

Cyp24a1 Cas9-KO Strategy

Designer:

Bingxuan Li

Reviewer:

Huimin Su

Design Date:

2019-11-1

Project Overview

Project Name

Cyp24a1

Project type

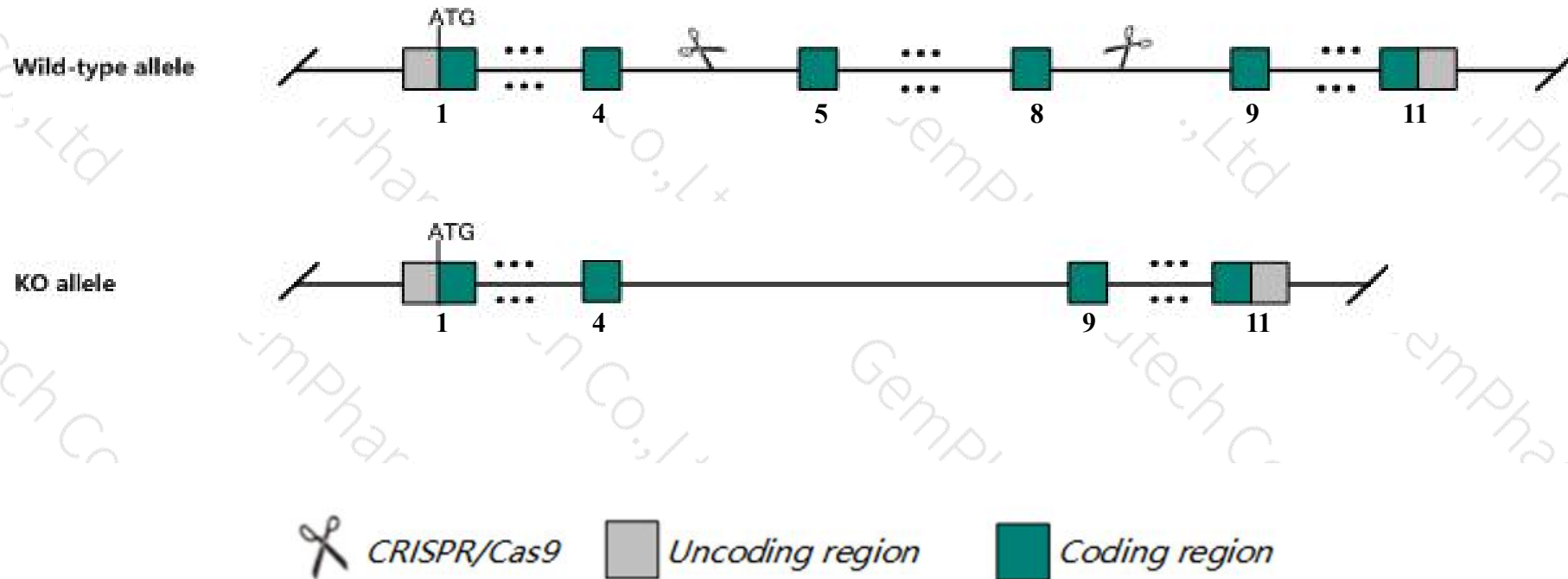
Cas9-KO

Strain background

C57BL/6JGpt

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cyp24a1* gene. The schematic diagram is as follows:



- The *Cyp24a1* gene has 1 transcript. According to the structure of *Cyp24a1* gene, exon5-exon8 of *Cyp24a1-201* (ENSMUST00000038824.5) transcript is recommended as the knockout region. The region contains 517bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Cyp24a1* gene. The brief process is as follows: gRNA was transcribed in vitro. Cas9 and gRNA 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.

- According to the existing MGI data, mice homozygous for disruption of this gene suffer a 50% mortality rate between birth and weaning. abnormalities are seen in the development of membranous bones.
- The *Cyp24a1* gene is located on the Chr2. 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 gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)

Cyp24a1 cytochrome P450, family 24, subfamily a, polypeptide 1 [Mus musculus (house mouse)]

Gene ID: 13081, updated on 31-Jan-2019

Summary



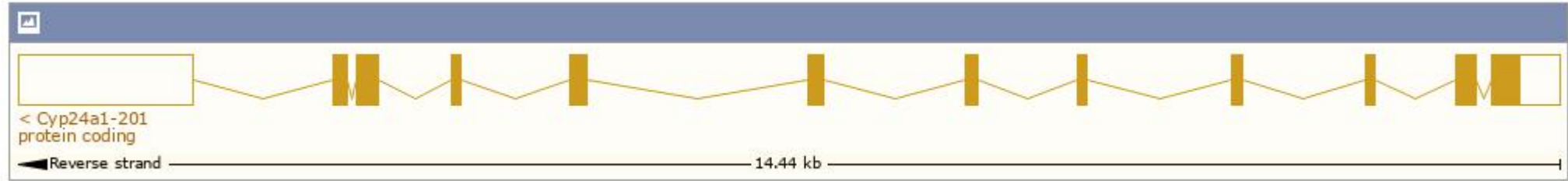
| | |
|---------------------------|--|
| Official Symbol | Cyp24a1 provided by MGI |
| Official Full Name | cytochrome P450, family 24, subfamily a, polypeptide 1 provided by MGI |
| Primary source | MGI:MGI:88593 |
| See related | Ensembl:ENSMUSG00000038567 |
| Gene type | protein coding |
| RefSeq status | REVIEWED |
| Organism | Mus musculus |
| Lineage | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| Also known as | 24-OHase, CP24, Cyp24 |
| Summary | The protein encoded by this gene localizes to the mitochondrion, where it degrades calcitriol to calcitriol. This gene is upregulated by binding of calcitriol to the upstream regulatory region and to a downstream enhancer region, thereby allowing calcitriol to autoregulate its concentration in the cell. The encoded protein may also play a role in calcium homeostasis. [provided by RefSeq, Aug 2015] |
| Expression | Restricted expression toward kidney adult (RPKM 11.8) See more |
| Orthologs | human all |

Transcript information (Ensembl)

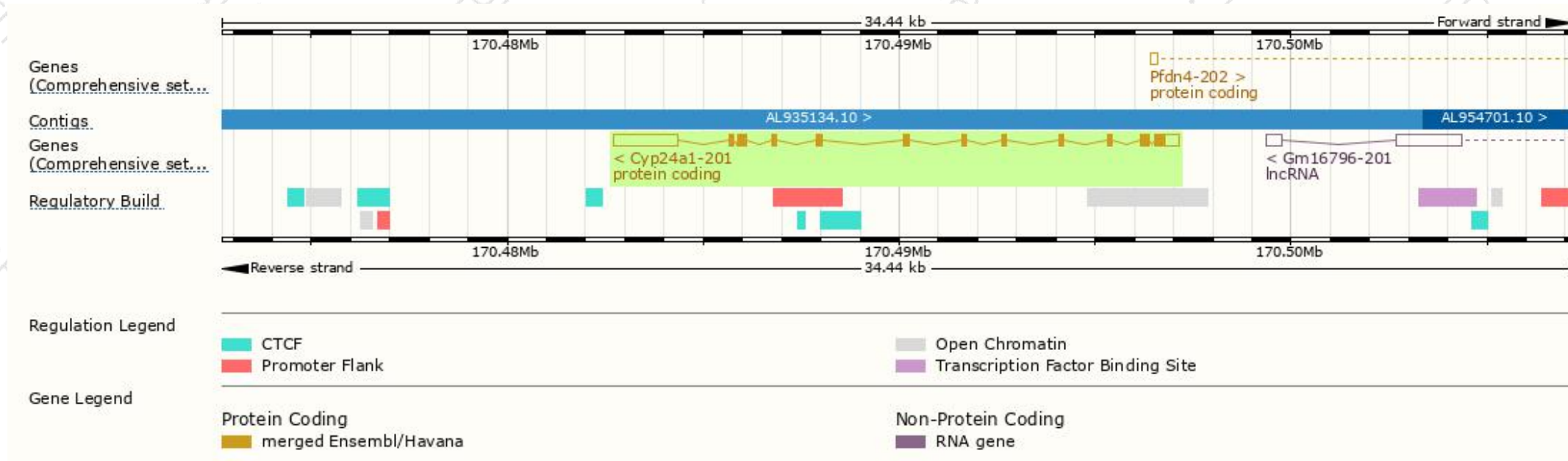
The gene has 1 transcript, and the transcript is shown below:

| Show/hide columns (1 hidden) | | | | | | | Filter | |
|------------------------------|--------------------------------------|------|-----------------------|----------------|---------------------------|---|--------|-------------------------|
| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags | |
| Cyp24a1-201 | ENSMUST00000038824.5 | 3560 | 514aa | Protein coding | CCDS17122 | Q3TWW0 Q64441 | TSL:1 | GENCODE basic APPRIS P1 |

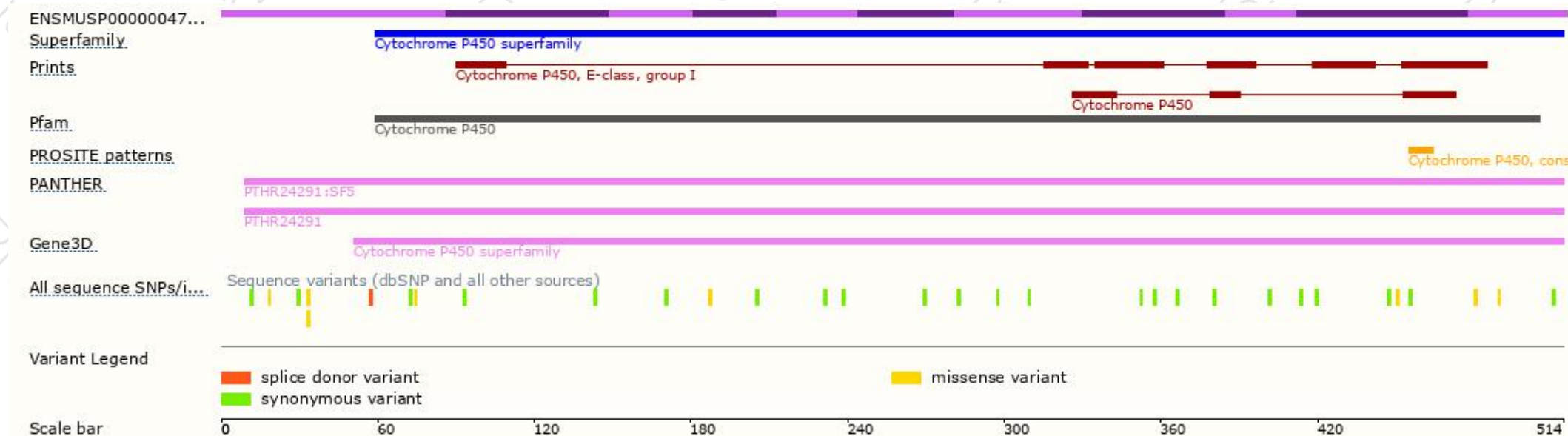
The strategy is based on the design of *Cyp24a1-201* transcript, the transcription is shown below



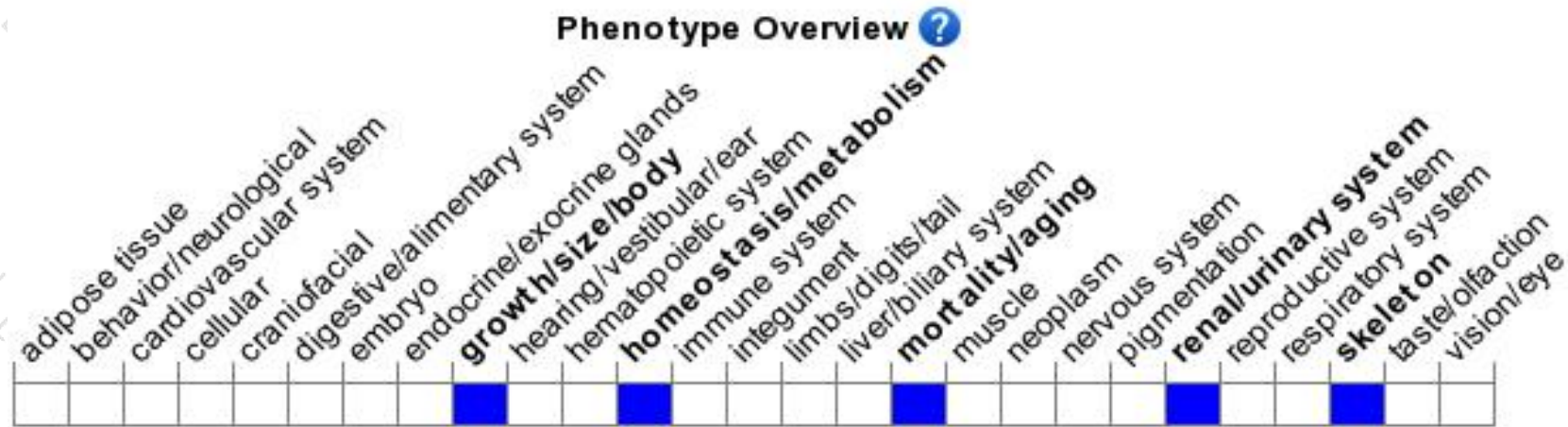
Genomic location distribution



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 disruption of this gene suffer a 50% mortality rate between birth and weaning. abnormalities are seen in the development of membranous bones.

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

Tel: 400-9660890

