

***Mlh3* Cas9-KO Strategy**

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Project Overview

Project Name

Mlh3

Project type

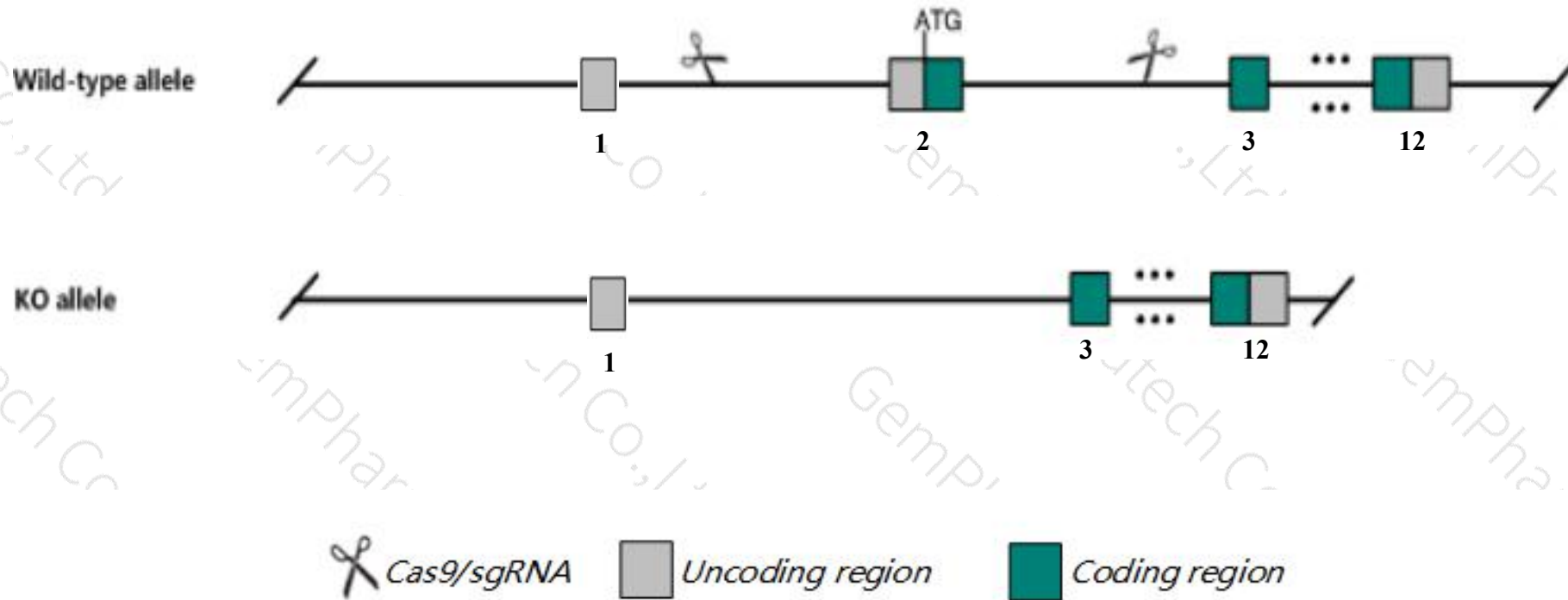
Cas9-KO

Strain background

C57BL/6JGpt

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Mlh3* gene. The schematic diagram is as follows:



Technical routes

- The *Mlh3* gene has 5 transcripts. According to the structure of *Mlh3* gene, exon2 of *Mlh3-201*(ENSMUST00000019378.7) 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 *Mlh3* gene. The brief process is as follows: CRISPR/Cas9 system 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.

- According to the existing MGI data, homozygotes for a targeted null mutation are sterile. Both oocytes and spermatocytes exhibit meiotic block and die.
- The *Mlh3* gene is located on the Chr12. 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)

MIh3 mutL homolog 3 [*Mus musculus* (house mouse)]

Gene ID: 217716, updated on 26-Jun-2020

Summary



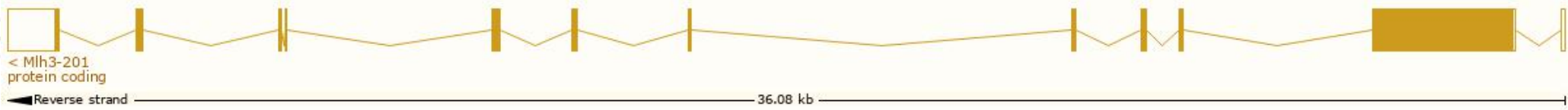
Official Symbol	MIh3 provided by MGI
Official Full Name	mutL homolog 3 provided by MGI
Primary source	MGI:MGI:1353455
See related	Ensembl:ENSMUSG00000021245
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AV125803; BB126472
Expression	Ubiquitous expression in bladder adult (RPKM 2.6), testis adult (RPKM 2.4) and 27 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

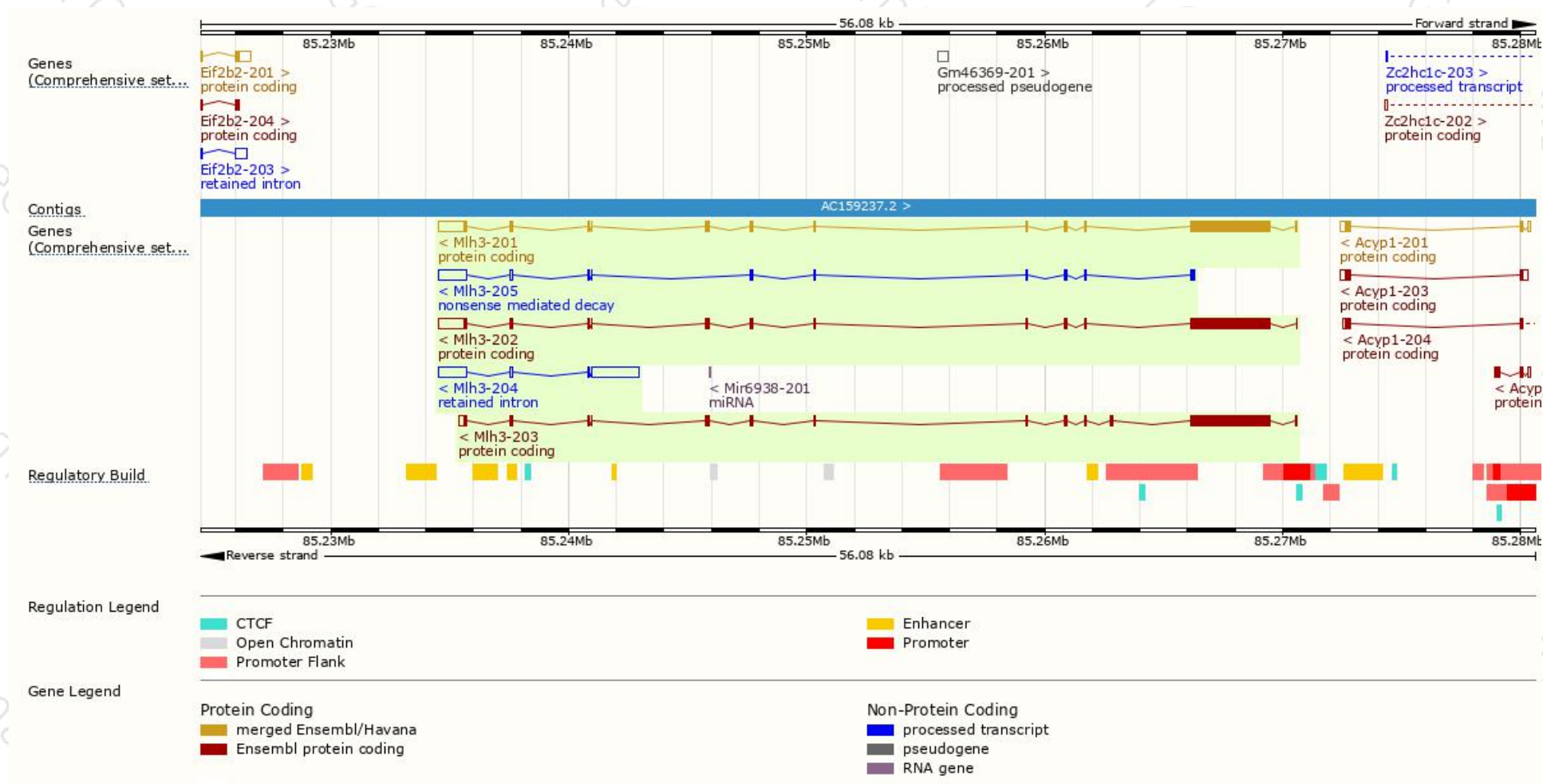
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
MIh3-205	ENSMUST00000223230.1	2052	197aa	Nonsense mediated decay	-	A0A1Y7VJX4	CDS 5' incomplete TSL:1
MIh3-201	ENSMUST00000019378.7	5463	1411aa	Protein coding	CCDS36497	Q68FG1	TSL:1 GENCODE basic APPRIS P2
MIh3-202	ENSMUST00000166821.7	5420	1411aa	Protein coding	CCDS36497	Q68FG1	TSL:1 GENCODE basic APPRIS P2
MIh3-203	ENSMUST00000220854.1	4699	1443aa	Protein coding	-	A0A1Y7VMP7	TSL:5 GENCODE basic APPRIS ALT2
MIh3-204	ENSMUST00000223005.1	3450	No protein	Retained intron	-	-	TSL:2

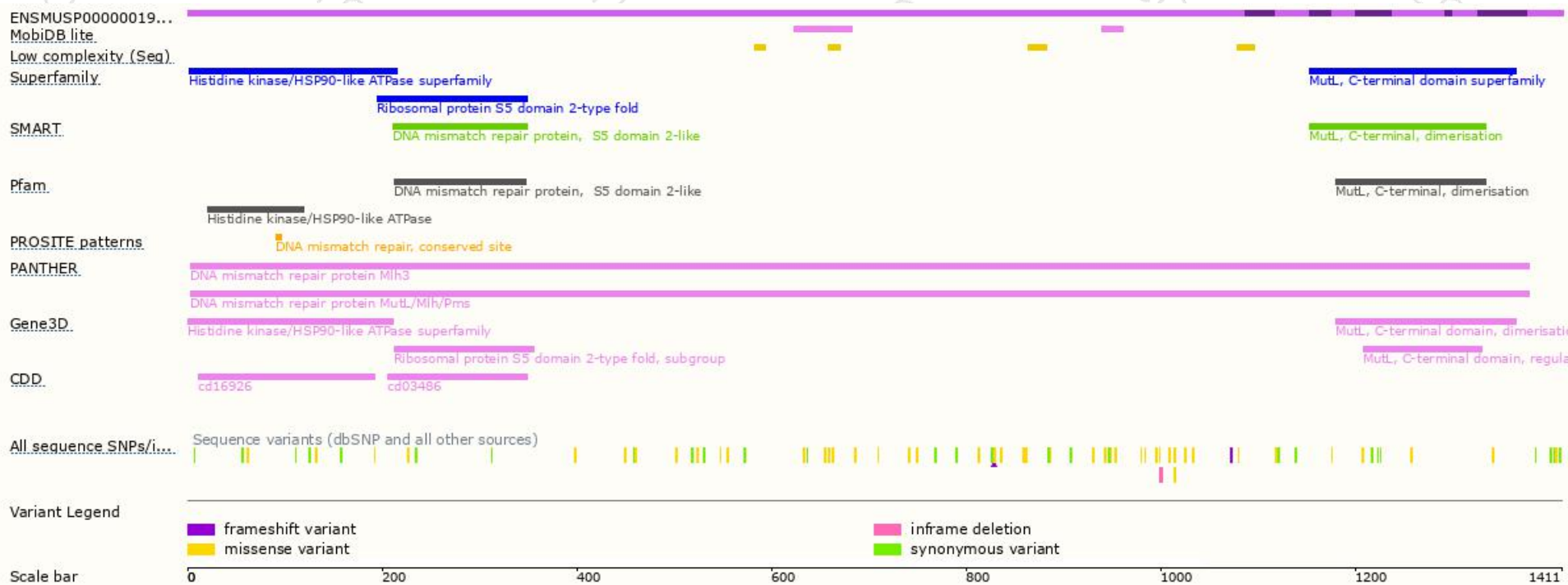
The strategy is based on the design of *MIh3-201* transcript,the transcription is shown below:



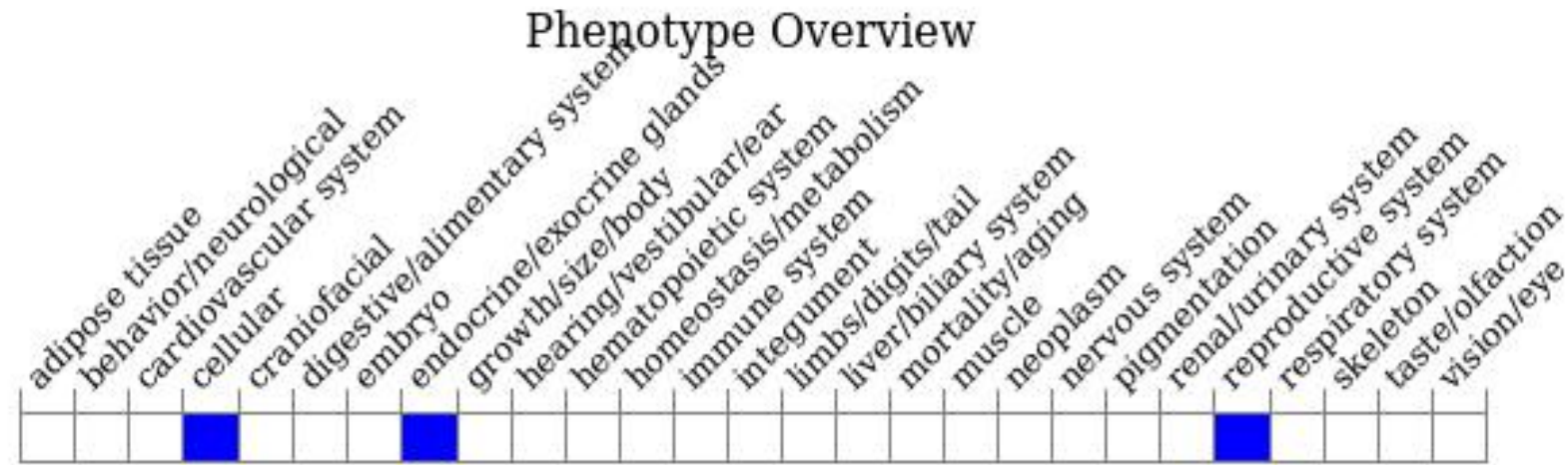
Genomic location distribution



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, homozygotes for a targeted null mutation are sterile. Both oocytes and spermatocytes exhibit meiotic block and die.

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

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