Sox2 Cas9-KO Strategy

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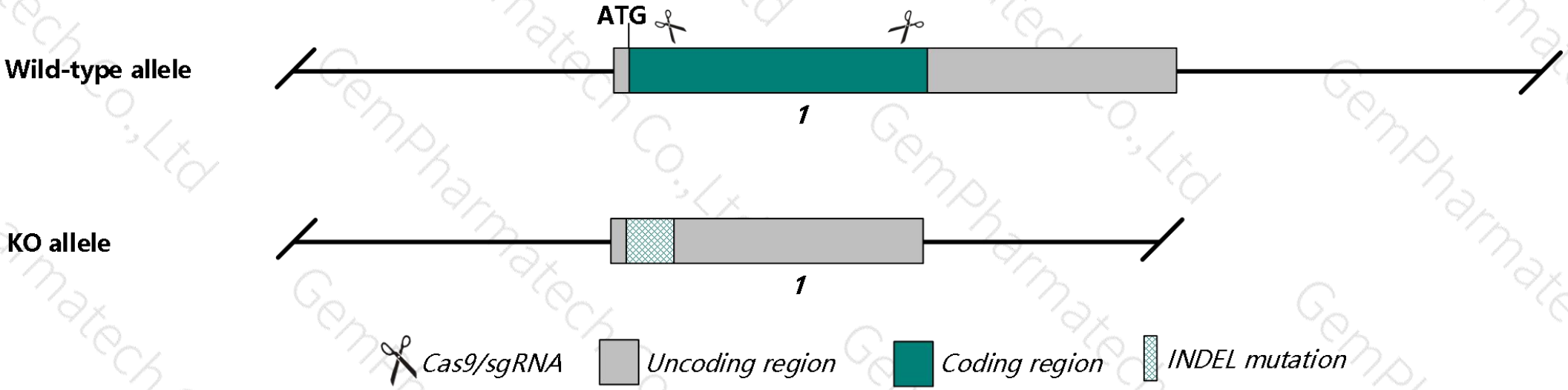
Project Overview

Project Name	***Sox2***
Project type	**Cas9-KO**
Strain background	**C57BL/6J**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Sox2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Sox2* gene has 1 transcript. According to the structure of *Sox2* gene, a part of exon1 of *Sox2-201* (ENSMUST00000099151.5) transcript is recommended as the knockout region. The INDEL mutation on Exon1 will result in frameshift mutation of *Sox2* gene. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Sox2* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.

Notice

➢ According to the existing MGI data, homozygotes for targeted null mutations implant but fail to develop an egg cylinder or epiblast, and die shortly thereafter. Other mutations that affect only regulatory elements show circling behavior and deafness, inner ear defects, and a yellow coat color.

- The distance between *Gm7611* gene and exon6 of *Sox2* is about 0.8 kb, this strategy may directly affect the regulation of *Gm7611* gene thereby affecting gene expression. *Sox2* gene overlaps with the introns of *Sox2ot*, which may have unknown effects on *Sox2ot*.

- The *Sox2* gene is located on the Chr3. 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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Sox2 SRY (sex determining region Y)-box 2 [*Mus musculus* (house mouse)]

Gene ID: 20674, updated on 25-Jun-2019

Summary

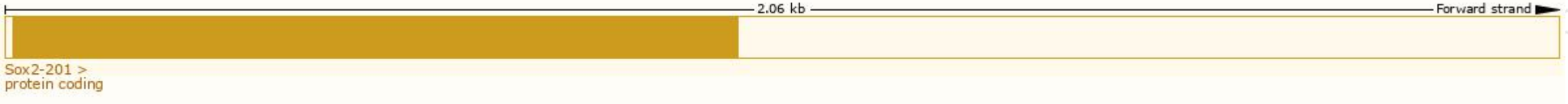
Official Symbol	Sox2 provided by MGI
Official Full Name	SRY (sex determining region Y)-box 2 provided by MGI
Primary source	MGI:MGI:98364
See related	Ensembl:ENSMUSG00000074637
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Icc; ysb; Sox-2
Summary	This intronless gene encodes a member of the SRY-related HMG-box (SOX) family of transcription factors involved in the regulation of embryonic development and in the determination of cell fate. The product of this gene is required for stem-cell maintenance in the central nervous system, and also regulates gene expression in the stomach. Mutations in a similar gene in human have been associated with optic nerve hypoplasia and with syndromic microphthalmia, a severe form of structural eye malformation. This gene lies within an intron of another gene called SOX2 overlapping transcript (Sox2ot). [provided by RefSeq, Sep 2015]
Orthologs	human all

Transcript information（Ensembl）

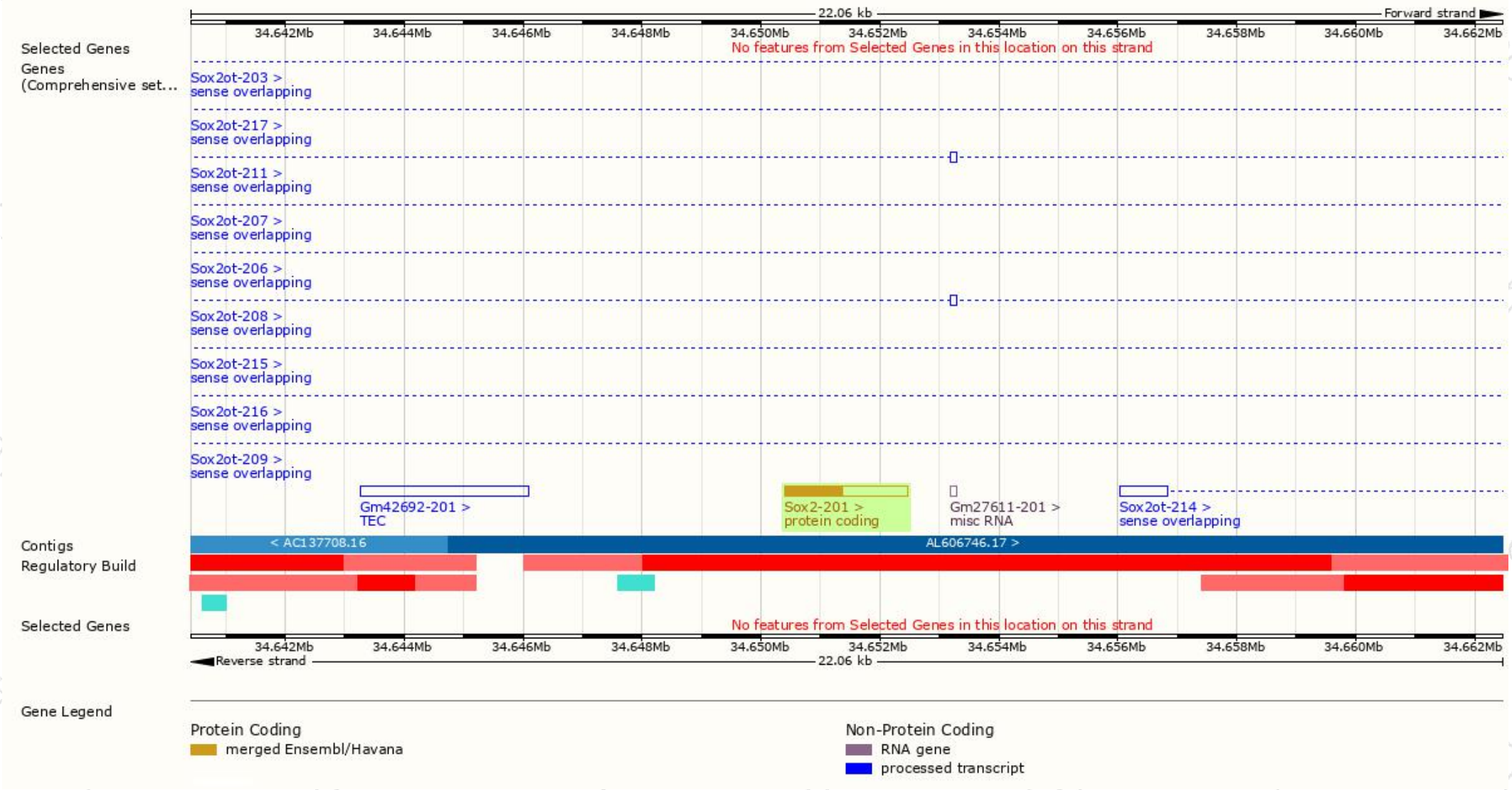
The gene has 1 transcripts,all transcripts are shown below（C57BL/6J）:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Sox2-201	ENSMUST00000099151.5	2057	319aa	Protein coding	CCDS38413	Q60I23	TSL:NA GENCODE basic APPRIS P1

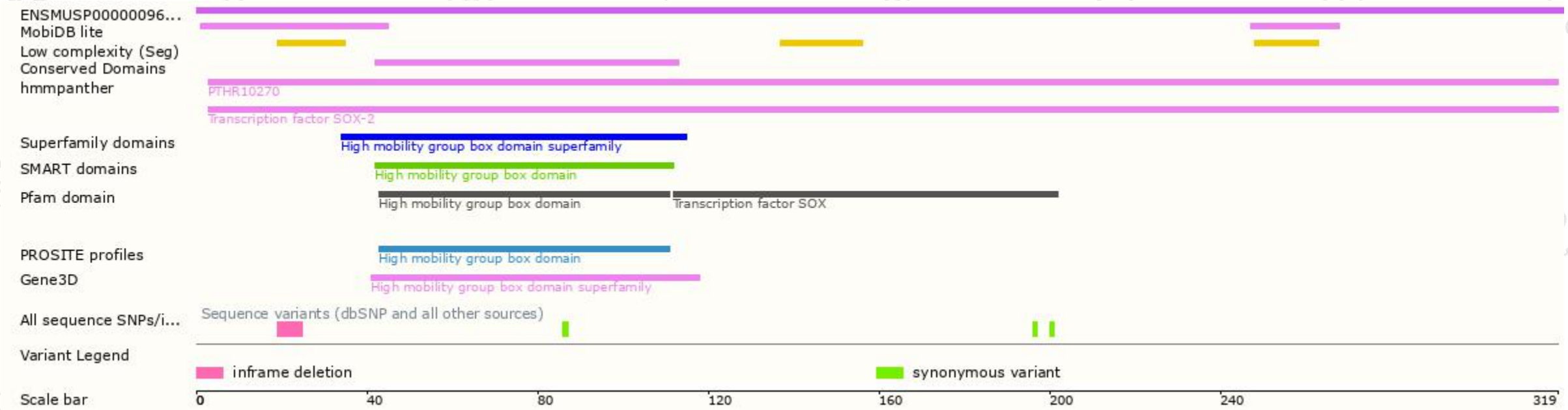
The strategy is based on the design of *Sox2-201* transcript, the transcription is shown below:



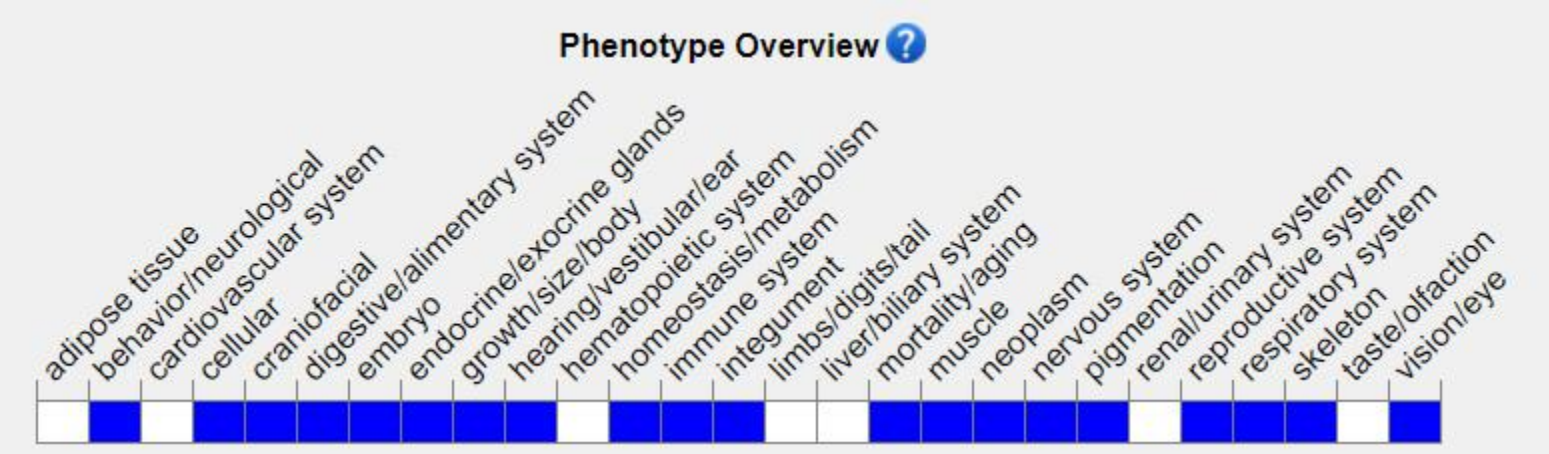
Genomic location distribution



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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