GemPharmatech™

Pax8 Cas9-KO Strategy

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Reviewer: Yanhua Shen

Design Date: 2022-12-6

Overview

Target Gene Name

- Pax8

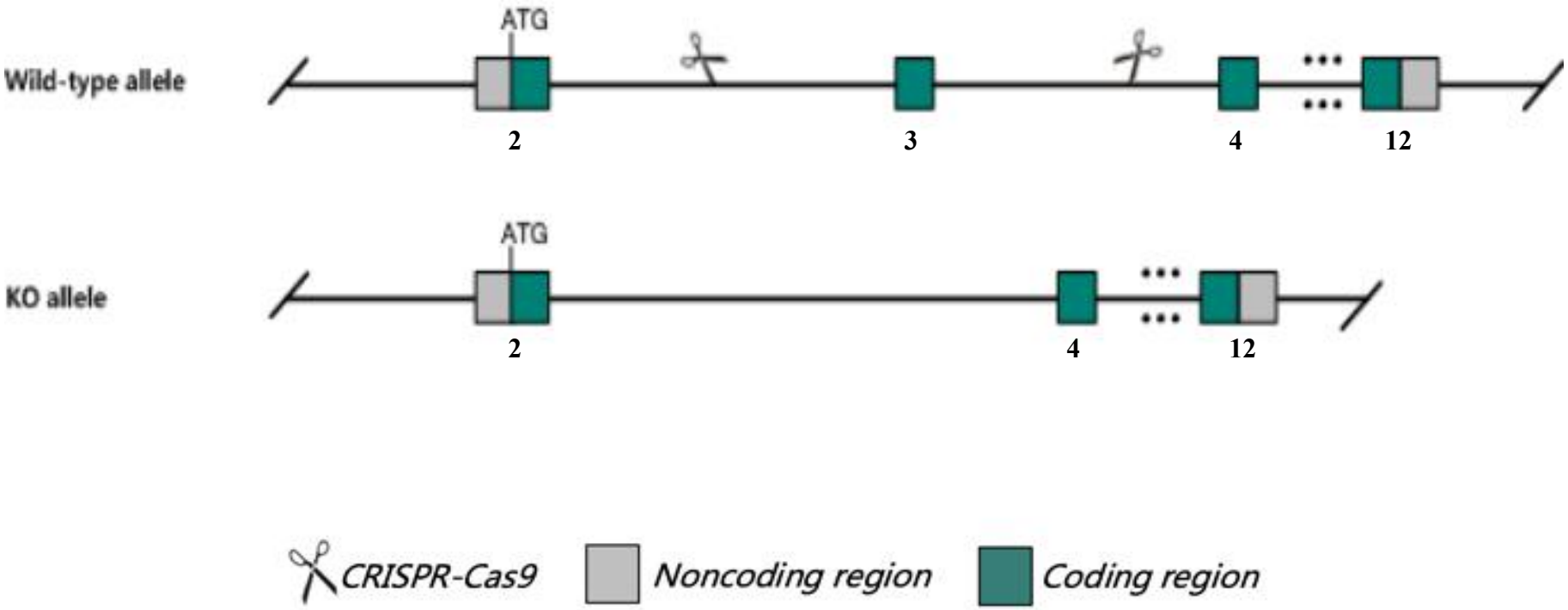
Project Type

- Cas9-KO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Pax8* gene.

Technical Information

- The *Pax8* gene has 8 transcripts. According to the structure of *Pax8* gene, exon3 of *Pax8-201* (ENSMUST00000028355.11) transcript is recommended as the knockout region. The region contains 166bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Pax8* gene. The brief process is as follows: gRNAs were transcribed in vitro. Cas9 and gRNAs 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.

Gene Information

Pax8 paired box 8 [*Mus musculus* (house mouse)]

Download Datasets

Gene ID: 18510, updated on 22-Nov-2022

Summary

Official Symbol	Pax8 provided by MGI
Official Full Name	paired box 8 provided by MGI
Primary source	MGI:MGI:97492
See related	Ensembl:ENSMUSG00000026976 AllianceGenome:MGI:97492
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	Pax-8
Summary	This gene encodes a member of a family of transcription factors that contain a characteristic N-terminal paired DNA-binding domain. The encoded protein is important for proper differentiation of the thyroid and the kidney. Alternatively spliced transcript variants of this gene have been described, but their full-length nature is not known. [provided by RefSeq, Mar 2013]
Expression	Biased expression in genital fat pad adult (RPKM 36.6), kidney adult (RPKM 27.4) and 5 other tissues See more
Orthologs	human all
NEW	Try the new Gene table Try the new Transcript table

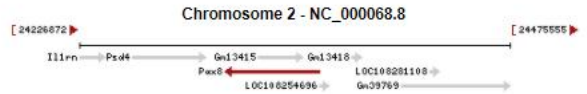
Genomic context

Location: 2 A3; 2 16.43 cM

See Pax8 in Genome Data Viewer

Exon count: 12

Annotation release	Status	Assembly	Chr	Location
109	current	GRCm39 (GCF_000001635.27)	2	NC_000068.8 (24310563..24365611, complement)
108.20200622	previous assembly	GRCm38.p6 (GCF_000001635.26)	2	NC_000068.7 (24420551..24475599, complement)



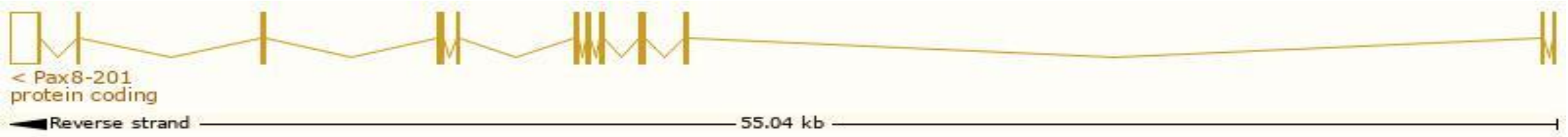
Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

The gene has 8 transcripts, all transcripts are shown below:

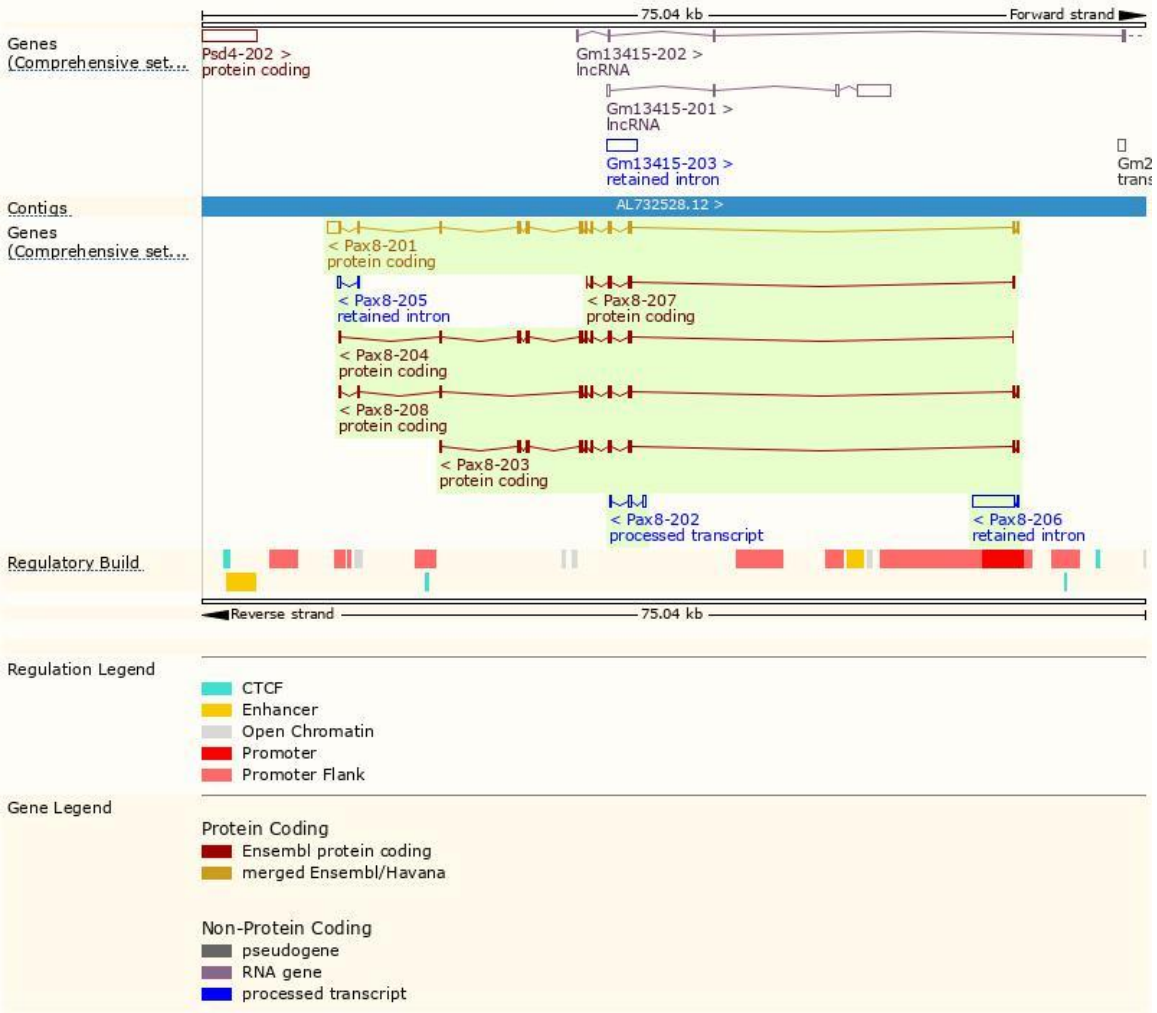
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pax8-201	ENSMUST00000028355.11	2560	457aa	Protein coding	CCDS15738		TSL:1 , GENCODE basic , APPRIS P1 ,
Pax8-203	ENSMUST00000136228.10	1381	404aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Pax8-204	ENSMUST00000149294.8	1236	412aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Pax8-208	ENSMUST00000153601.8	1226	322aa	Protein coding	-		TSL:5 , GENCODE basic ,
Pax8-207	ENSMUST00000153535.2	626	169aa	Protein coding	-		CDS 3' incomplete , TSL:3 ,
Pax8-202	ENSMUST00000133746.2	554	No protein	Processed transcript	-		TSL:2 ,
Pax8-206	ENSMUST00000152614.2	3386	No protein	Retained intron	-		TSL:2 ,
Pax8-205	ENSMUST00000149860.2	345	No protein	Retained intron	-		TSL:3 ,

The strategy is based on the design of *Pax8-201* transcript, the transcription is shown below:



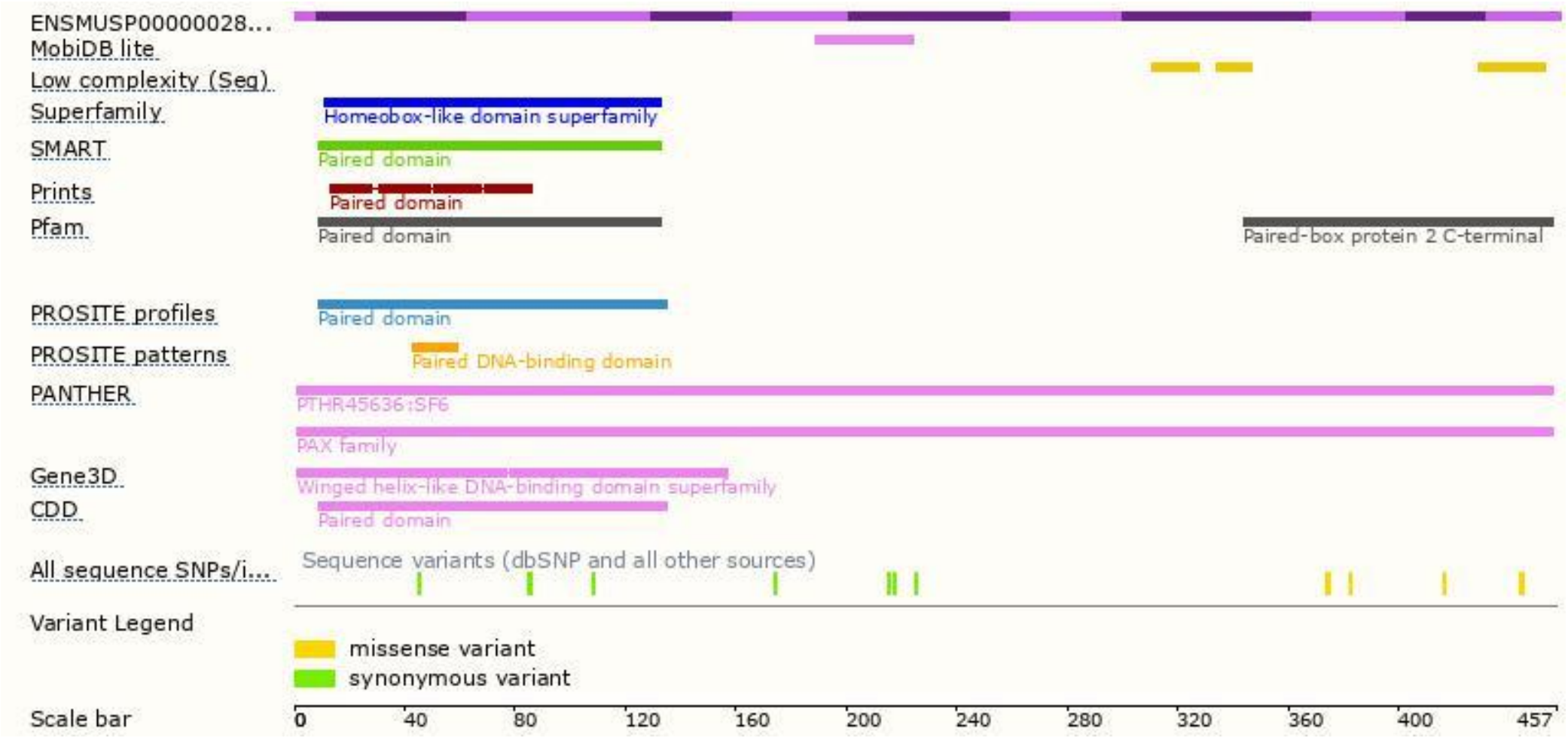
Source: https://www.ensembl.org

Genomic Information



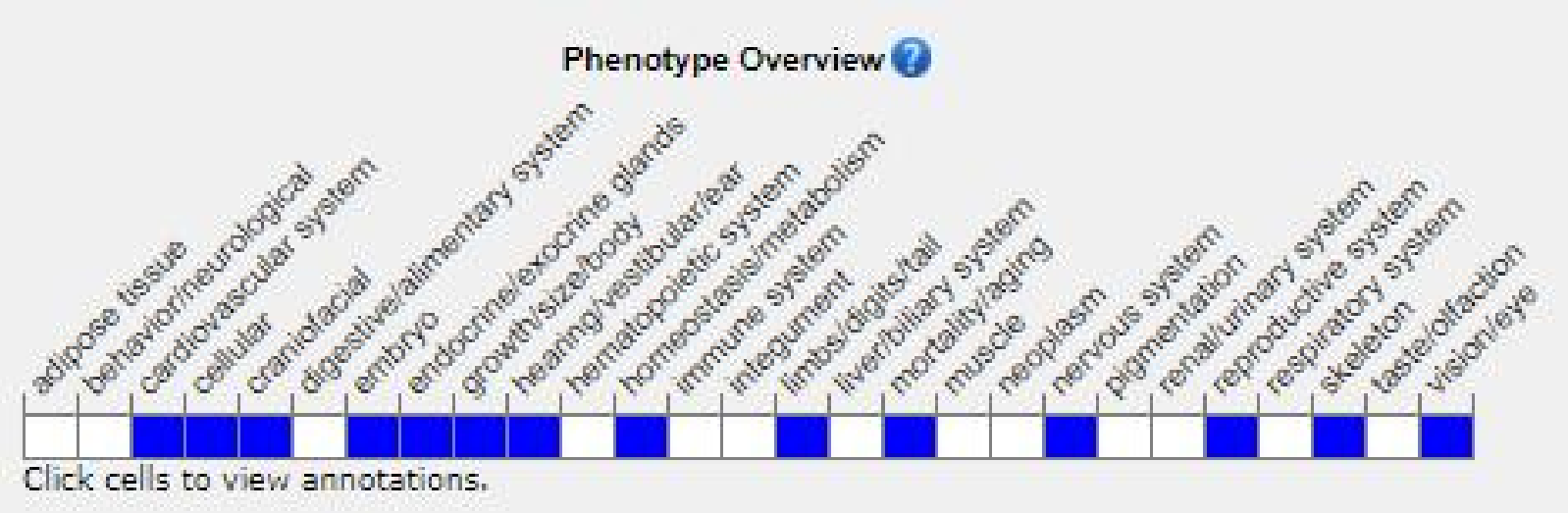
Source: : https://www.ensembl.org

Protein Information



Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



- Homozygotes for targeted mutations exhibit severe hypothyroidism due to thyroid follicular cell aplasia, male infertility, deafness, ataxia, growth retardation, tiny spleens, impaired ossification of long bones and maturation of the small intestine, fatty livers, and lethality around weaning age.

Source: https://www.informatics.jax.org

Important Information

- According to the breeding data, the gene knockout homozygous mice died at the embryonic stage.
- When the target gene is knocked out, the *Gm13415* gene will also be destroyed, and the risk is unknown.
- *Pax8* is located on Chr2. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.