

Piwil2 Cas9-CKO Strategy

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Reviewer:

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Design Date:

2019-9-19

Project Overview

Project Name

Piwil2

Project type

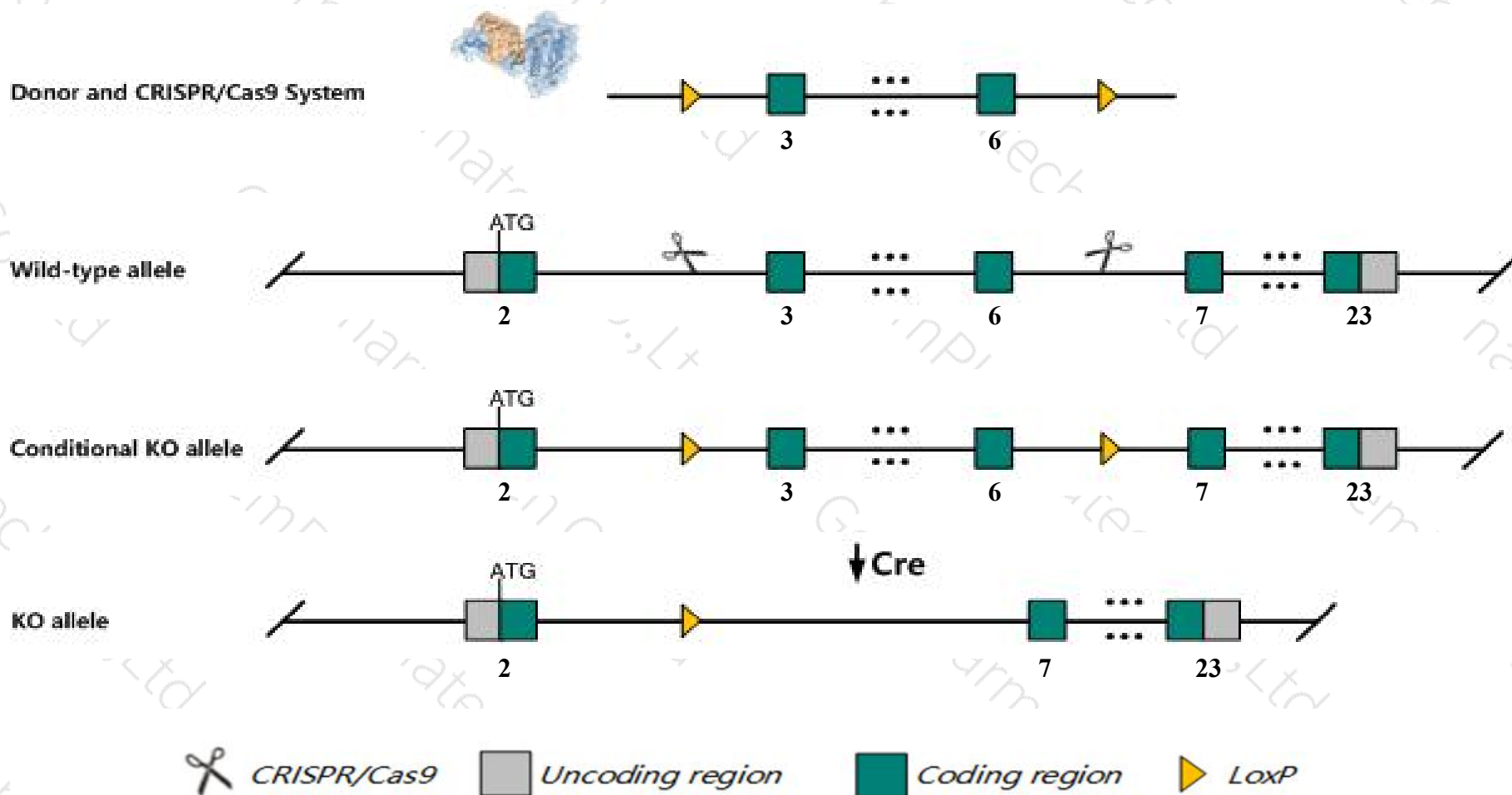
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Piwi2* gene. The schematic diagram is as follows:



- The *Piwil2* gene has 3 transcripts. According to the structure of *Piwil2* gene, exon3-exon6 of *Piwil2-201* (ENSMUST00000048129.5) transcript is recommended as the knockout region. The region contains 545bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Piwil2* 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.

- According to the existing MGI data, Mice homozygous for a knock-out allele exhibit decreased testis weight, azoospermia, and male infertility associated with a complete arrest of spermatogenesis and increased apoptotic cell death during the early prophase of the first meiosis.
- The KO region contains functional region of the *Gm22725* gene. Knockout the region may affect the function of *Gm22725* gene.
- The *Piwi2* gene is located on the Chr14. 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)

Piwi2 piwi-like RNA-mediated gene silencing 2 [*Mus musculus* (house mouse)]

Gene ID: 57746, updated on 17-Sep-2019

Summary

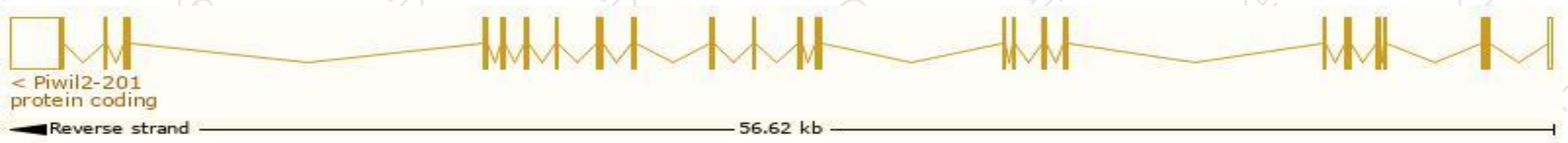
Official Symbol	Piwi2 provided by MGI
Official Full Name	piwi-like RNA-mediated gene silencing 2 provided by MGI
Primary source	MGI:MGI:1930036
See related	Ensembl:ENSMUSG00000033644
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	mili; Piwil1l
Expression	Biased expression in testis adult (RPKM 14.5) and genital fat pad adult (RPKM 0.5) See more
Orthologs	human all

Transcript information (Ensembl)

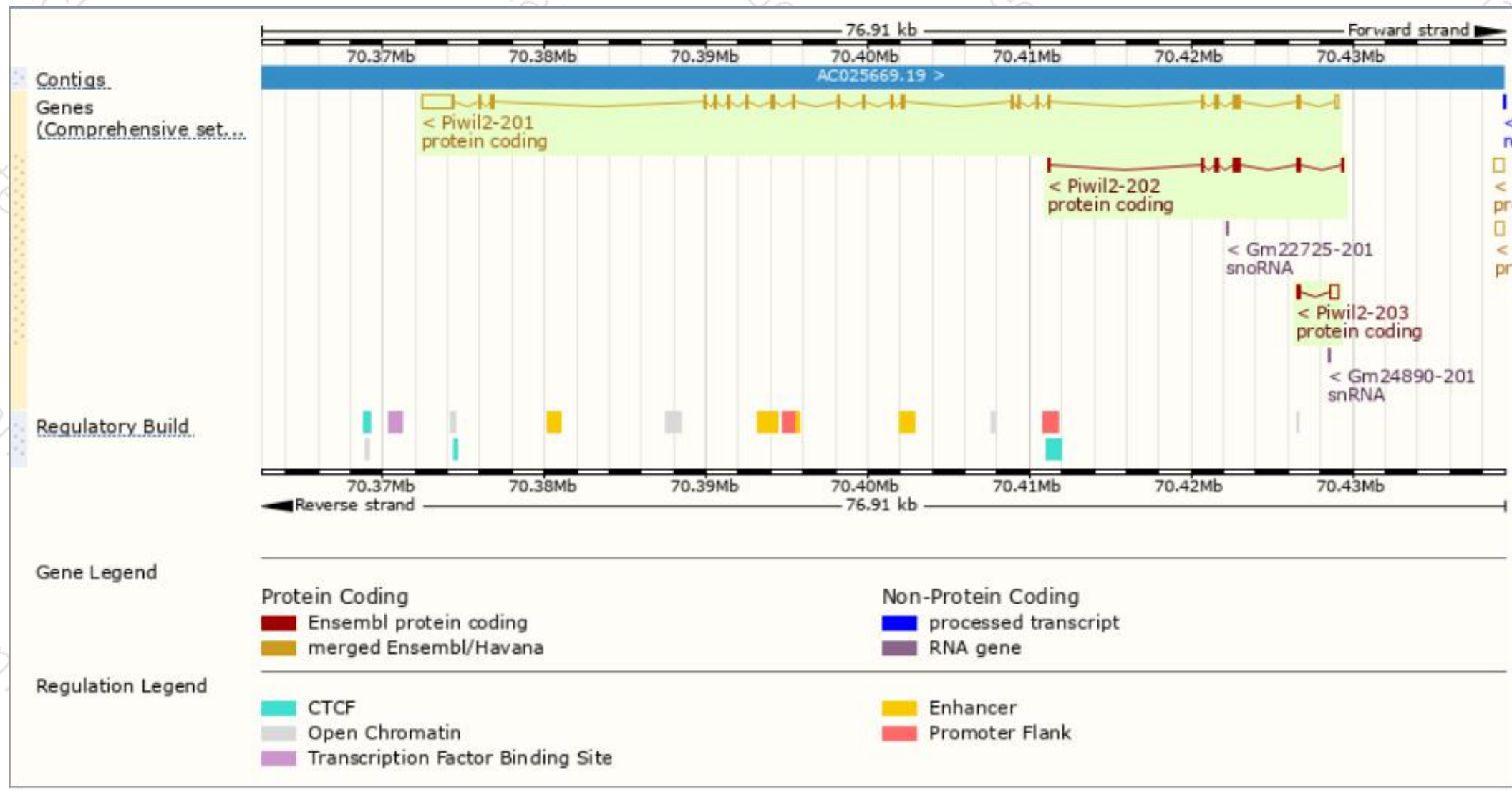
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Piwil2-201	ENSMUST00000048129.5	4916	971aa	Protein coding	CCDS27252	Q8CDG1	TSL:1 GENCODE basic APPRIS P1
Piwil2-202	ENSMUST00000226229.1	962	285aa	Protein coding	-	A0A2I3BRK1	CDS 3' incomplete
Piwil2-203	ENSMUST00000226426.1	666	63aa	Protein coding	-	A0A2I3BRD3	CDS 3' incomplete

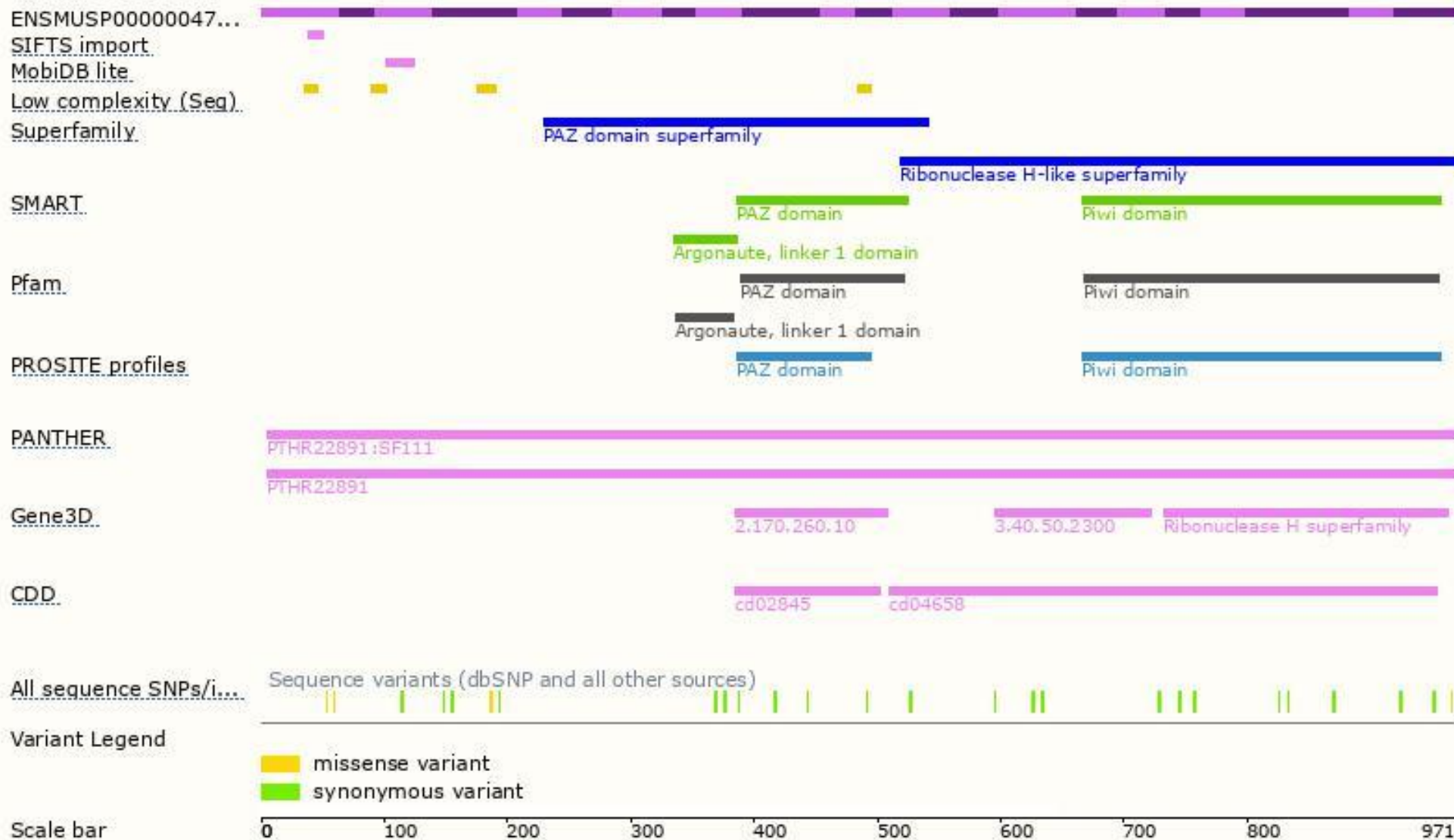
The strategy is based on the design of *Piwil2-201* transcript,The transcription is shown below



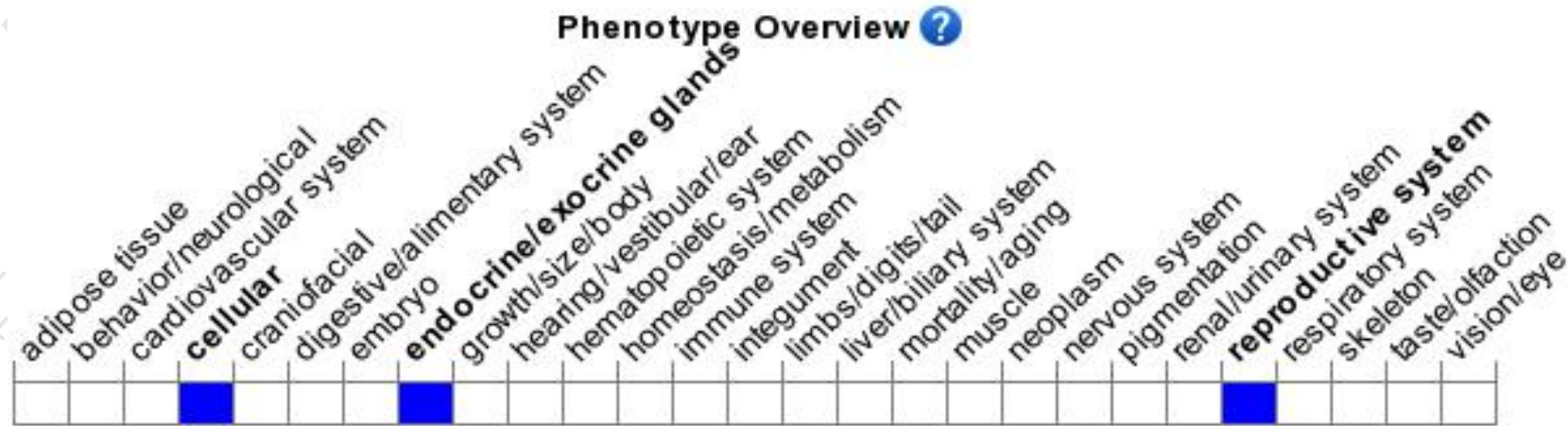
Genomic location distribution



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 knock-out allele exhibit decreased testis weight, azoospermia, and male infertility associated with a complete arrest of spermatogenesis and increased apoptotic cell death during the early prophase of the first meiosis.

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

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