Ophn1 Cas9-CKO *Strategy*

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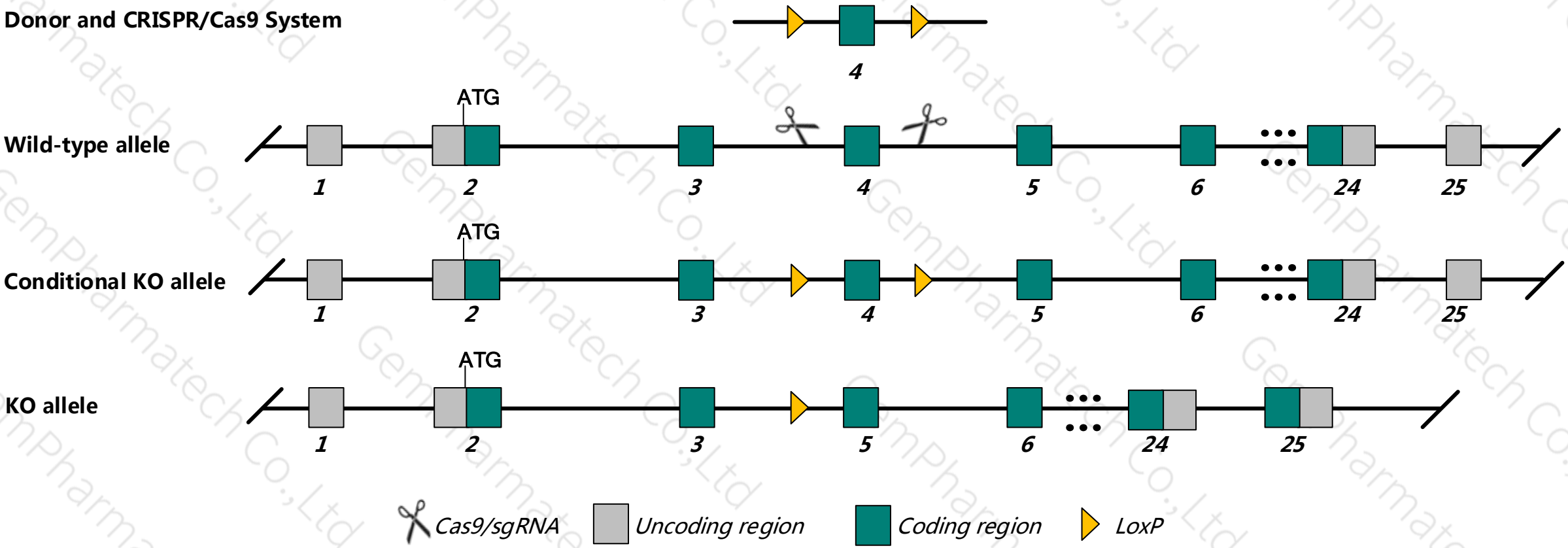
Design Date: **2020-4-2**

Project Overview

Project Name	***Ophn1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ophn1* gene. The schematic diagram is as follows:



Technical routes

- The *Ophn1* gene has 10 transcripts.According to the structure of *Ophn1* gene, exon4 of *Ophn1*-201 (ENSMUST00000033560.8)transcript is recommended as the knockout region.The region contains 62bp coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Ophn1* 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.

Notice

- According to the existing MGI data，homozygous and hemizygous mice exhibit strain background-dependent premature death, dilated brain ventricles, impaired sperm motility, and abnormal behavior including decreased anxiety and increased activity. Heterozygotes have a wavy coat and vibrissae,are small and have decreased litter sizes.
- Transcript *NM_001313756.1* may not be affected.
- The *Ophn1* gene is located on the ChrX. 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）

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Ophn1 oligophrenin 1 [*Mus musculus* (house mouse)]

Gene ID: 94190, updated on 13-Mar-2020

Summary

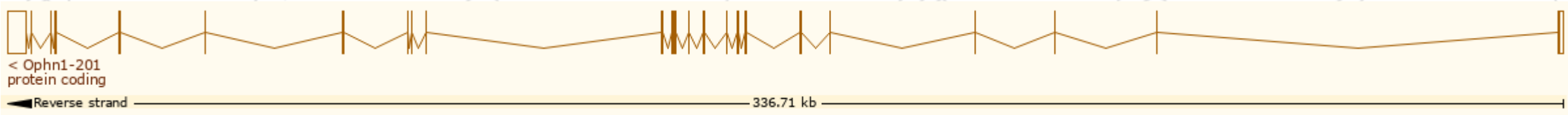
Official Symbol	Ophn1 provided by MGI
Official Full Name	oligophrenin 1 provided by MGI
Primary source	MGI:MGI:2151070
See related	Ensembl:ENSMUSG00000031214
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Wtgr; C130037N19Rik
Expression	Broad expression in CNS E18 (RPKM 5.4), frontal lobe adult (RPKM 4.2) and 16 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

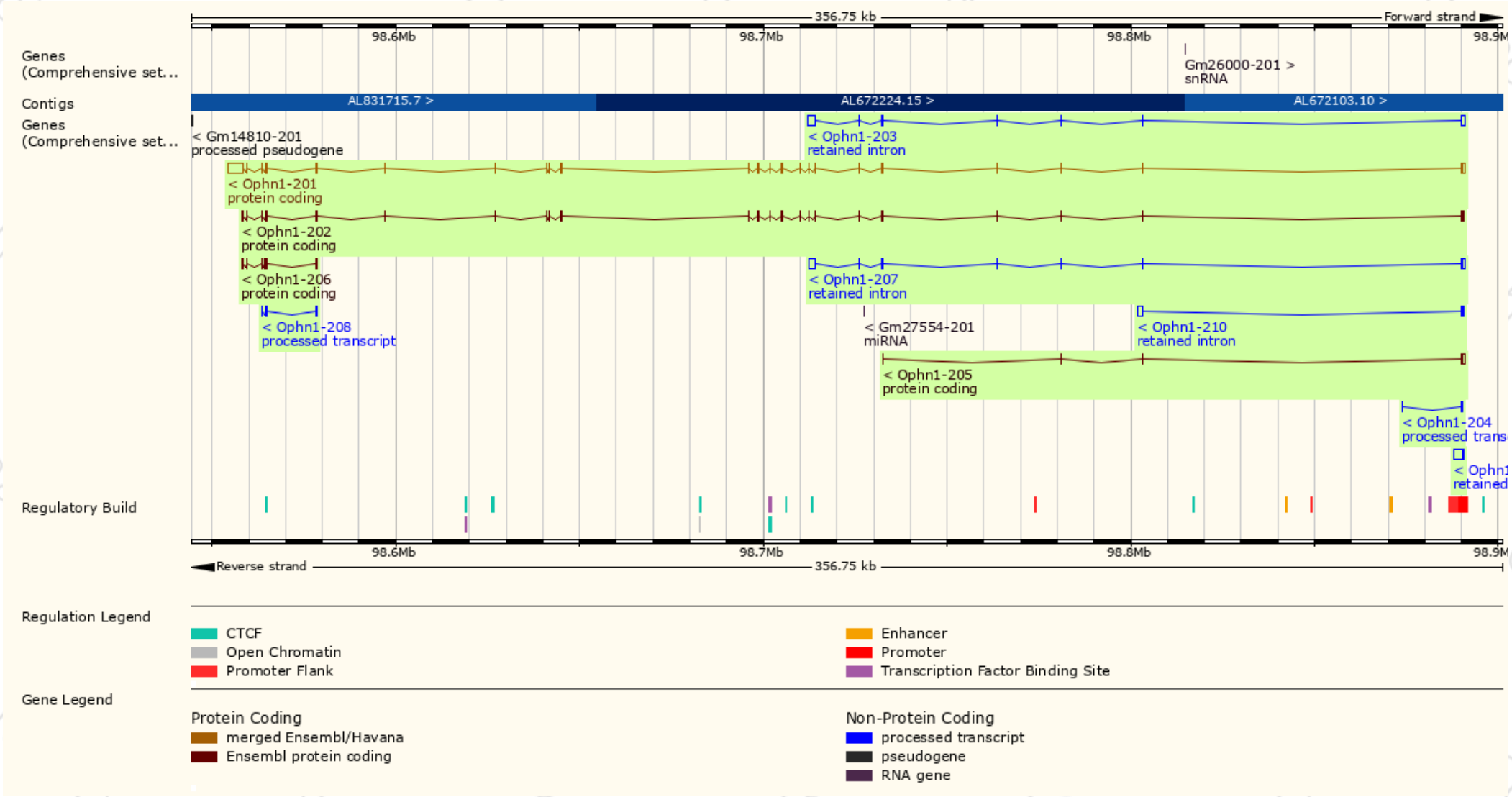
The gene has 10 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ophn1-203	ENSMUST00000125150.7	3400	No protein	Retained intron	-	-	TSL:1
Ophn1-207	ENSMUST00000147805.7	3006	No protein	Retained intron	-	-	TSL:1
Ophn1-209	ENSMUST00000156634.1	2720	No protein	Retained intron	-	-	TSL:1
Ophn1-210	ENSMUST00000156917.7	1782	No protein	Retained intron	-	-	TSL:1
Ophn1-201	ENSMUST00000033560.8	7170	802aa	Protein coding	CCDS30295	Q99J31	TSL:1 GENCODE basic APPRIS P1
Ophn1-202	ENSMUST00000113826.7	2794	802aa	Protein coding	CCDS30295	Q99J31	TSL:1 GENCODE basic APPRIS P1
Ophn1-205	ENSMUST00000142267.1	1011	129aa	Protein coding	-	B1AV64	CDS 3' incomplete TSL:1
Ophn1-206	ENSMUST00000147529.1	556	132aa	Protein coding	-	B1AXS4	CDS 5' incomplete TSL:3
Ophn1-204	ENSMUST00000140598.1	474	No protein	Processed transcript	-	-	TSL:1
Ophn1-208	ENSMUST00000154920.1	330	No protein	Processed transcript	-	-	TSL:3

The strategy is based on the design of *Ophn1*-201 transcript, the transcription is shown below:

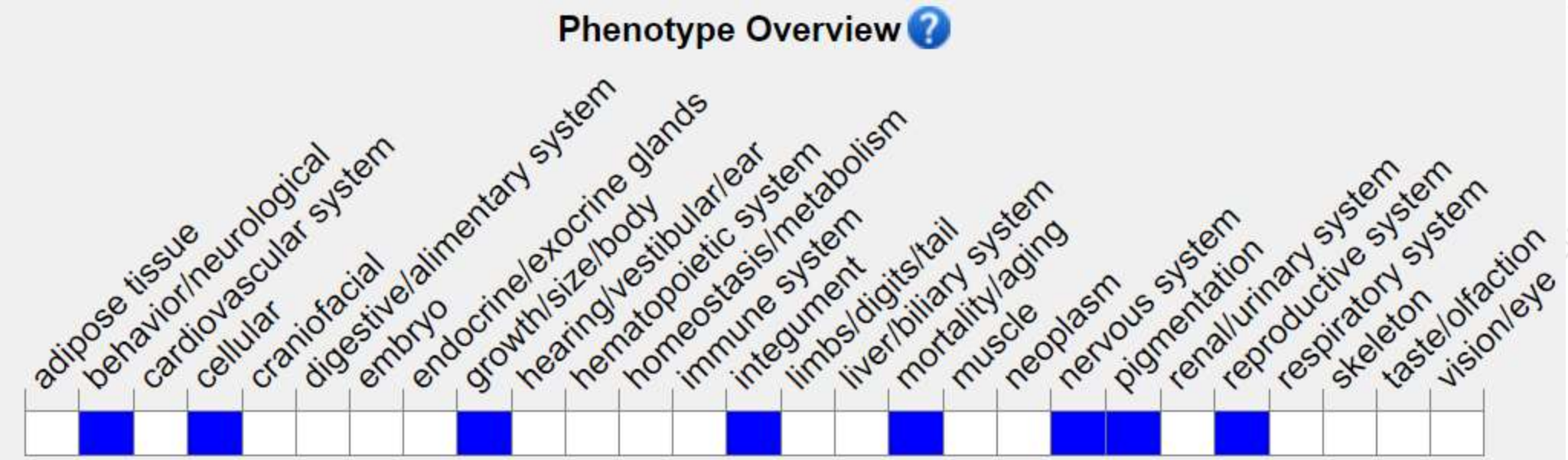


Genomic location distribution



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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