

# ***Piwil2-EGFP* Cas9-KI Strategy**

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**Reviewer**

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# Project Overview

**Project Name**

***Piwil2-EGFP***

**Project type**

**Cas9-KI**

**Strain background**

**C57BL/6JGpt**

# Knockin strategy

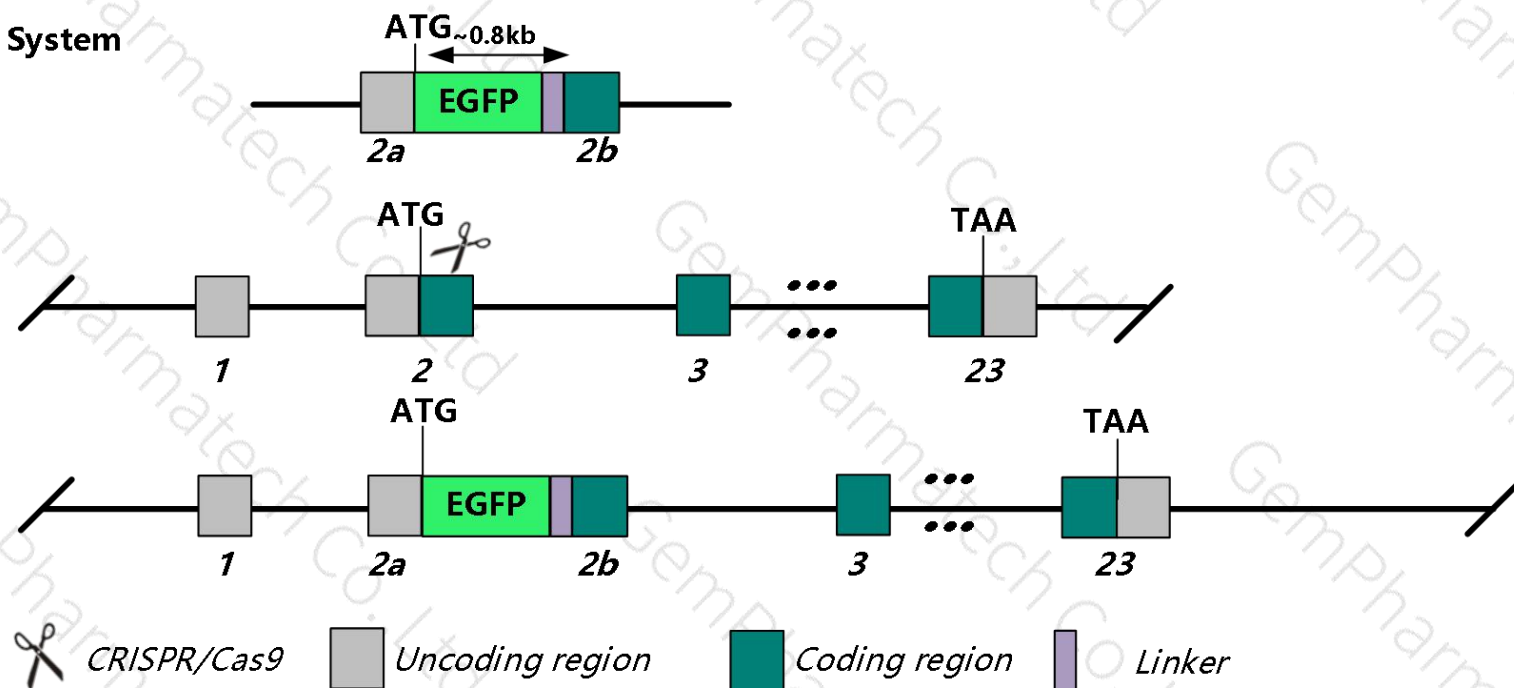
This model will use CRISPR/Cas9 technology to edit the *Piwi2* gene. The schematic diagram is as follows:

## Donor and CRISPR/Cas9 System

Piwi2-201  
ENSMUST00000048129.6

Wild-type allele

Targeted allele



- The *Piwil2* gene has 3 transcripts. According to structure of *Piwil2* gene, *Piwil2-201*(ENSMUST00000048129.6) is selected for presentation of the recommended strategy.
- *Piwil2-201* gene has 23 exons, with the ATG start codon in exon2 and TAA stop codon in exon23.
- We make *Piwil2-EGFP* knockin mice via CRISPR/Cas9 system. CRISPR/Cas9 system and donor will be co-injected into zygotes. Cas9 endonuclease cleavage near start codon(ATG) of exon2 of *Piwil2* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in *EGFP* after start coding(ATG) of *Piwil2* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

- 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 effect of *Gm22725-201* and *Gm24890-201* gene is unknown.
- Insertion of *EGFP* may affect the regulation of the 5' end of the *Piwil2* gene.
- There may be 1 to 2 amino acid synonymous mutation in exon2 of *Piwil2* gene in this strategy.
- The *Piwil2* gene is located on the Chr14. If the knockin mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- The scheme is designed according to the genetic information in the existing database. Inserting a foreign gene after the gene coding region may affect the expression of endogenous and foreign genes. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.



# Gene information (NCBI)

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

Download Datasets

Gene ID: 57746, updated on 2-Oct-2021

### Summary

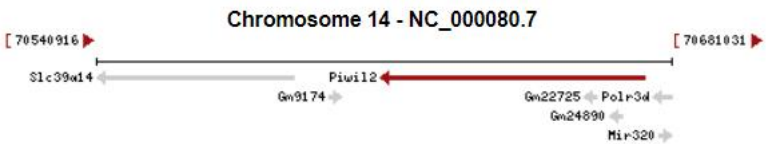
Official Symbol	Piwi2 provided by MGI
Official Full Name	piwi-like RNA-mediated gene silencing 2 provided by MGI
Primary source	MGI: MGI:1930036
See related	Ensembl: ENSMUSG00000033644
Gene type	protein coding
RefSeq status	VALIDATED
Organism	<i>Mus musculus</i>
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	mili; Piwi1l
Expression	Biased expression in testis adult (RPKM 14.5) and genital fat pad adult (RPKM 0.5) <a href="#">See more</a>
Orthologs	<a href="#">human</a> <a href="#">all</a>
<div>NEW</div>	<a href="#">Try the new Gene table</a> <a href="#">Try the new Transcript table</a>

### Genomic context

Location: 14; 14 D2

See Piwi2 in [Genome Data Viewer](#)

Exon count: 25

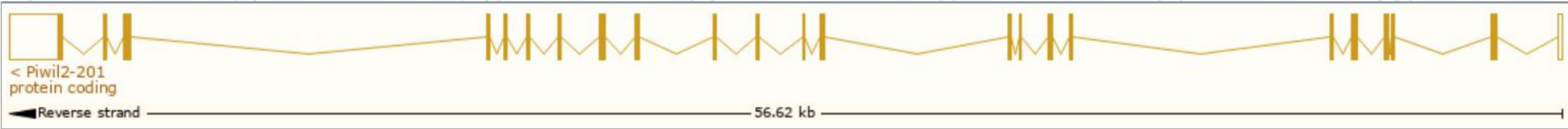


# Transcript information (Ensembl)

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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Piwil2-201	<a href="#">ENSMUST00000048129.6</a>	4916	<a href="#">971aa</a>	Protein coding	<a href="#">CCDS27252</a>	<a href="#">Q8CDG1</a>	GENCODE basic APPRIS P1 TSL:1
Piwil2-202	<a href="#">ENSMUST00000226229.2</a>	962	<a href="#">285aa</a>	Protein coding	-	<a href="#">A0A2I3BRK1</a>	CDS 3' incomplete
Piwil2-203	<a href="#">ENSMUST00000226426.2</a>	666	<a href="#">63aa</a>	Protein coding	-	<a href="#">A0A2I3BRD3</a>	CDS 3' incomplete

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

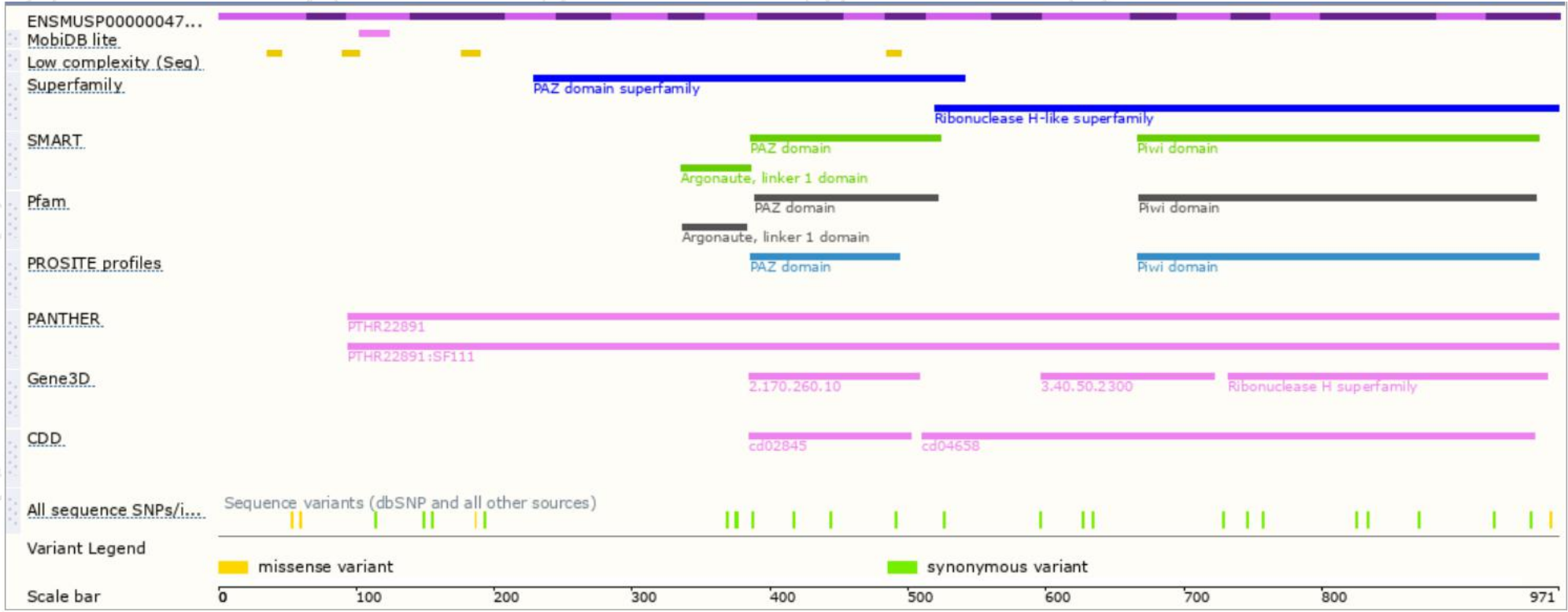


# Genomic location distribution

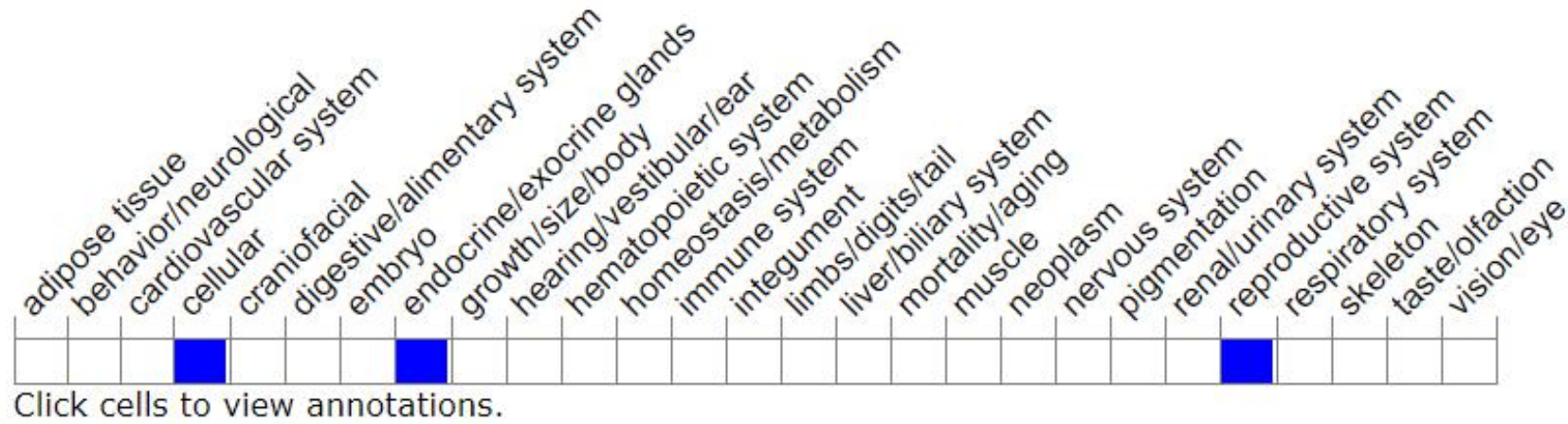




# Protein domain



# Mouse phenotype description(MGI)



*Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(<http://www.informatics.jax.org/marker/MGI:1930036>) .*

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.

# 3xGGGGS(from addgene)

GGAGGCGGCGGTAGCGGAGGAGGCGGGTCCGGCGGC GGCGGTAGT



<http://www.addgene.org/browse/sequence/131121/>



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