Nr0b1 Cas9-CKO *Strategy*

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Reviewer. **Huimin Su**
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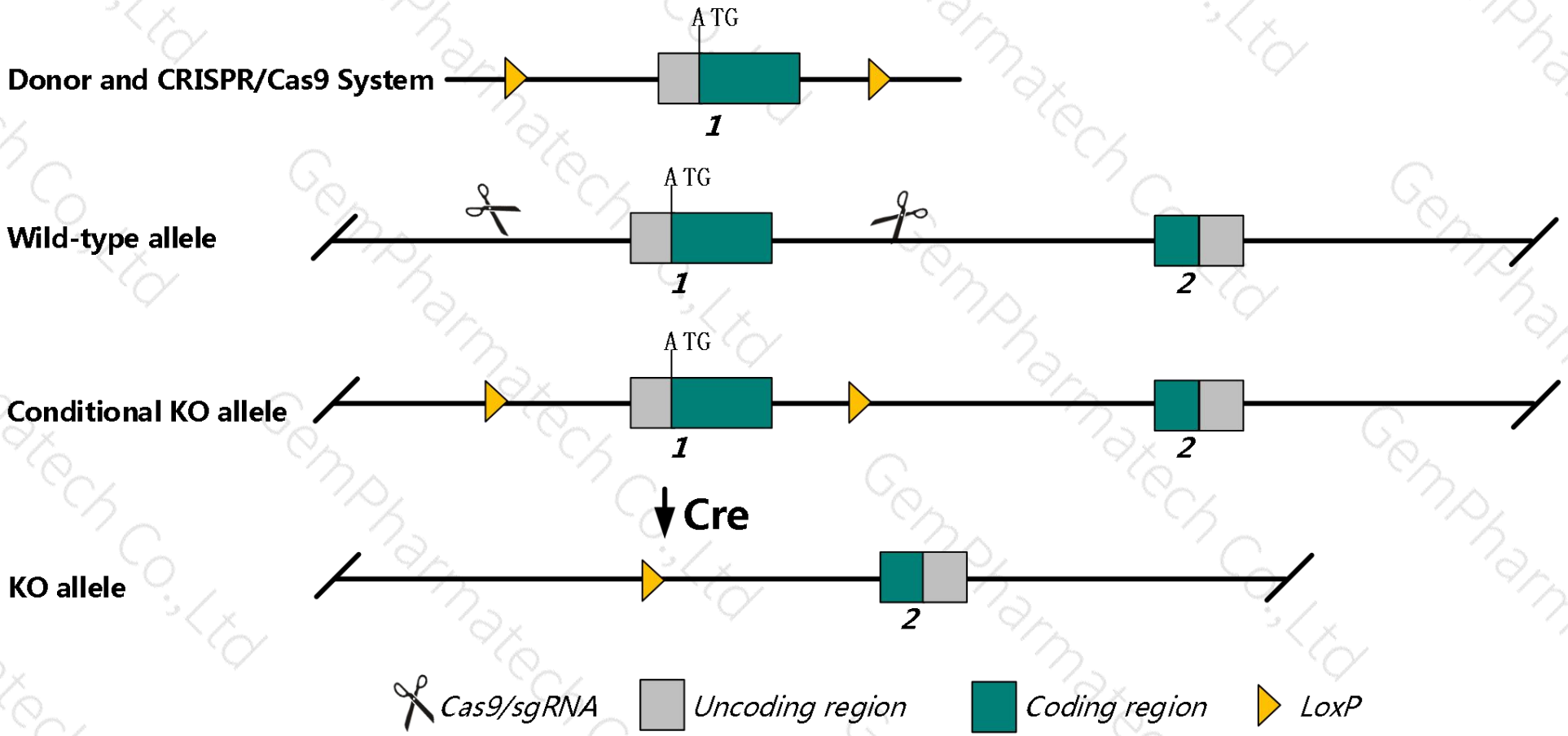
Project Overview

Project Name	***Nr0b1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Nr0b1* gene. The schematic diagram is as follows:



Technical routes

- The *Nr0b1* gene has 1 transcript1. According to the structure of *Nr0b1* gene, exon1 of *Nr0b1*-201 (ENSMUST00000026036.4)transcript is recommended as the knockout region.The region contains start codon ATG .Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Nr0b1* 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 or cell types.

Notice

- According to the existing MGI data，mutations that inactivate this X-linked gene result in abnormal repoductive development in the hemizygote, ranging from defects in testes development and spermatogenesis to complete male to female sex reversal depending on genetic background.
- The *Nr0b1* 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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Nr0b1 nuclear receptor subfamily 0, group B, member 1 [*Mus musculus* (house mouse)]

Gene ID: 11614, updated on 12-Aug-2019

Summary

Official Symbol	Nr0b1 provided by MGI
Official Full Name	nuclear receptor subfamily 0, group B, member 1 provided by MGI
Primary source	MGI:MGI:1352460
See related	Ensembl:ENSMUSG00000025056
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	AHX; Ahc; Ahch; Dax1; DAX-1
Summary	This gene encodes an orphan nuclear receptor protein that plays a key role in differentiation of the gonads. This protein regulates steroidogenic factor 1 (Sf-1) in a dose-dependent manner, sometimes functioning as a repressor of SF-1 target genes, and sometimes functioning as a co-activator. Overexpression of this gene can cause feminization of the XY male gonads. This gene is also involved in the maintenance of embryonic stem cell pluripotancy. Mutations in the related gene in human cause congenital adrenal hypoplasia and hypogonadotropic hypogonadism. [provided by RefSeq, May 2015]
Expression	Biased expression in adrenal adult (RPKM 34.4), ovary adult (RPKM 8.3) and 1 other tissue See more
Orthologs	human all

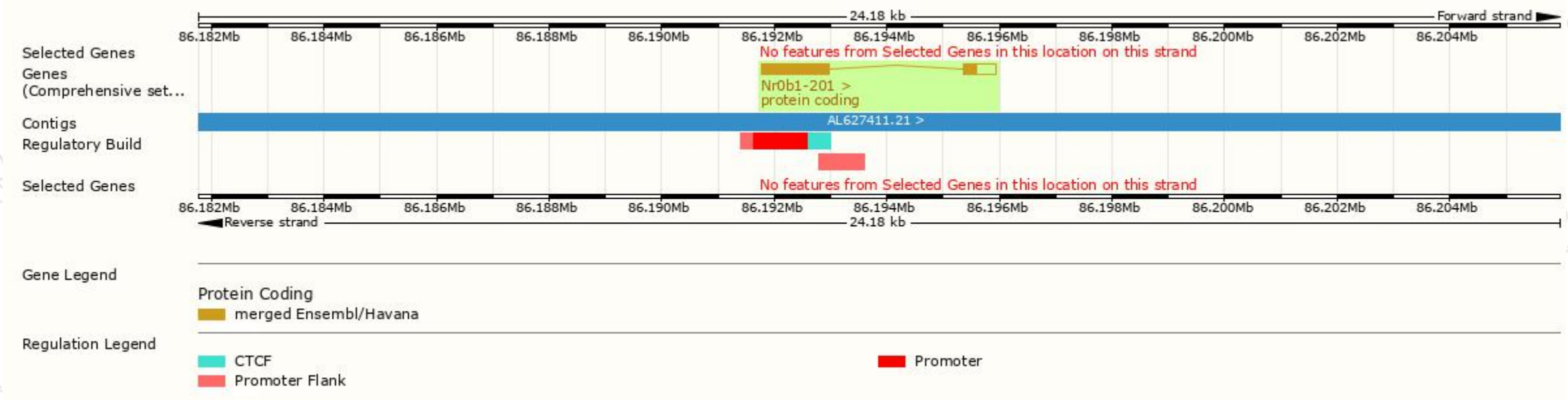
Transcript information（Ensembl）

The gene has 1 transcript, and all transcripts are shown below:

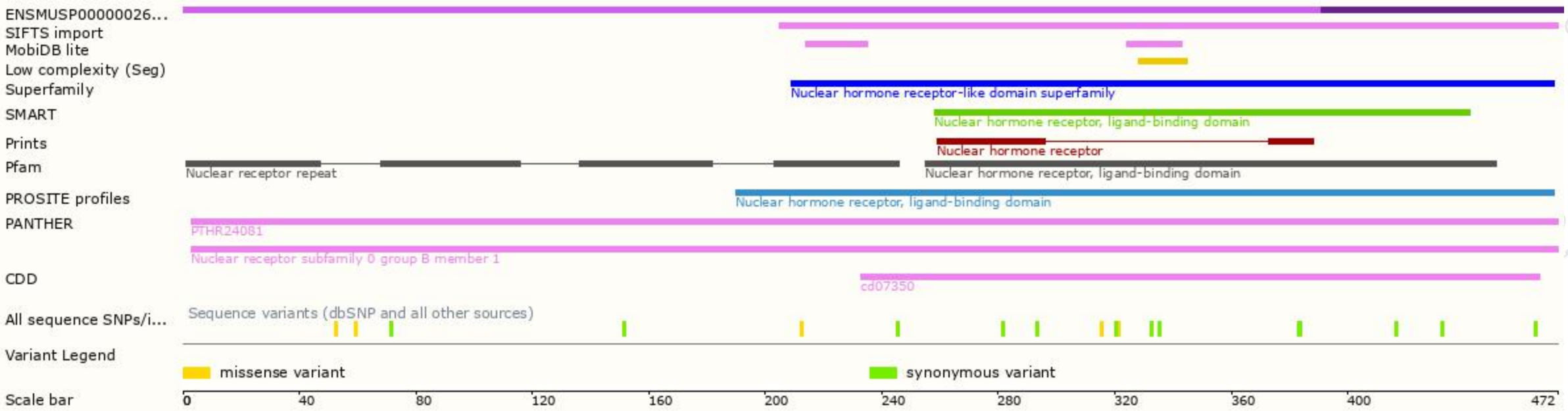
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nr0b1-201	ENSMUST00000026036.4	1808	472aa	Protein coding	CCDS30260	Q53ZY9 Q61066	TSL:1 GENCODE basic APPRIS P1

The strategy is based on the design of *Nr0b1*-201 transcript,The transcription is shown below

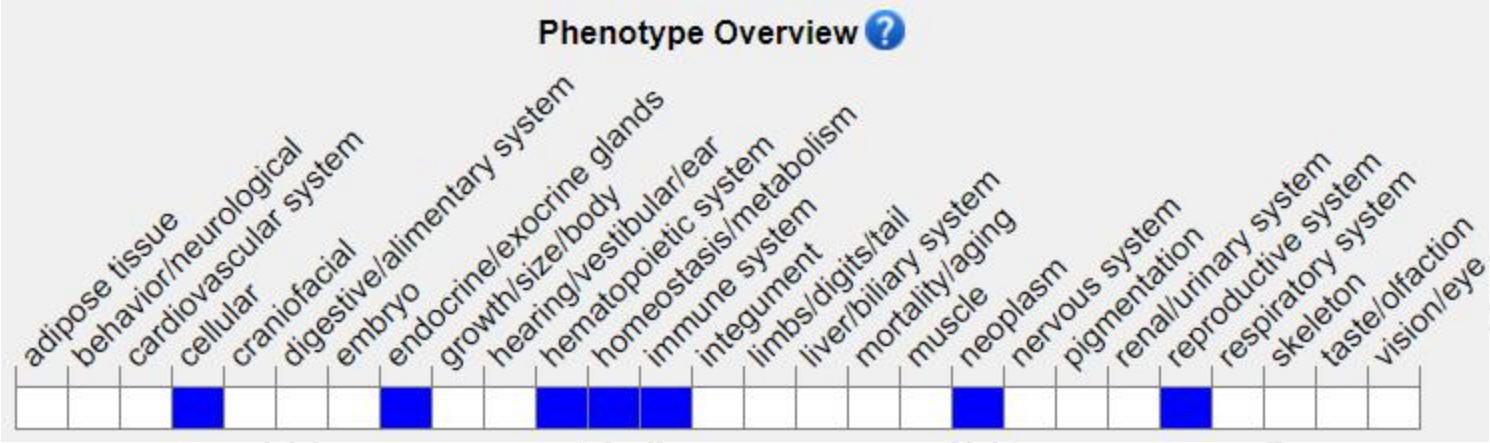
Genomic location distribution



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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