Cyp2r1 Cas9-KO Strategy

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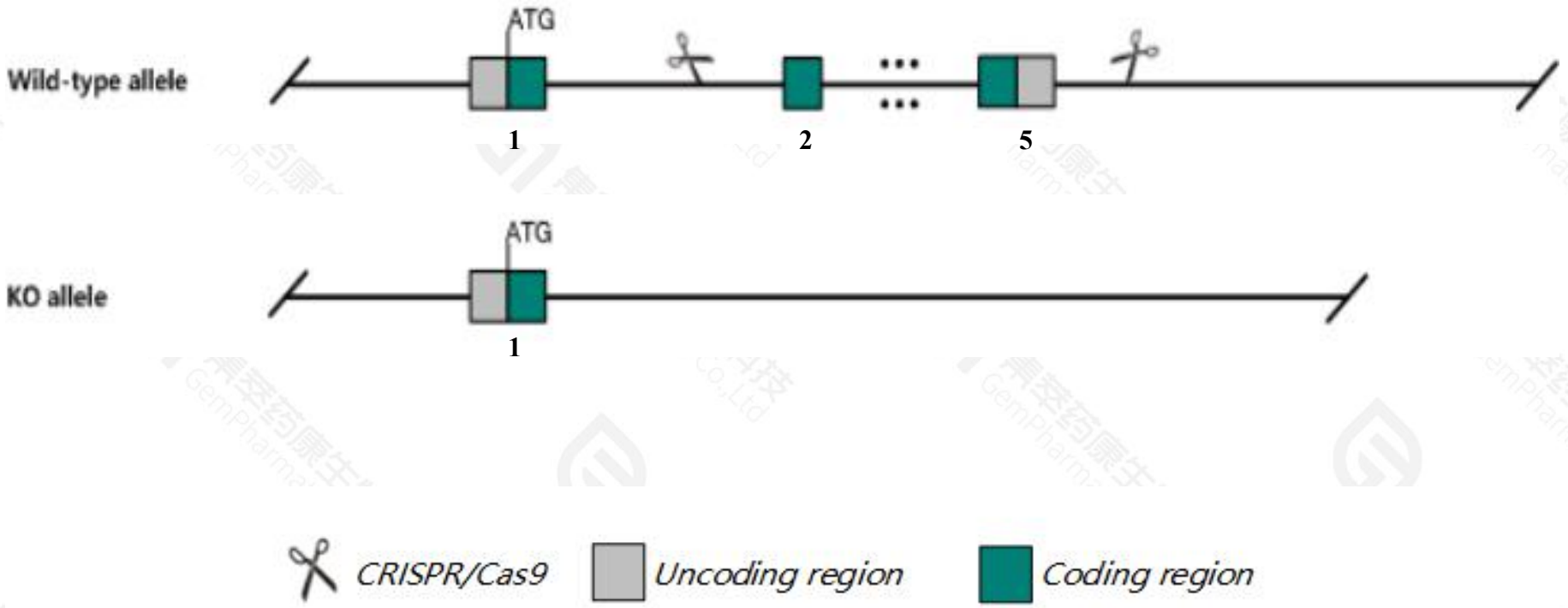
Project Overview

Project Name	***Cyp2r1***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cyp2r1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Cyp2r1* gene has 7 transcripts. According to the structure of *Cyp2r1* gene, exon2-exon5 of *Cyp2r1-201*(ENSMUST00000032908.15) transcript is recommended as the knockout region.The region contains most of the coding sequence.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Cyp2r1* gene. The brief process is as follows: CRISPR/Cas9 system 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.

Notice

➢ According to the existing MGI data,mice homozygous for a knock-out allele exhibit more than a 50% reduction in serum 25-hydroxyvitamin D3 levels but remain healthy and show normal serum 1alpha,25-dihydroxyvitamin D3 levels.

➢ The *Cyp2r1* 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

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Cyp2r1 cytochrome P450, family 2, subfamily r, polypeptide 1 [Mus musculus (house mouse)]

Gene ID: 244209, updated on 14-Jan-2021

Summary

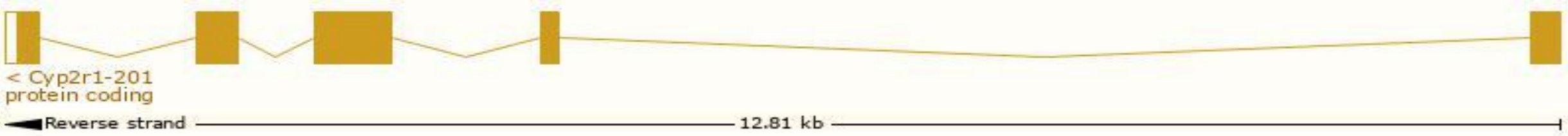
Official Symbol	Cyp2r1 provided by MGI
Official Full Name	cytochrome P450, family 2, subfamily r, polypeptide 1 provided byMGI
Primary source	MGI:MGI:2449771
See related	Ensembl:ENSMUSG00000030670
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Biased expression in liver adult (RPKM 2.6), adrenal adult (RPKM 1.1) and 13 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

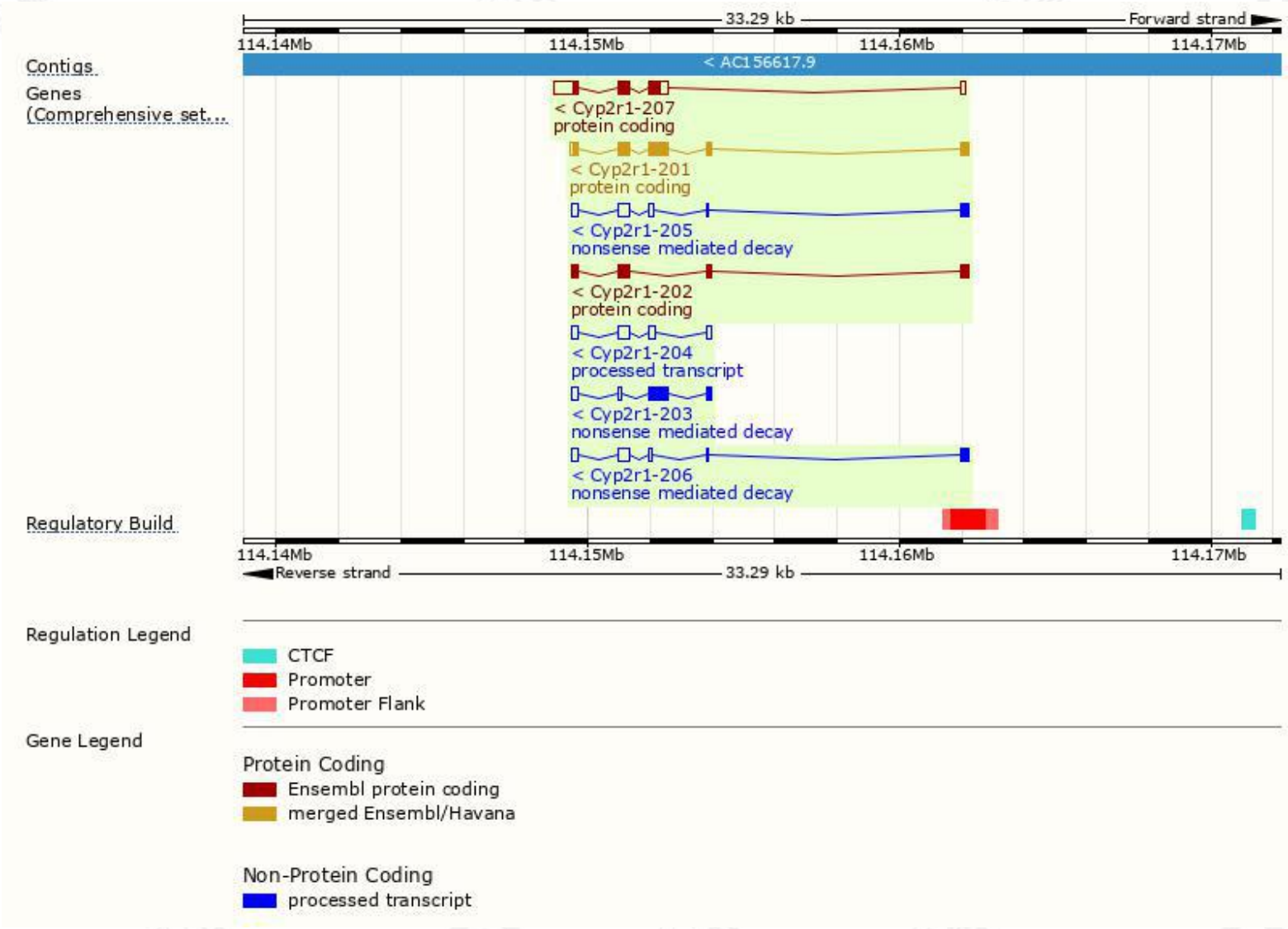
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cyp2r1-201	ENSMUST00000032908.15	1627	501aa	Protein coding	CCDS21761		TSL:1 , GENCODE basic , APPRIS P1 ,
Cyp2r1-207	ENSMUST00000211506.2	1887	292aa	Protein coding	-		TSL:5 , GENCODE basic ,
Cyp2r1-202	ENSMUST00000119712.8	917	290aa	Protein coding	-		TSL:5 , GENCODE basic ,
Cyp2r1-203	ENSMUST00000128587.8	1070	268aa	Nonsense mediated decay	-		CDS 5' incomplete , TSL:5 ,
Cyp2r1-205	ENSMUST00000138712.8	995	83aa	Nonsense mediated decay	-		TSL:1 ,
Cyp2r1-206	ENSMUST00000147428.2	894	83aa	Nonsense mediated decay	-		TSL:1 ,
Cyp2r1-204	ENSMUST00000133484.8	901	No protein	Processed transcript	-		TSL:5 ,

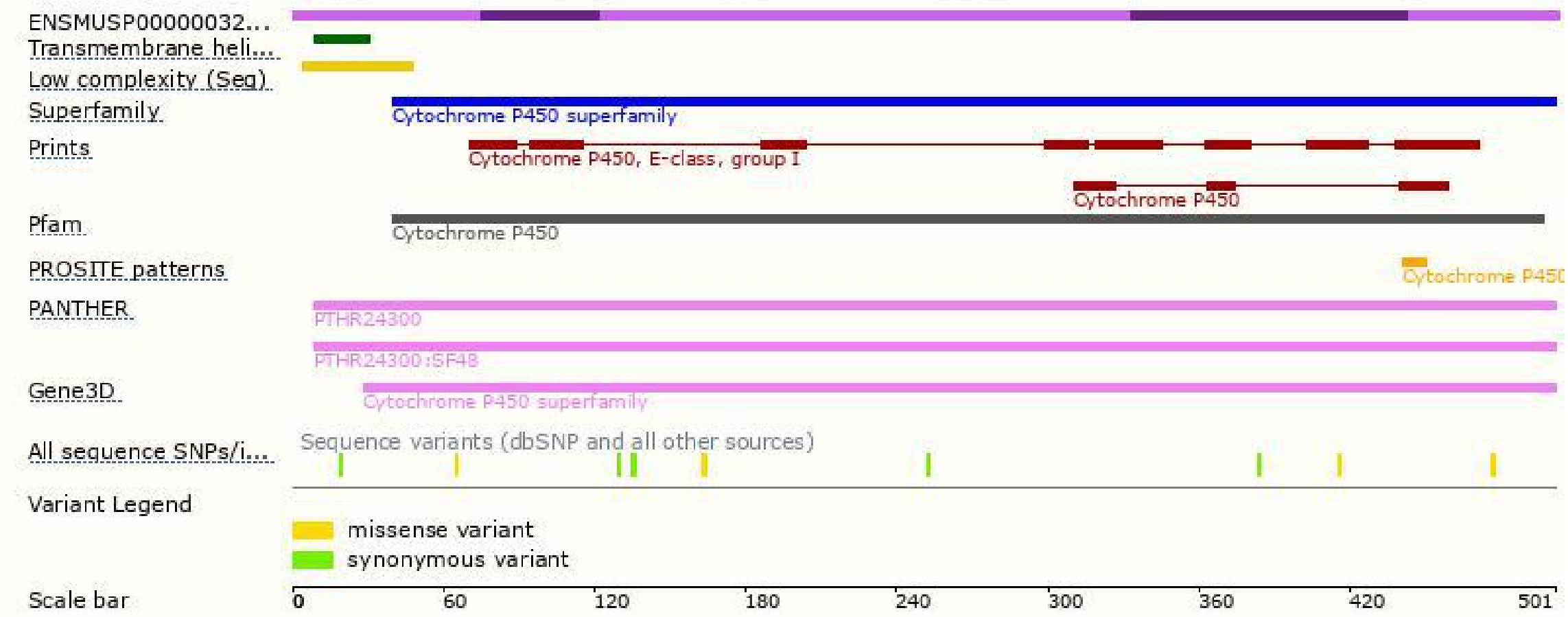
The strategy is based on the design of *Cyp2r1-201* transcript,the transcription is shown below:



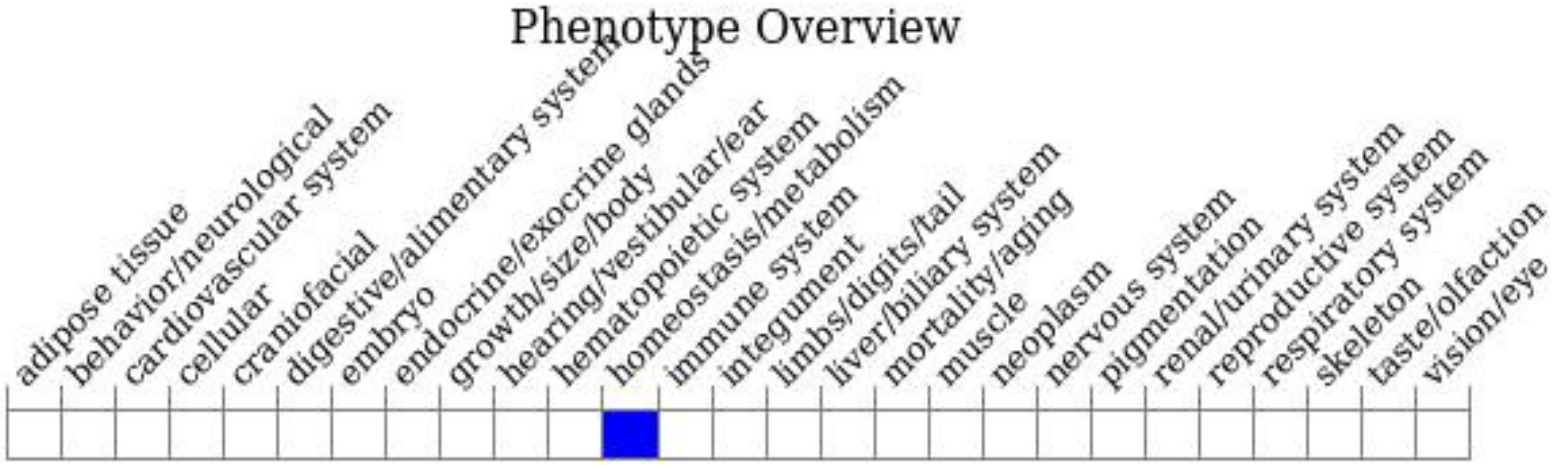
Genomic location distribution



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/).

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