

# *Cyp26a1* Cas9-CKO Strategy

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**Reviewer:**

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**Design Date:**

**2019-11-1**

# Project Overview

**Project Name**

*Cyp26a1*

**Project type**

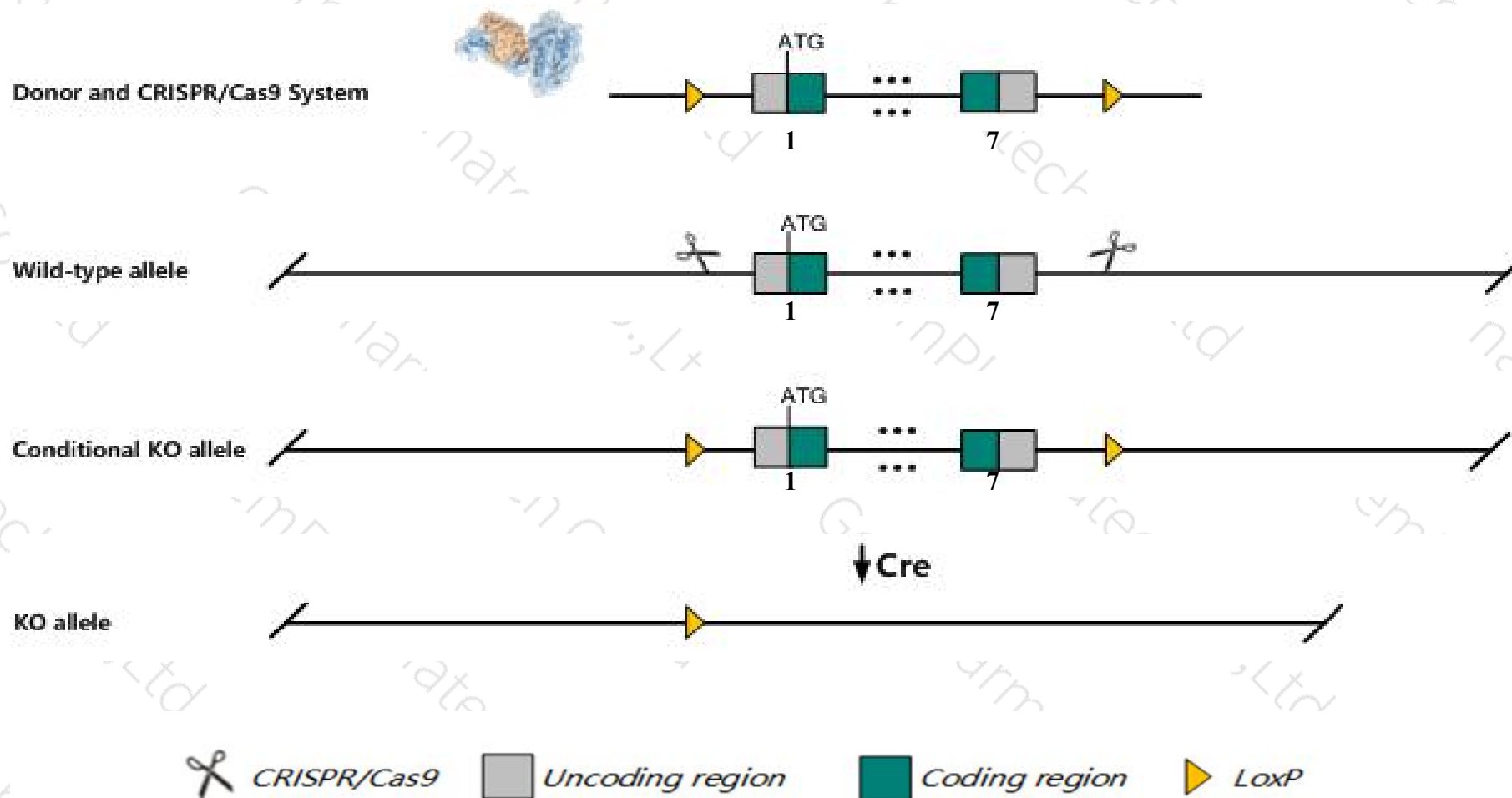
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cyp26a1* gene. The schematic diagram is as follows:



- The *Cyp26a1* gene has 2 transcripts. According to the structure of *Cyp26a1* gene, exon1-exon7 of *Cyp26a1-201* (ENSMUST00000025946.6) transcript is recommended as the knockout region. The region contains all 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 *Cyp26a1* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed. Cas9, gRNA 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.

- According to the existing MGI data, homozygotes for targeted null mutations die during mid-late gestation and exhibit spina bifida, caudal agenesis, and abnormalities of the kidneys, urogenital tract, hindgut, cervical vertebrae, and rostral hindbrain.
- The insertion position of 5-terminal Loxp is in the promoter region of *Cyp26a1*, which may affect the 5-terminal regulation of *Cyp26a1*.
- The *Cyp26a1* gene is located on the Chr19. 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)

## Cyp26a1 cytochrome P450, family 26, subfamily a, polypeptide 1 [Mus musculus (house mouse)]

Gene ID: 13082, updated on 31-Jan-2019

### Summary



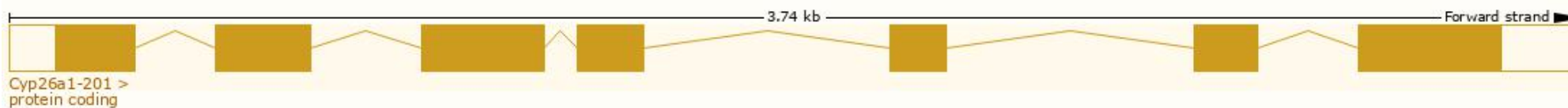
<b>Official Symbol</b>	Cyp26a1 provided by <a href="#">MGI</a>
<b>Official Full Name</b>	cytochrome P450, family 26, subfamily a, polypeptide 1 provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:1096359</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000024987</a>
<b>Gene type</b>	protein coding
<b>RefSeq status</b>	VALIDATED
<b>Organism</b>	<a href="#">Mus musculus</a>
<b>Lineage</b>	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
<b>Also known as</b>	Cyp26, P450RA, P450RAI, RAH
<b>Expression</b>	Biased expression in liver adult (RPKM 38.3) and ovary adult (RPKM 6.7) <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>

# Transcript information (Ensembl)

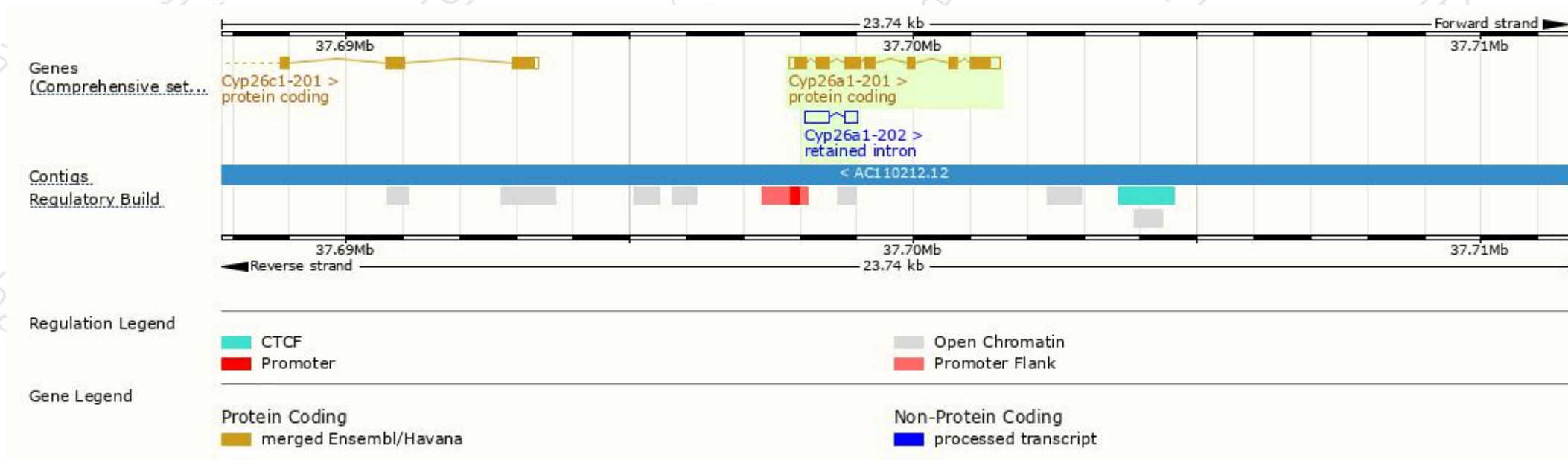
The gene has 2 transcripts, all transcripts are shown below:

Show/hide columns (1 hidden)		Filter					
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cyp26a1-201	<a href="#">ENSMUST00000025946.6</a>	1791	<a href="#">497aa</a>	Protein coding	<a href="#">CCDS29780</a>	<a href="#">A0A0R4J061</a>	TSL:1 GENCODE basic APPRIS P1
Cyp26a1-202	<a href="#">ENSMUST000000236500.1</a>	659	No protein	Retained intron	-	-	-

The strategy is based on the design of *Cyp26a1-201* transcript, the transcription is shown below

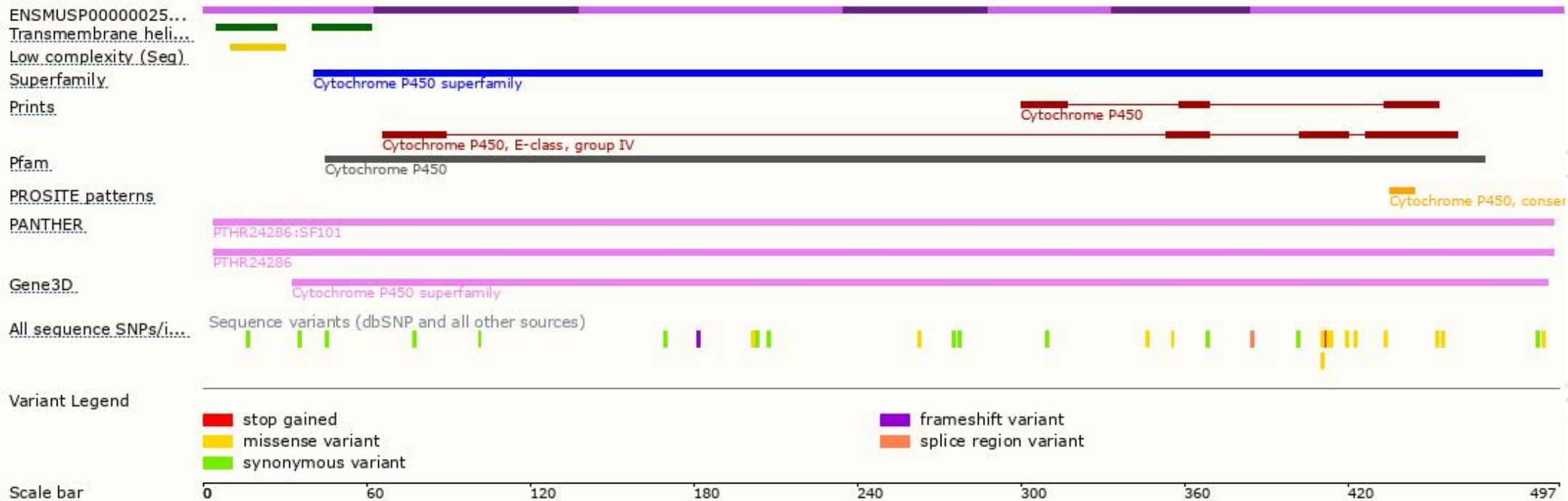


# Genomic location distribution

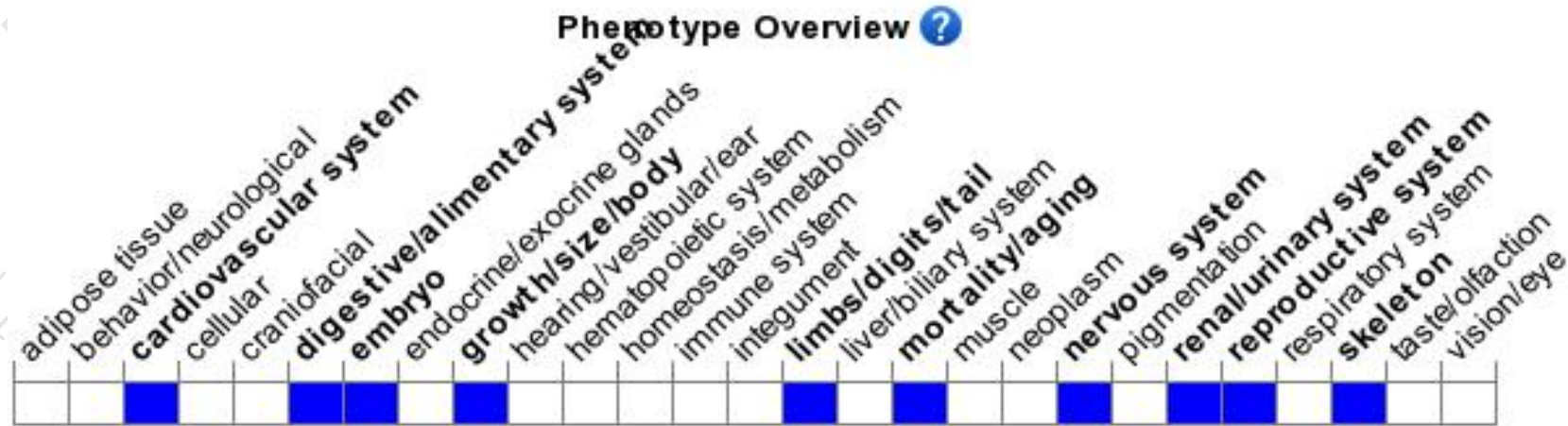




# Protein domain



# 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, homozygotes for targeted null mutations die during mid-late gestation and exhibit spina bifida, caudal agenesis, and abnormalities of the kidneys, urogenital tract, hindgut, cervical vertebrae, and rostral hindbrain.

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

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