

# *Wwox* Cas9-CKO Strategy

**Designer: Rui Xiong**

**Reviewer: Longyun Hu**

**Design Date: 2021-6-19**

# Project Overview

---

**Project Name**

*Wwox*

---

**Project type**

**Cas9-CKO**

---

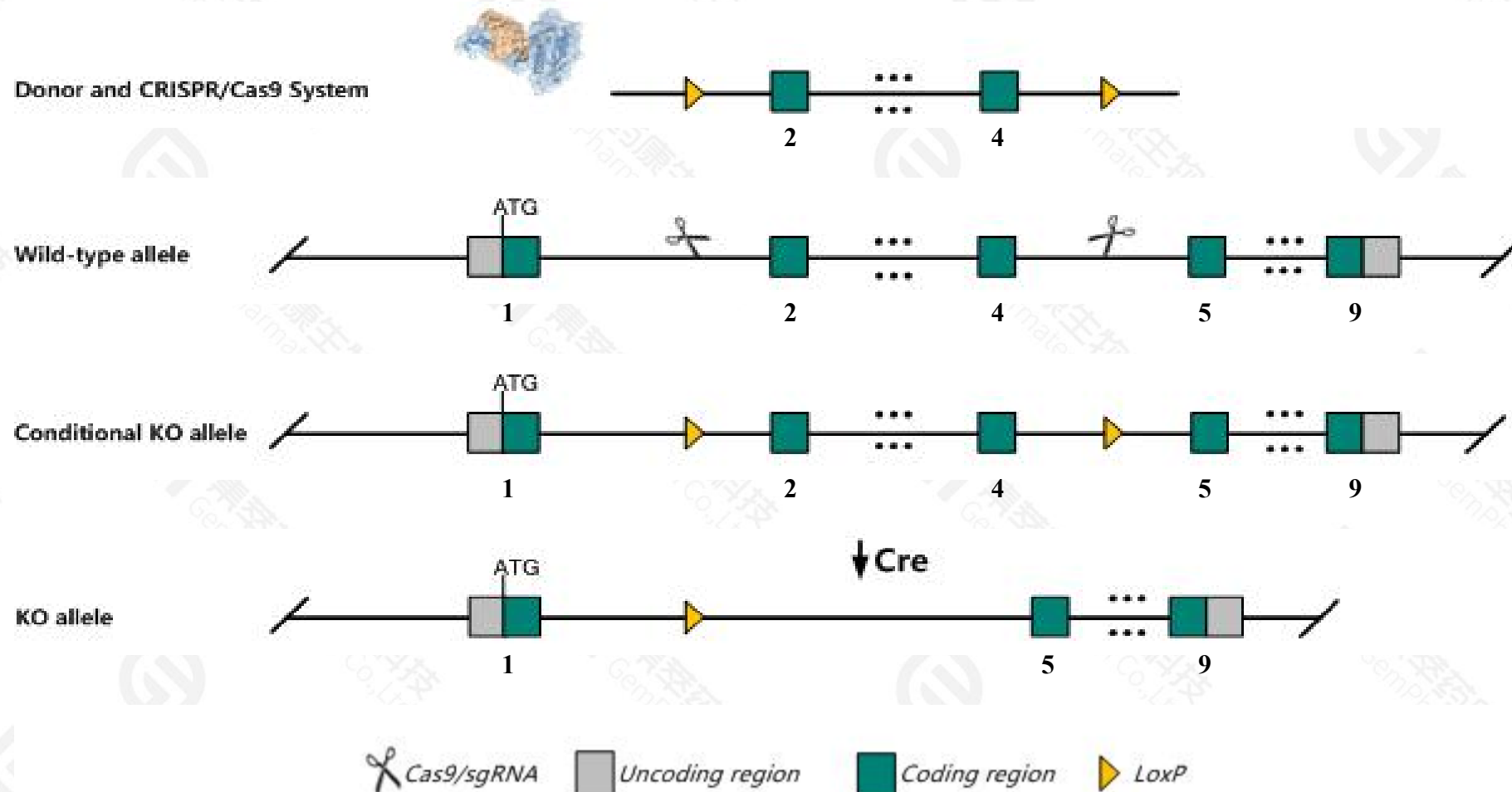
**Strain background**

**C57BL/6JGpt**

---

# Conditional Knockout strategy

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



# Technical routes

- The *Wwox* gene has 9 transcripts. According to the structure of *Wwox* gene, exon2-exon4 of *Wwox*-201(ENSMUST00000004756.14) transcript is recommended as the knockout region. The region contains 302bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Wwox* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor 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 and cell types.



- According to the existing MGI data, homozygous mutation of this gene results in premature death and increased incidence of tumor development. Reduced male fertility and testicular atrophy are also observed in mice with a hypomorphic allele.
- The *Wwox* gene is located on the Chr8. 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)

## Wwox WW domain-containing oxidoreductase [Mus musculus (house mouse)]

Gene ID: 80707, updated on 14-Feb-2021

### Summary



**Official Symbol** Wwox provided by [MGI](#)

**Official Full Name** WW domain-containing oxidoreductase provided by [MGI](#)

**Primary source** [MGI:MGI:1931237](#)

**See related** [Ensembl:ENSMUSG00000004637](#)

**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** 5330426P09Rik, 9030416C10Rik, WOX, WOX1

**Expression** Ubiquitous expression in cerebellum adult (RPKM 2.7), adrenal adult (RPKM 2.5) and 28 other tissues [See more](#)

**Orthologs** [human](#) [all](#)

# Transcript information (Ensembl)

The gene has 9 transcripts,all transcripts are shown below:

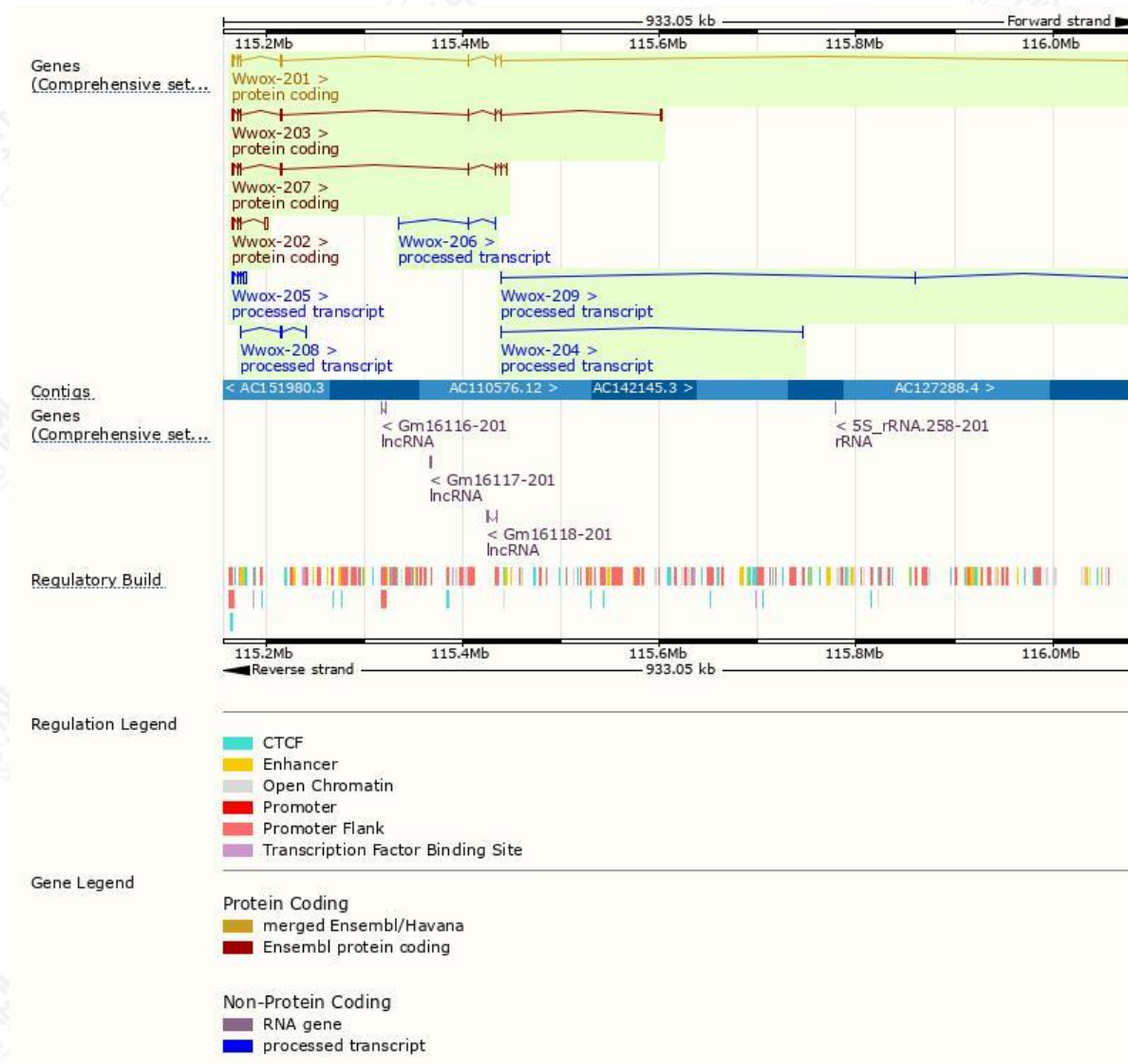
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Wwox-201	<a href="#">ENSMUST00000004756.14</a>	2244	<a href="#">414aa</a>	Protein coding	<a href="#">CCDS22691</a>		TSL:1 , GENCODE basic , APPRIS P1 ,
Wwox-202	<a href="#">ENSMUST00000109107.3</a>	2383	<a href="#">158aa</a>	Protein coding	-		TSL:1 , GENCODE basic ,
Wwox-203	<a href="#">ENSMUST00000109108.9</a>	1771	<a href="#">367aa</a>	Protein coding	-		TSL:1 , GENCODE basic ,
Wwox-207	<a href="#">ENSMUST00000160862.8</a>	1715	<a href="#">354aa</a>	Protein coding	-		TSL:1 , GENCODE basic ,
Wwox-205	<a href="#">ENSMUST00000160005.2</a>	3781	No protein	Processed transcript	-		TSL:1 ,
Wwox-208	<a href="#">ENSMUST00000161753.2</a>	953	No protein	Processed transcript	-		TSL:2 ,
Wwox-209	<a href="#">ENSMUST00000162052.8</a>	683	No protein	Processed transcript	-		TSL:3 ,
Wwox-204	<a href="#">ENSMUST00000159870.2</a>	560	No protein	Processed transcript	-		TSL:3 ,
Wwox-206	<a href="#">ENSMUST00000160205.2</a>	432	No protein	Processed transcript	-		TSL:3 ,

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



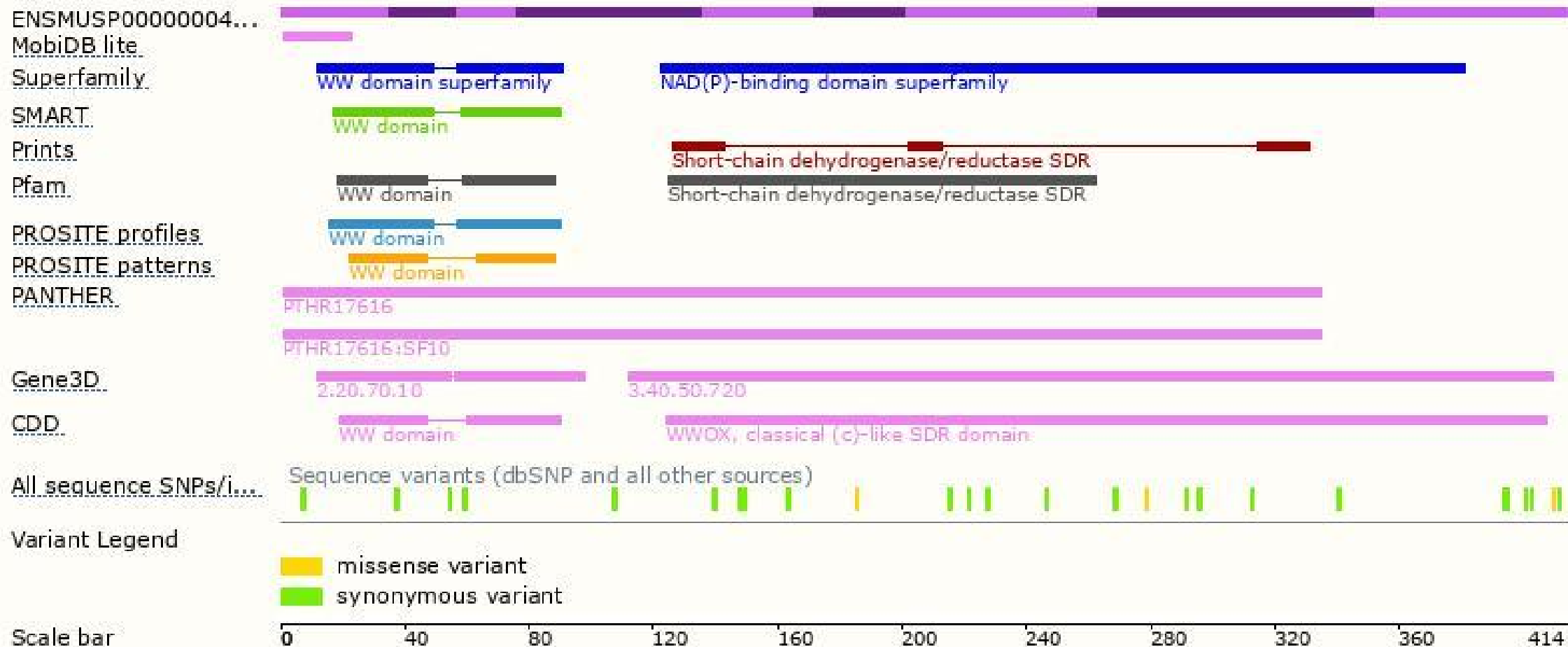


# Genomic location distribution

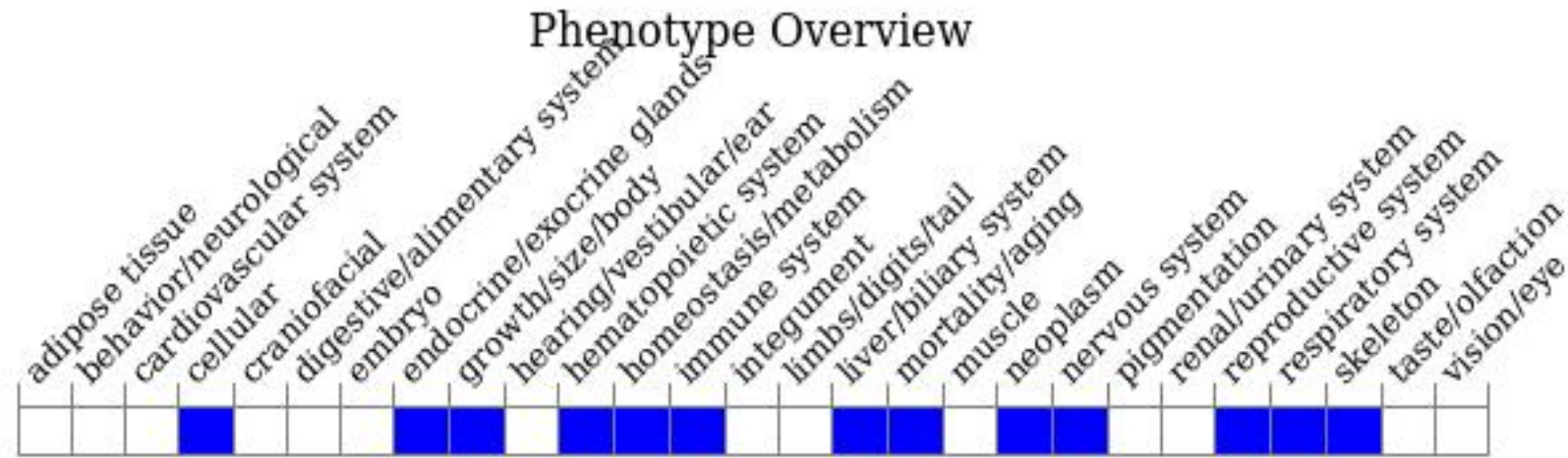




# Protein domain



# 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, homozygous mutation of this gene results in premature death and increased incidence of tumor development. Reduced male fertility and testicular atrophy are also observed in mice with a hypomorphic allele.

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

Tel: 025-5864 1534

