Piwil4 Cas9-KO Strategy

Designer: Huimin Su

Reviewer: Ruiuri Zhang

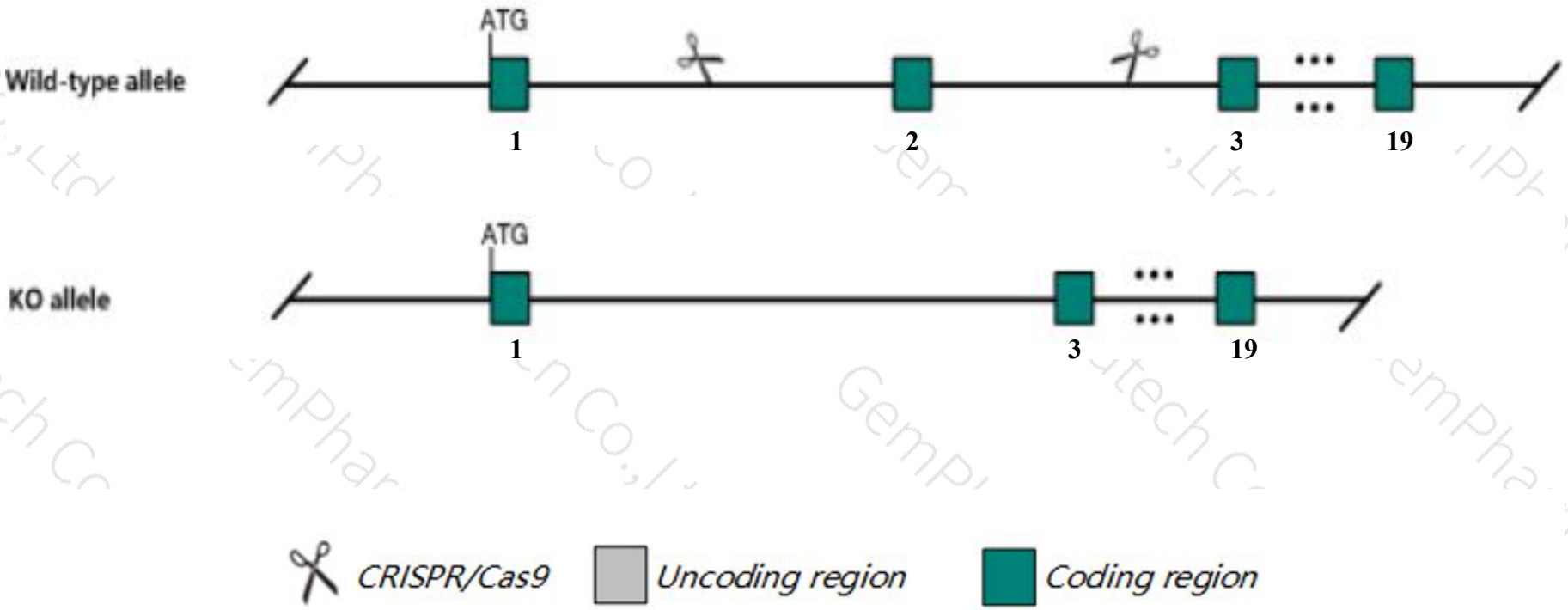
Design Date: 2020-5-12

Project Overview

Project Name	***Piwil4***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Piwil4* gene. The schematic diagram is as follows:



Technical routes

➢ The *Piwil4* gene has 4 transcripts. According to the structure of *Piwil4* gene, exon2 of *Piwil4-201* (ENSMUST00000076946.3) transcript is recommended as the knockout region.The region contains 64bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Piwil4* gene. The brief process is as follows: CRISPR/Cas9 system

Notice

- According to the existing MGI data,mice homozygous for a null allele exhibit male specific-infertility with a progressive loss of male germ cells, reduced testis size, abnormal male meiosis and increased apoptosis of spermatocytes in seminiferous tubules.
- Transcript *Piwil4-204* is incomplete, so the effect on it is unknown.
- The *Piwil4* gene is located on the Chr9. 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）

集萃药康 GemPharmatech

Piwil4 piwi-like RNA-mediated gene silencing 4 [*Mus musculus* (house mouse)]

Gene ID: 330890, updated on 3-May-2020

Summary

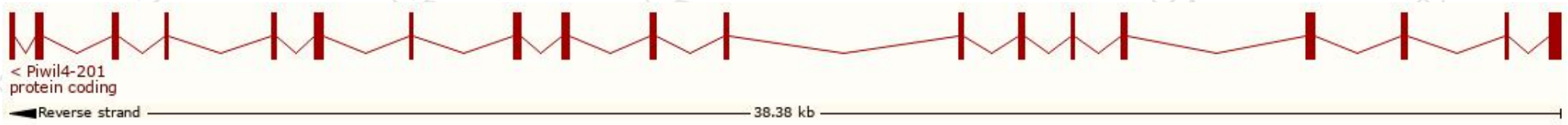
Official Symbol	Piwil4 provided by MGI
Official Full Name	piwi-like RNA-mediated gene silencing 4 provided by MGI
Primary source	MGI:MGI:3041167
See related	Ensembl:ENSMUSG00000036912
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Miwi2; mAgo5; 9230101H05Rik
Expression	Biased expression in genital fat pad adult (RPKM 2.0), colon adult (RPKM 1.2) and 10 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

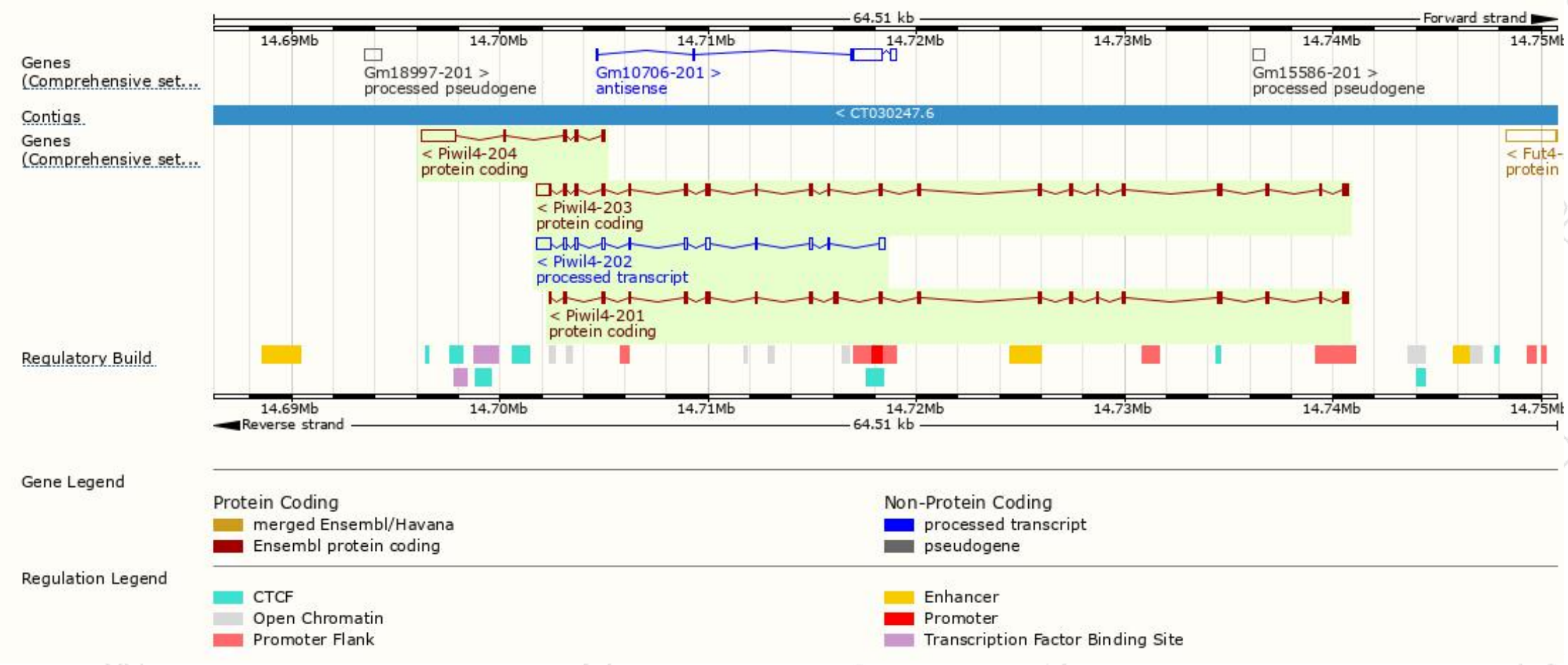
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Piwil4-201	ENSMUST00000076946.3	2637	878aa	Protein coding	CCDS22824	A0A0R4J0Y7	TSL:2 GENCODE basic
Piwil4-203	ENSMUST00000115644.9	3323	911aa	Protein coding	-	Q8CGT6	TSL:5 GENCODE basic APPRIS P1
Piwil4-204	ENSMUST00000136399.7	2190	174aa	Protein coding	-	F6VRE5	CDS 5' incomplete TSL:1
Piwil4-202	ENSMUST00000115643.7	2111	No protein	Processed transcript	-	-	TSL:1

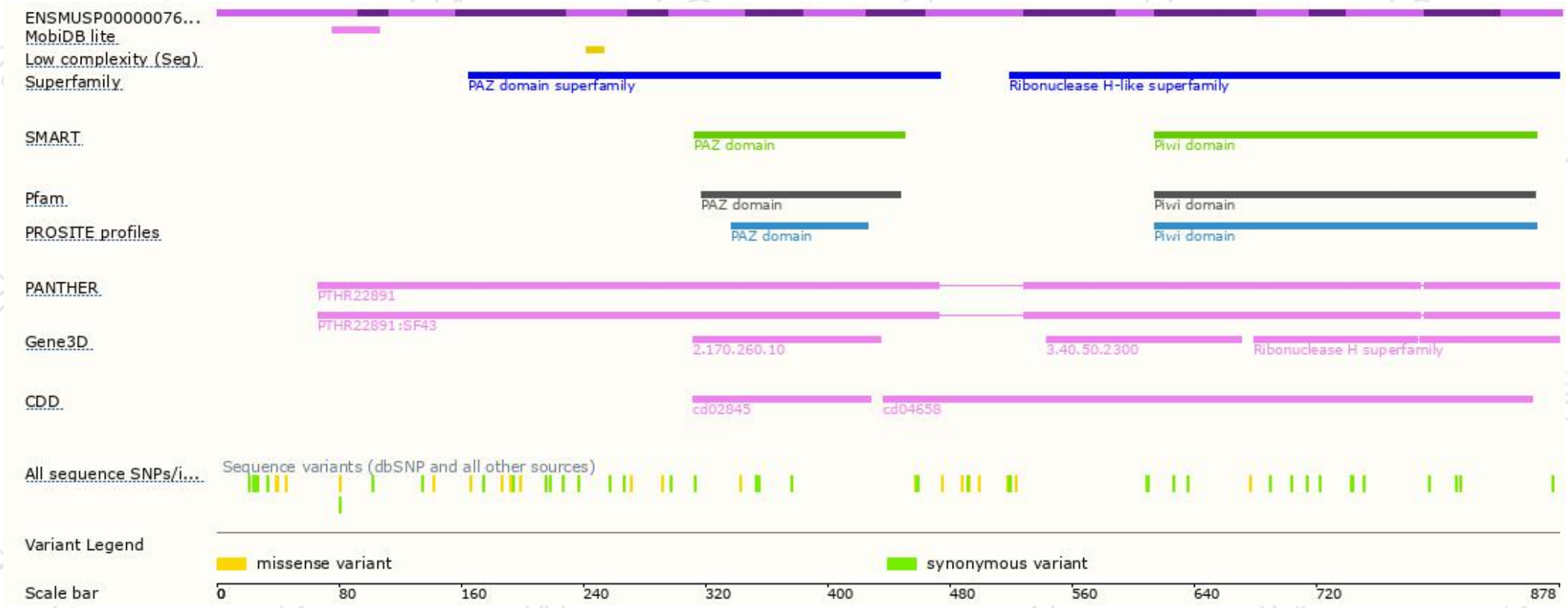
The strategy is based on the design of *Piwil4-201* transcript, the transcription is shown below



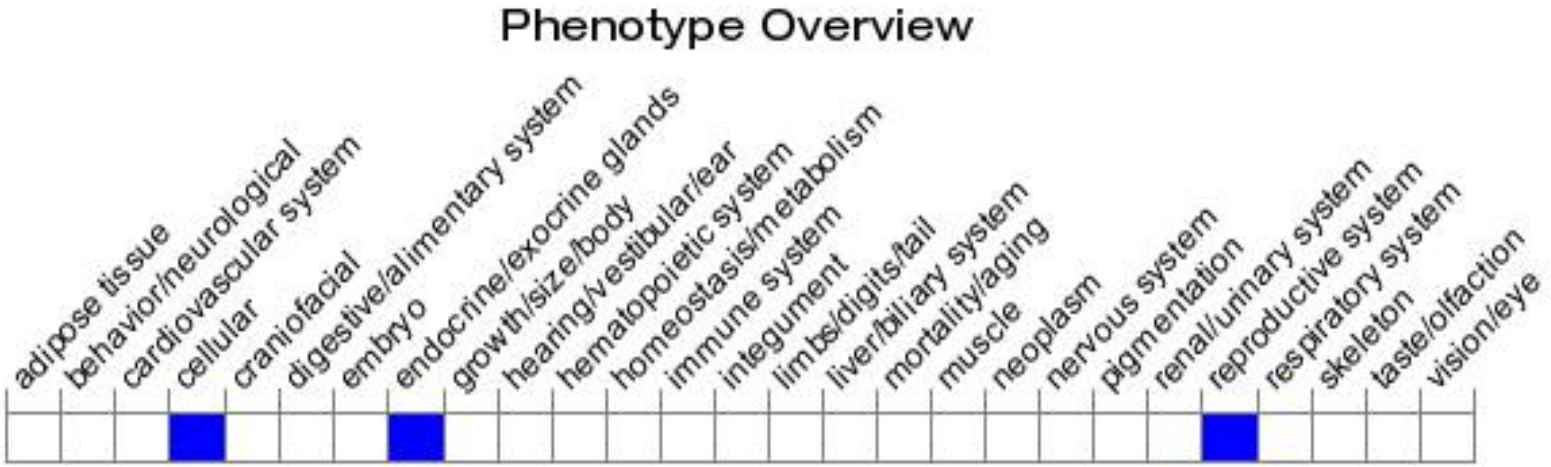
Genomic location distribution



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, mice homozygous for a null allele exhibit male specific-infertility with a progressive loss of male germ cells, reduced testis size, abnormal male meiosis and increased apoptosis of spermatocytes in seminiferous tubules.

集萃药康
GemPharmatech

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd