

***Cyp8b1* Cas9-CKO Strategy**

Designer:

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Project Overview

Project Name

Cyp8b1

Project type

Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

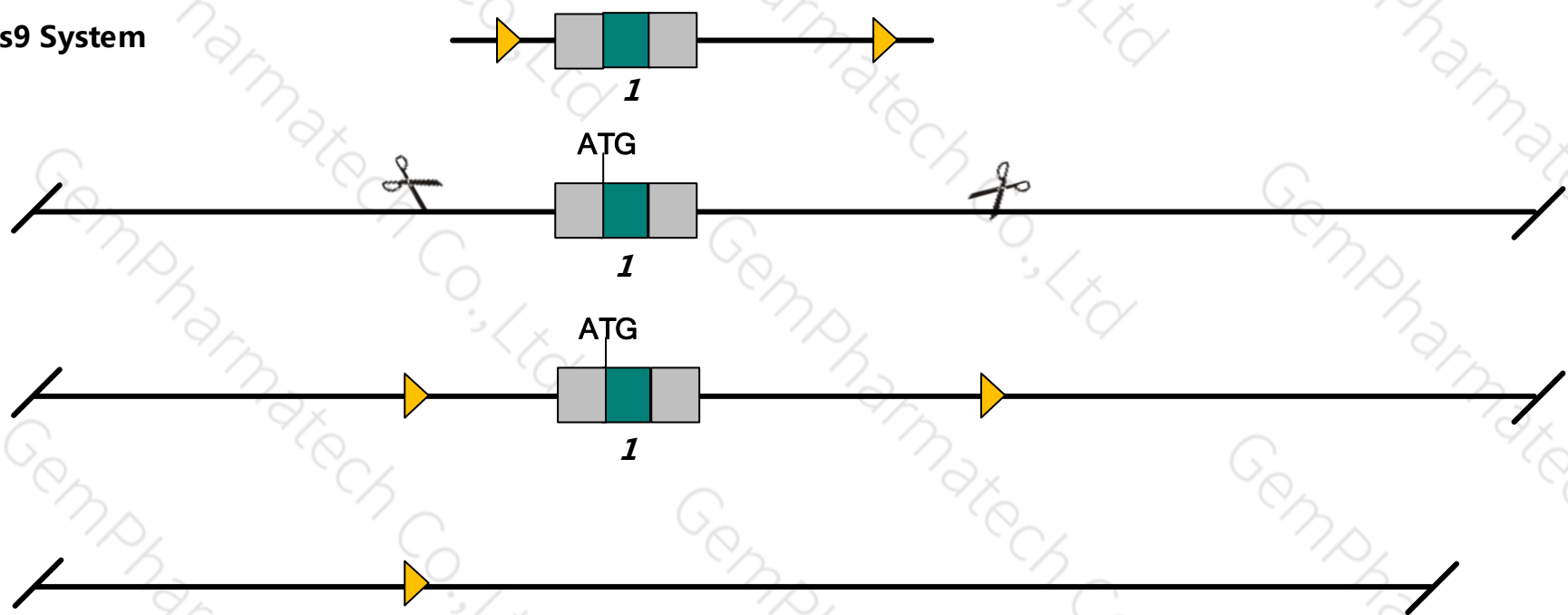
This model will use CRISPR/Cas9 technology to edit the *Cyp8b1* gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System

Wild-type allele

Conditional KO allele

KO allele



 Cas9/sgRNA  Uncoding region  Coding region  LoxP

- The *Cyp8b1* gene has 1 transcript. According to the structure of *Cyp8b1* gene, exon1 of *Cyp8b1*-201 (ENSMUST00000062474.4) transcript is recommended as the knockout region. The region contains all coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Cyp8b1* 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 knock-out allele lack synthesis of cholate (a primary bile acid) and its metabolites and display an increased bile acid pool and alterations in cholesterol metabolism.
- The *Cyp8b1* gene is located on the Chr9. 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)

Cyp8b1 cytochrome P450, family 8, subfamily b, polypeptide 1 [*Mus musculus* (house mouse)]

Gene ID: 13124, updated on 23-Feb-2019

Summary

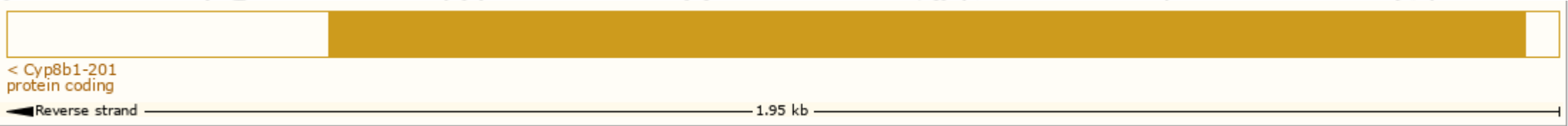
Official Symbol	Cyp8b1 provided by MGI
Official Full Name	cytochrome P450, family 8, subfamily b, polypeptide 1 provided by MGI
Primary source	MGI:MGI:1338044
See related	Ensembl:ENSMUSG00000050445
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Orthologs	human all

Transcript information (Ensembl)

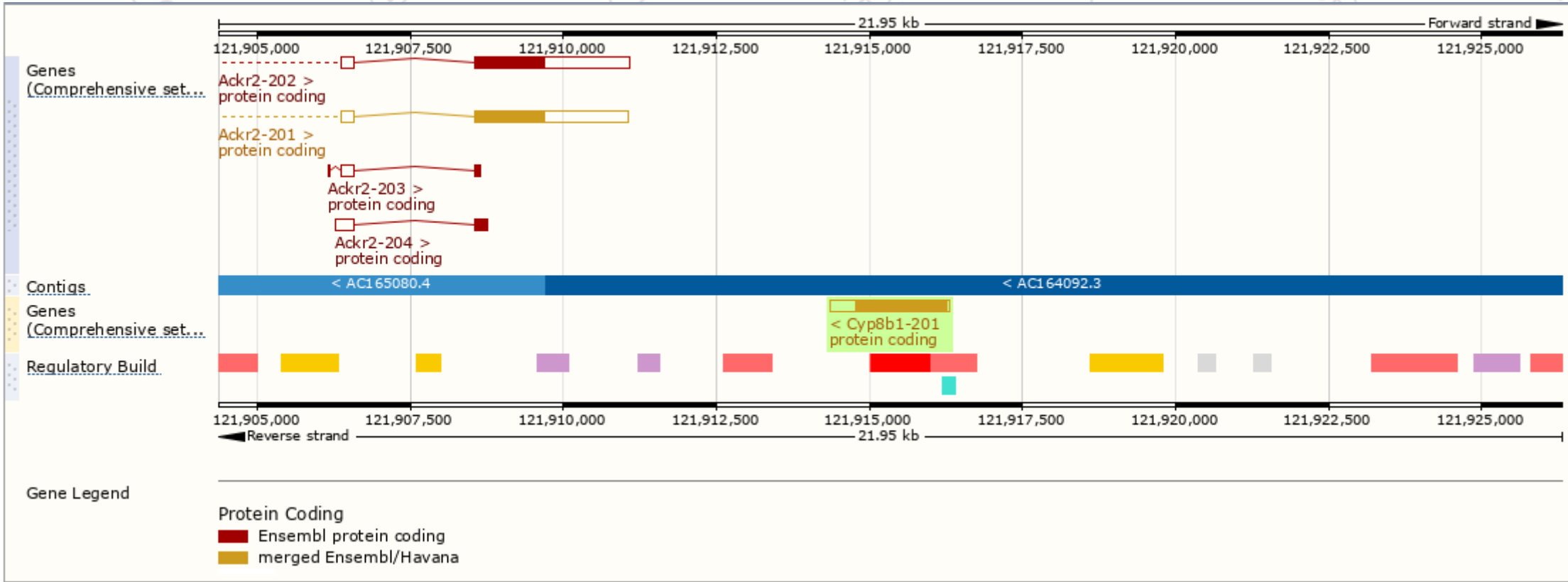
The gene has 1 transcript, and the transcript is shown below:

Show/hide columns (1 hidden)							Filter	
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
Cyp8b1-201	ENSMUST00000062474.4	1950	500aa	Protein coding	CCDS23641	A0A0R4J0N7	TSL:NA	GENCODE basic APPRIS P1

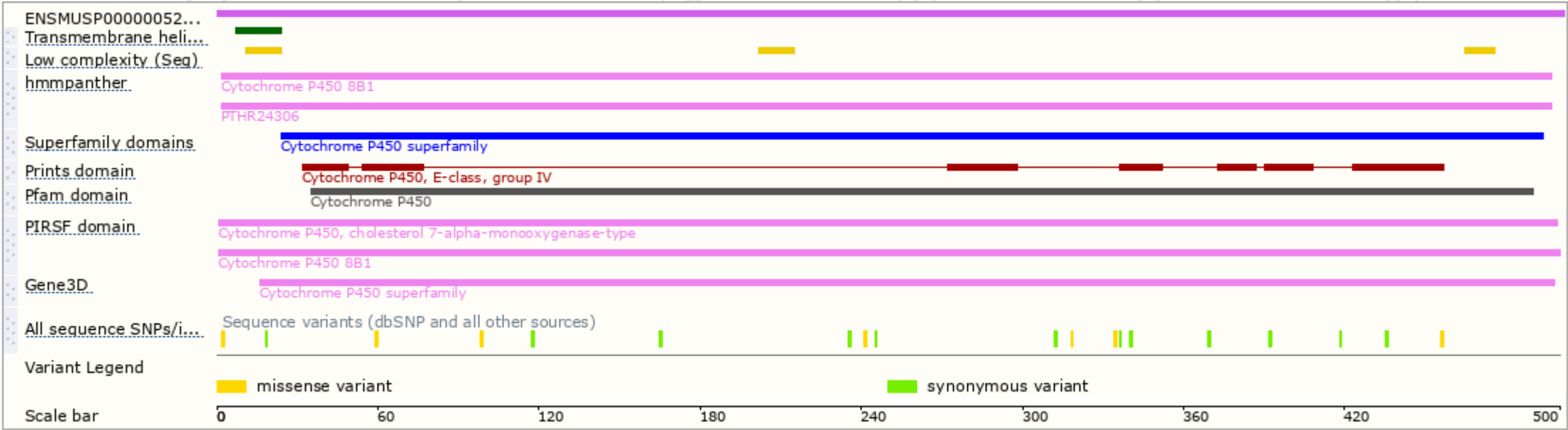
The strategy is based on the design of Cyp8b1-201 transcript,The transcription is shown below



Genomic location distribution



Protein domain



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GemPharmatech



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