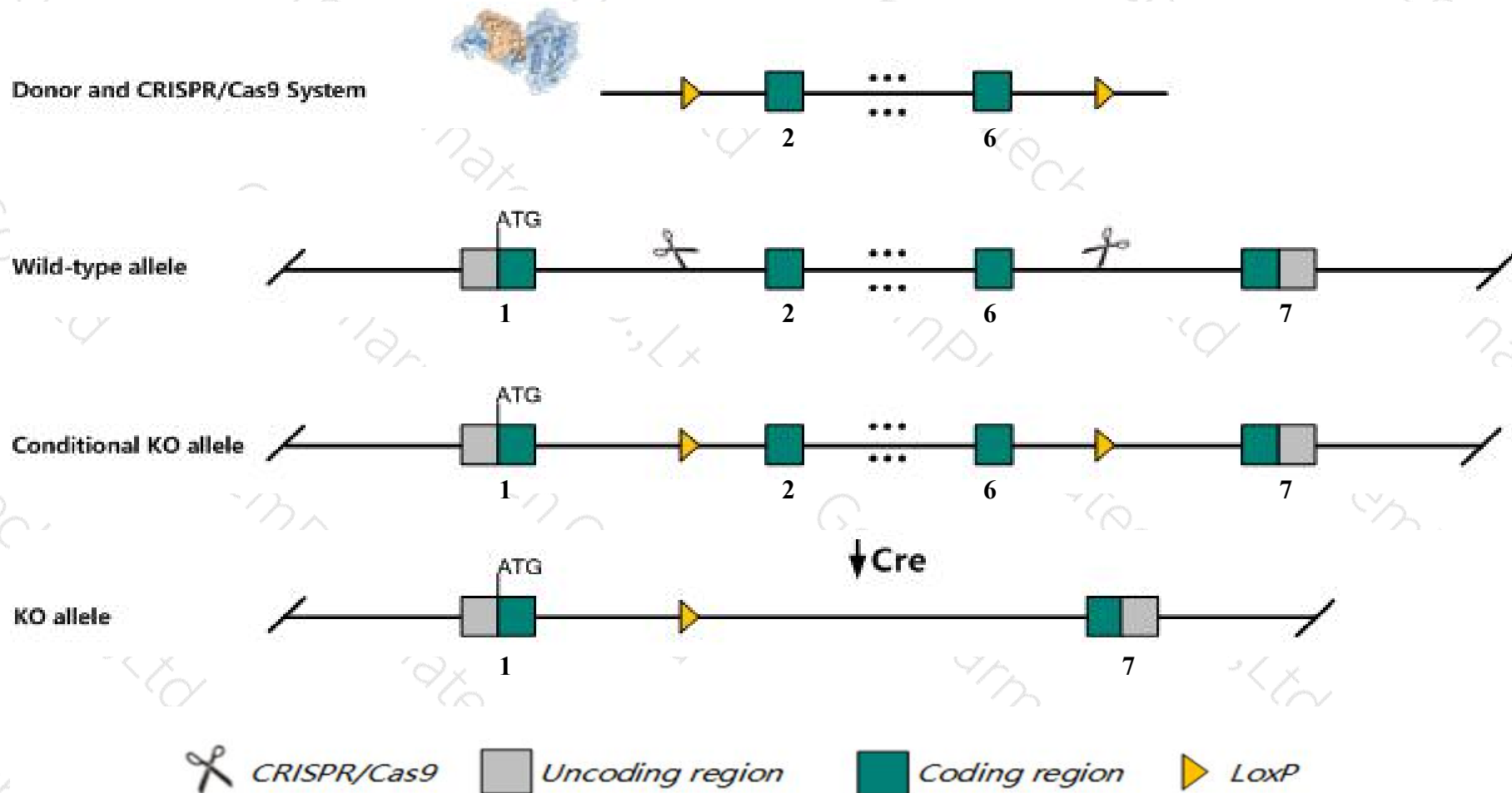|	
Project Name	*Car2*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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

Technical routes

➤ 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 Ens 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: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,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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing

   technological level.

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
108	current	GRCm38.p6 (GCF_000001635.26)	3	NC_000069.6 (14886269..14900770)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	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	ENSMUST00000029078.8	1788	260aa	Protein coding	CCDS17251	P00920	TSL:1 GENCODE basic APPRIS P1
Car2-202	ENSMUST00000192609.5	587	115aa	Protein coding	-	A0A0A6YX78	CDS 3' incomplete TSL:3
Car2-203	ENSMUST00000195520.1	881	No protein	Retained intron	-	-	TSL:2

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

14.34 kb ── Forward strand
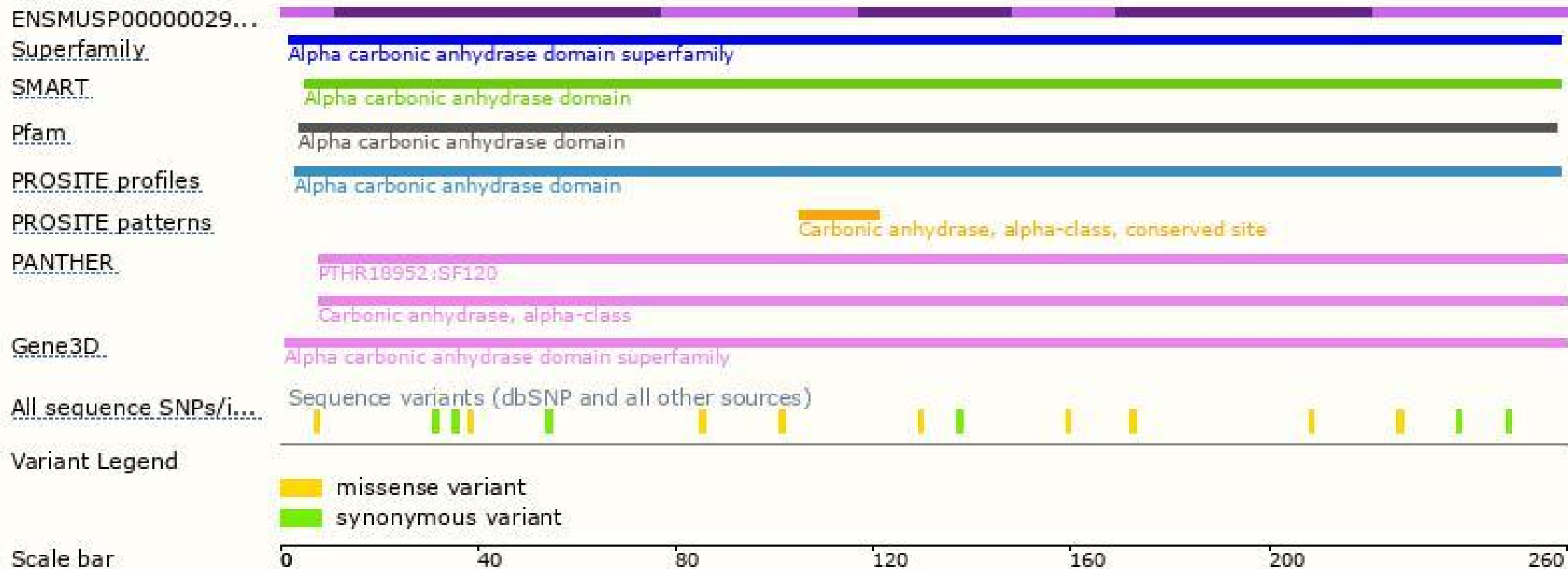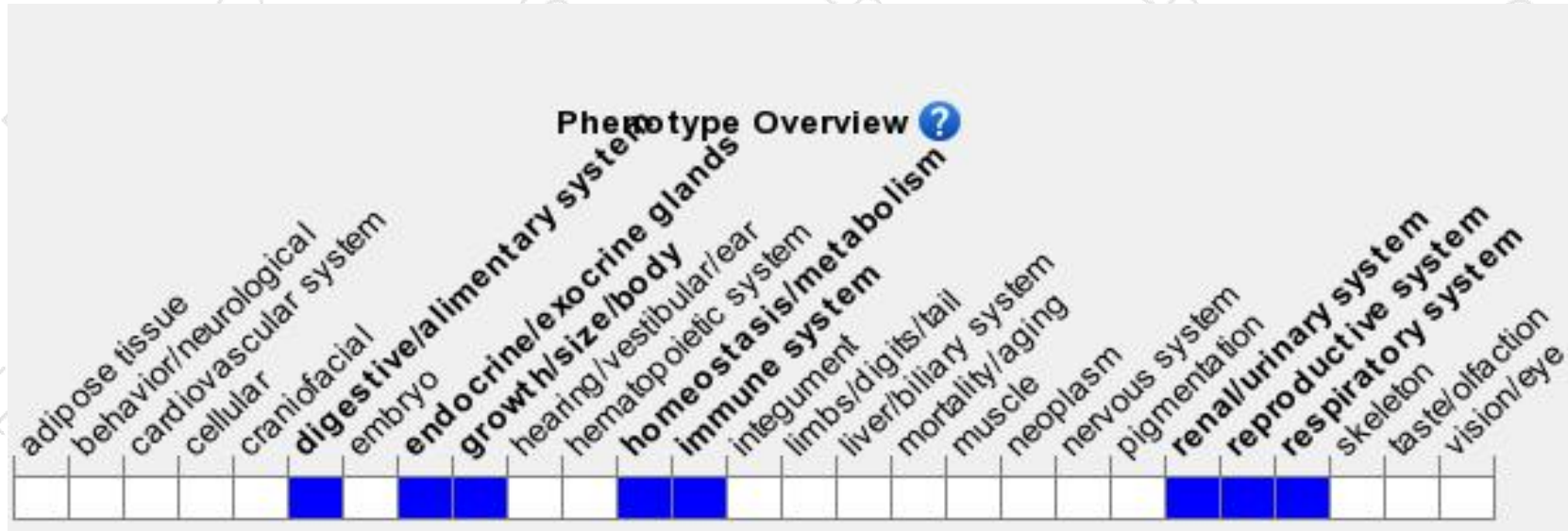
Car2-201 >
protein coding
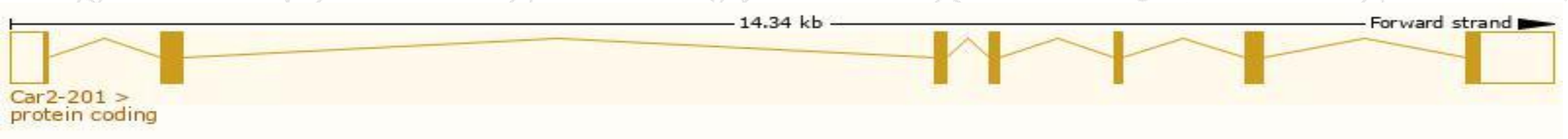
Genomic location distribution
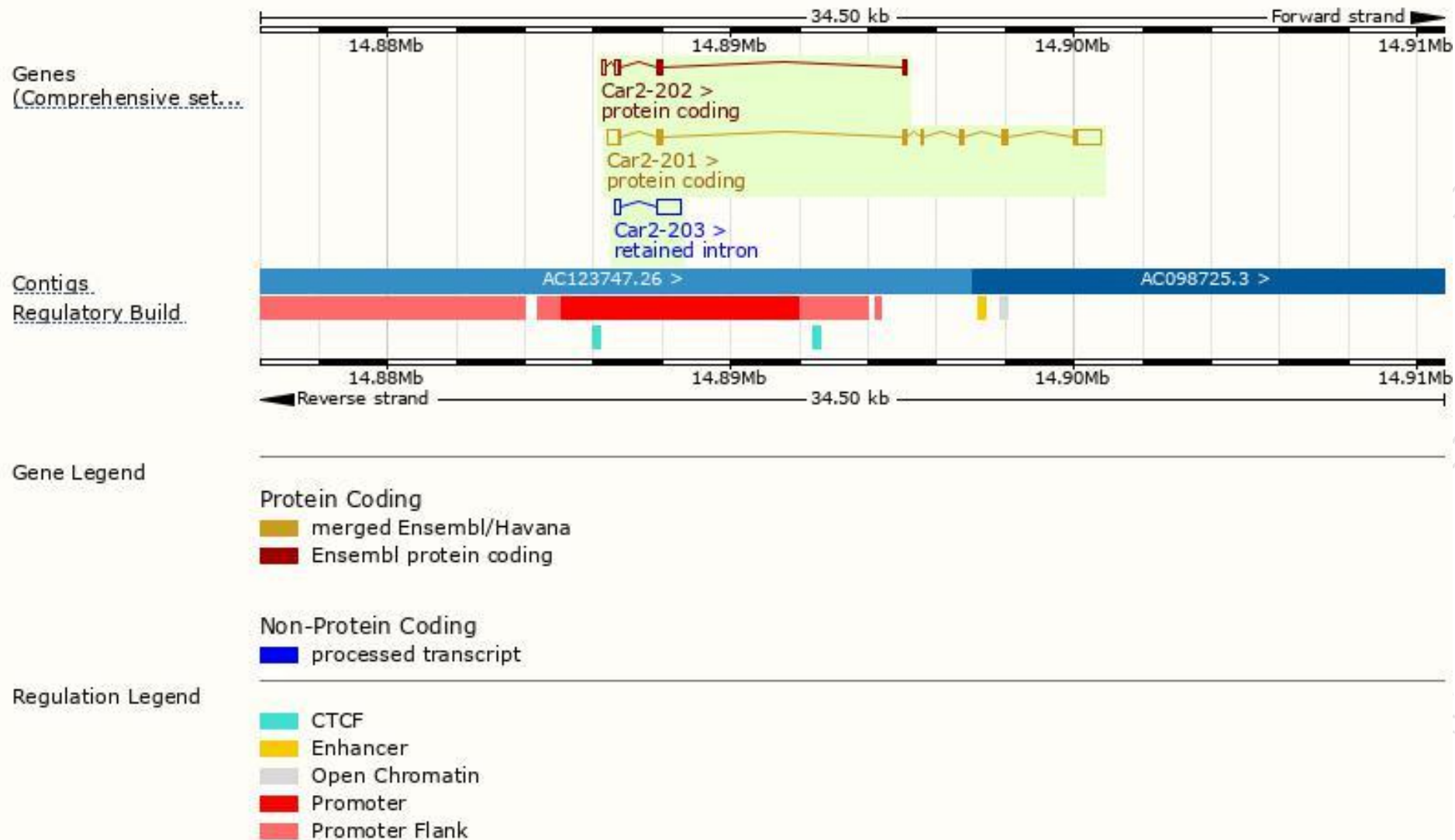
Protein domain

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,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.

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