Pax1 Cas9-CKO Strategy

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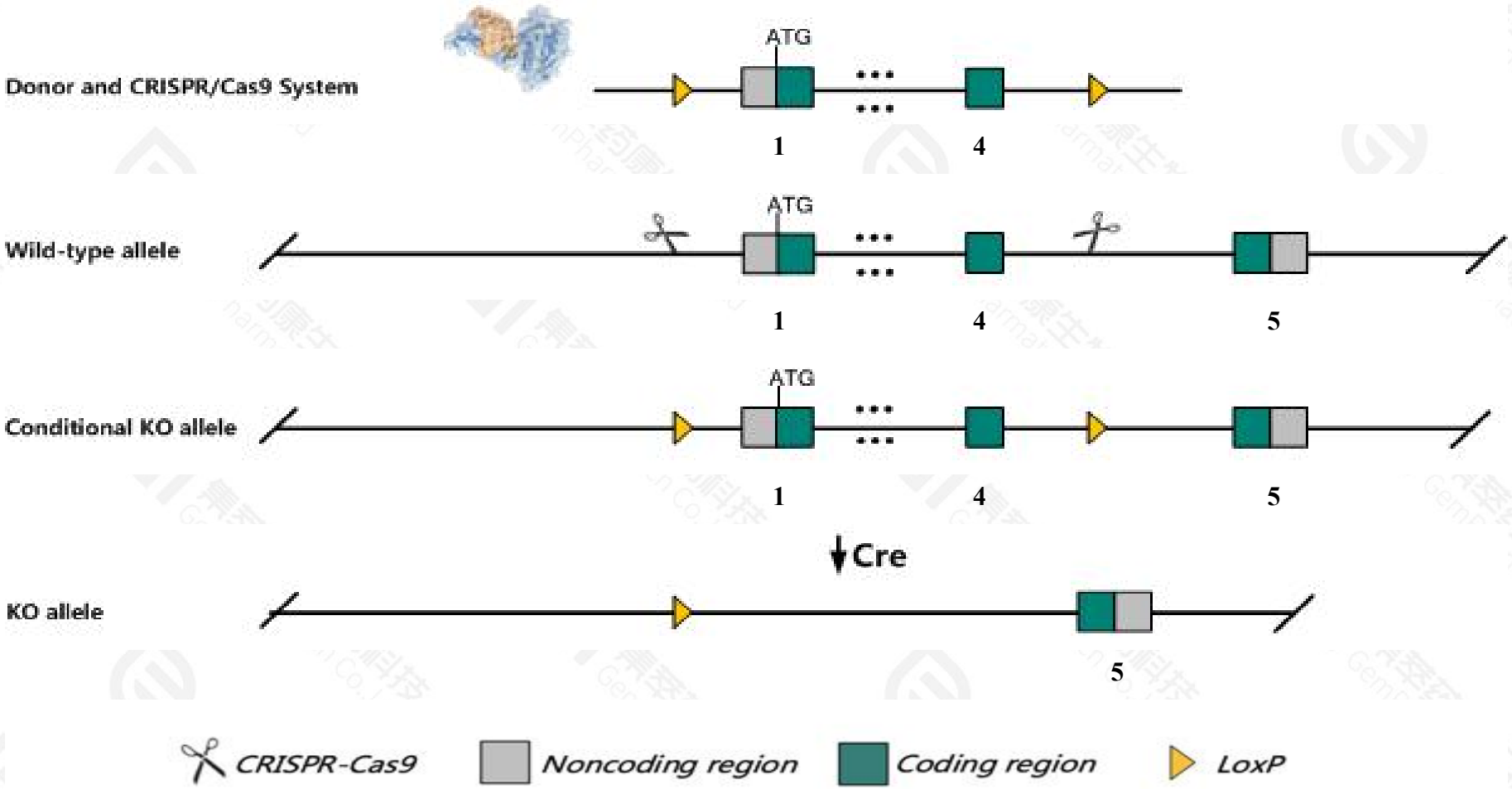
Project Overview

Project Name	***Pax1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR-Cas9 technology to edit the *Pax1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Pax1* gene has 3 transcripts. According to the structure of *Pax1* gene, exon1-exon4 of *Pax1-201*(ENSMUST00000109968.3) 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 *Pax1* gene. The brief process is as follows: CRISPR-Cas9 system 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.

Notice

➢ According to the existing MGI data,homozygotes for several mutations exhibit variably severe morphological alterations of vertebral column, sternum, scapula, skull, and thymus, with reduced adult survival and fertility. Some heterozygotes show milder skeletal abnormalities.

➢ The insertion of 5-terminal Loxp may affect the 5-terminal regulation of *AI646519* and *Pax1* gene.

➢ This strategy may affect the regulation of the 5 terminus of *AI646519* gene.

➢ The *Pax1* gene is located on the Chr2. 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）

集萃药康 GemPharmatech

Pax1 paired box 1 [Mus musculus (house mouse)]

Gene ID: 18503, updated on 13-Mar-2020

Summary

Official Symbol	Pax1 provided by MGI
Official Full Name	paired box 1 provided byMGI
Primary source	MGI:MGI:97485
See related	Ensembl:ENSMUSG00000037034
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	Pax-1, hbs, hunchback, un, undulated, wt
Expression	Biased expression in thymus adult (RPKM 22.9), testis adult (RPKM 3.3) and 2 other tissuesSee more
Orthologs	human all

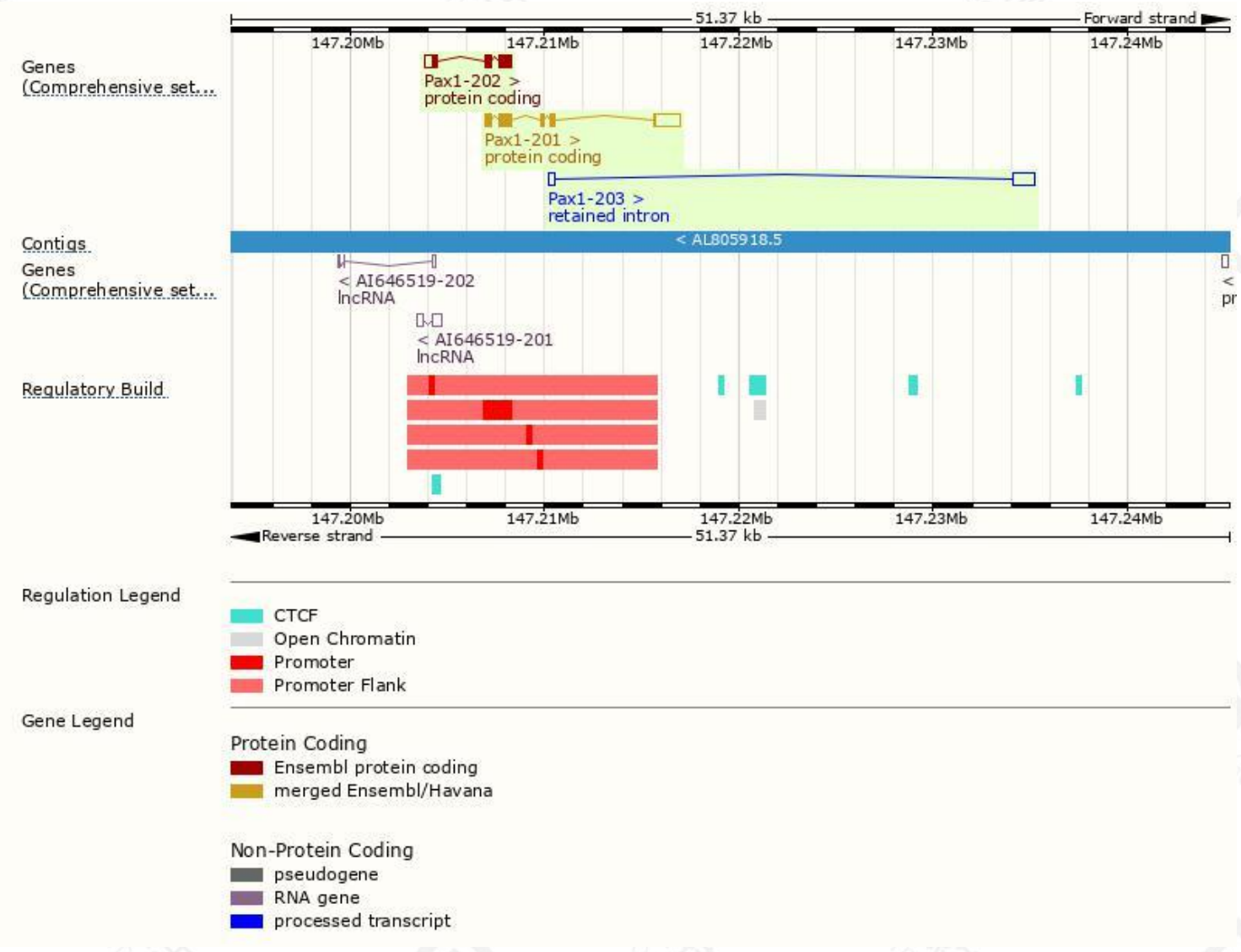
Transcript information（Ensembl）

The gene has 3 transcripts,all transcripts are shown below:

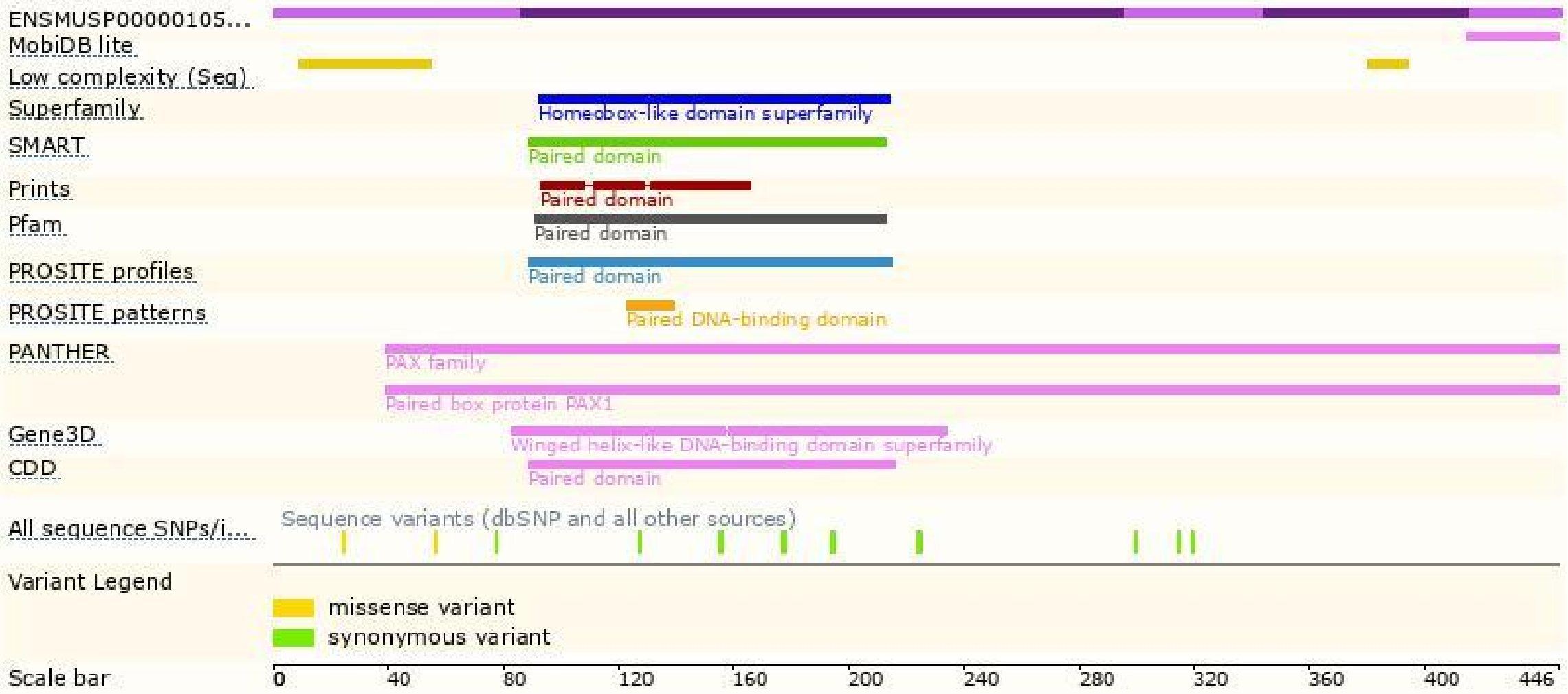
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pax1-201	ENSMUST00000109968.2	2643	446aa	Protein coding	CCDS16835	P09084	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Pax1-202	ENSMUST00000126068.8	1529	384aa	Protein coding	-	A2ALK0	CDS 3' incomplete TSL:5
Pax1-203	ENSMUST00000156584.1	1457	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Pax1-201* transcript,the transcription is shown below:

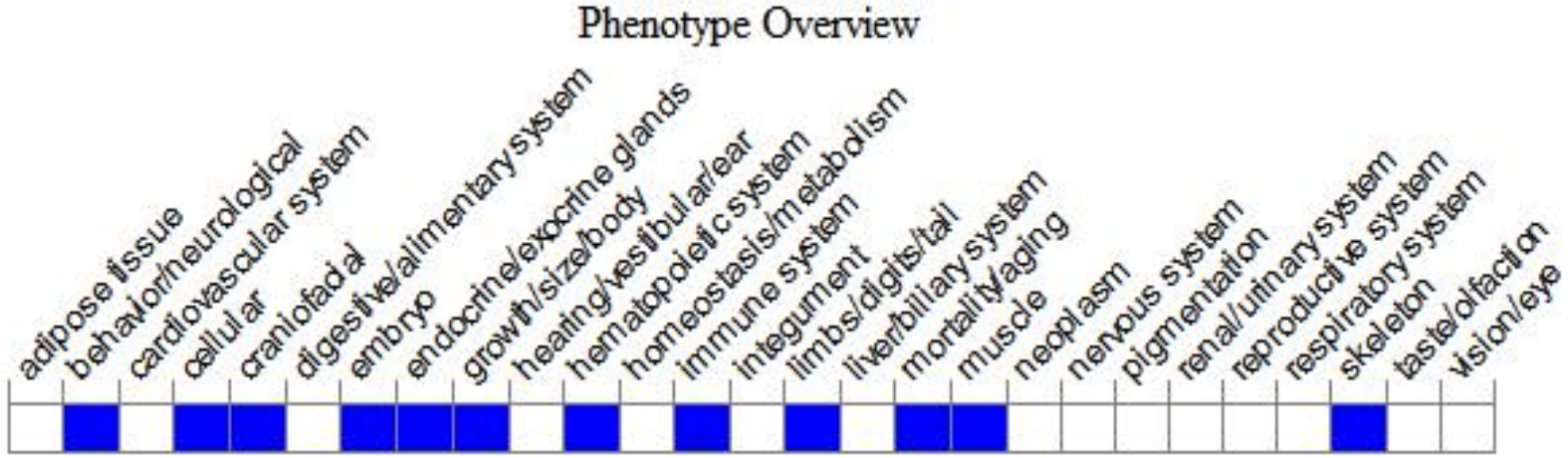
Genomic location distribution



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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